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VALORISATION OF FOOD INDUSTRY BY-PRODUCTS THROUGH THEIR USE AS FUNCTIONAL INGREDIENTS AND FOR SHELF-LIFE EXTENSION OF FOOD PRODUCTS

Ph.D. Thesis

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To my precious family.

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ABSTRACT

Food industry produces huge amounts of waste, which are characterised by significant cost of management and environmental impact. Therefore, in recent years, great attention has been focused on fruits and vegetables by-products valorisation, by their utilization to produce value-added foodstuffs, also achieving economic and environmental benefits.

Based on this awareness, the aim of this Ph.D. research project consisted in developing efficient and sustainable approaches for the valorisation of food industry by-products, to produce new food products enriched with bioactive compounds, such as bakery goods, and to develop strategies to extend the shelf life of minimally processed vegetables, such as edible coating. Indeed, the addition of bioactive compounds in food matrices could be a valid alternative to the use of synthetic preservatives.

For this purpose, the scientific approach used was divided in three steps: by-products characterisation and extraction of bioactive compounds, production of enriched bakery goods, formulation of polysaccharide enriched edible coating and their application in minimally processed vegetables.

This study has been evaluated on waste from the citrus processing industry (lemon peel, pulp and seed) and red onion processing (outer dry and semi-dry layers and apical and basal trimmings).

In particular, the characterisation and valorisation step showed that these wastes are rich in bioactive compounds, such as eriocitrin and hesperidin for lemon by-products (1167.28±0.25 and 1694.98±0.36 mg kg^{-1} dry weight, respectively) and quercetin for the onion ones (5322.61 ± 0.32 mg kg^{-1} dry weight), with significant antioxidant and antimicrobial properties.

Based on these results, specific processes have been designed that exploit the application of these by-products in food production: (i) biscuits formulated with the addition of bioactive compounds obtained from lemon by-products; (ii) antioxidant extract from onion processing by-products used as food ingredients in bread production. The obtained bakery goods were evaluated for their phenolic content, antioxidant activity, phisico-chemical parameters and sensory acceptability. Obtained results pointed out the possibility to produce new functional food products promoting at the same time the utilization of waste derived food.

Finally, different formulations of enriched edible coatings (alginate, pectin and gellan gum based) were studied and applied on minimally processed vegetables, to estimate their shelf life in terms of respiration rate, polyphenol and bioactive compounds content, antioxidant activity, enzyme activity (PPO, POD, PME) and maintenance of quality parameters, such as colour and texture.

The approaches studied in this research for the valorisation of food industry by-products could constitute an innovative and sustainable way of food production, with relevant economic and

environmental impacts at the industrial level. They could be considered as a starting point for more informed decisions to invest in more sustainable production strategies.

RIASSUNTO

L'industria alimentare produce enormi quantità di rifiuti, caratterizzati da significativi costi di gestione e impatto ambientale. Pertanto, negli ultimi anni, grande attenzione è stata incentrata sulla valorizzazione dei sottoprodotti di frutta e verdura, mediante il loro utilizzo nella produzione di alimenti a valore aggiunto, ottenendo anche benefici di carattere economico e ambientale.

Sulla base di tali consapevolezze, l'obiettivo di questo progetto di dottorato è stato quello di sviluppare approcci efficienti e sostenibili per la valorizzazione dei sottoprodotti dell'industria alimentare, al fine di produrre nuovi prodotti alimentari arricchiti di composti bioattivi, come ad esempio i prodotti da forno, e sviluppare nuove strategie per estendere la shelf life di vegetali minimamente processati, come ad esempio coating edibili. L'addizione di composti bioattivi nelle matrici alimentari, infatti, potrebbe essere una valida alternativa all'uso di conservanti sintetici.

Per tale scopo, l'approccio scientifico utilizzato è stato suddiviso in tre fasi principali: caratterizzazione dei sottoprodotti ed estrazione dei composti bioattivi, produzione di prodotti da forno funzionali, formulazione di coating edibili arricchiti di composti utili e loro applicazione in vegetali minimamente processati.

Questo studio è stato effettuato considerando scarti dell'industria di trasformazione degli agrumi (buccia, polpa e semi di limone) e della lavorazione della cipolla rossa (strati esterni secchi e semisecchi e rifilature apicali e basali). In particolare, la fase di caratterizzazione ed estrazione ha mostrato che questi scarti sono ricchi di composti bioattivi come l'eriocitrina e l'esperidina, per i sottoprodotti del limone (1167.28±0.25 e 1694.98±0.36 mg kg^{-1} peso secco, rispettivamente), e la quercetina, per quelli di cipolla (5322.61±0.32 mg kg^{-1} peso secco), con significative proprietà antiossidanti e antimicrobiche.

Sulla base dei risultati ottenuti, sono stati progettati formulazioni di prodotti da forno che sfruttano l'applicazione di questi sottoprodotti nel processo produttivo: (i) biscotti formulati con l'aggiunta di composti bioattivi ottenuti dai sottoprodotti del limone; (ii) aggiunta di estratto antiossidante ottenuto dai sottoprodotti della lavorazione della cipolla nella produzione di pane in cassetta. I prodotti da forno ottenuti sono stati valutati in merito al loro contenuto fenolico, attività antiossidante, parametri fisico-chimici e accettabilità sensoriale. I risultati hanno evidenziato la possibilità di produrre nuovi prodotti alimentari funzionali promuovendo allo stesso tempo l'utilizzo di scarti alimentari.

Infine, diverse formulazioni di coating edibili arricchiti (a base di alginato, pectina e gomma di gellano) sono state studiate e applicate su vegetali minimamente processati, per stimarne la loro shelf life in termini di tasso di respirazione, contenuto di polifenoli e composti bioattivi, attività

antiossidante, attività enzimatica (PPO, POD, PME) e mantenimento dei parametri di qualità, come colore e consistenza.

Gli approcci studiati nella presente ricerca per la valorizzazione dei sottoprodotti dell'industria alimentare potrebbero costituire una modalità innovativa e sostenibile di produzione alimentare, con positivi impatti economici e ambientali rilevanti. Potrebbero essere considerati come un punto di partenza per decisioni più consapevoli nei riguardi di strategie di produzione più sostenibili.

KEYWORDS

Lemon by-products; onion solid waste; bioactive compounds; bakery products; edible coating; minimally processed vegetables; ready-to-eat.

INTRODUCTION

Every year food industry generates a considerable amount of by-products and critical concerns are correlated with its disposal (Rusu et al., 2018). One third of fruit and vegetables in such a way as peels, pulps, seeds, steams or skins may be removed during preparation and processing of fruit and vegetables, thus creating 'waste', leading to a reduction in the nutritional potential of the final products (O'Shea et al., 2012). In this context, by-products could be defined as the organic matrices remaining after the production of fruit and vegetable-based products, consisting of those parts of the raw material that are not used in the production process of the main foodstuffs. Currently, by-products are disposed of through animal feed, landfill or incineration, causing potentially negative environmental effects and representing an additional expense for the producer (Angulo et al., 2012; Leroy et al., 2007). Moreover, the production of these wastes is often closely linked to a defined annual seasonality depending on the type of food matrix, so that a large quantity of a specific by-products is generated in a short period of time. This makes it very difficult to dispose of and recycle industrial food residues, which is why it is necessary to look for different alternatives for their valorisation, to reduce their environmental impact and obtain a clear economic benefit. In fact, the priority is to develop new commercial perspectives that aim, through careful, sustainable and cost-effective management, at an advantageous view of a material that is mostly considered food waste, such as obtaining energy, application to agricultural land and use in the livestock and food sectors.

Nowadays, consumers are increasingly aware of and interested in a healthy lifestyle. In this respect, researchers are more and more involved in discovering alternative uses of these food waste as sources of potential added-value ingredients (O'Shea et al., 2012). Schieber et al. (2001) highlighted the high availability of bioactive compounds, such as carotenoids, polyphenols and tocopherols, in food industry by-products and their potential to be easily used as functional ingredients in food production. Specifically, waste obtained from fruit and vegetable processing is becoming increasingly prominent as a new and economical source of healthy functional ingredients. (Ayala-Zavala et al., 2011), as well as for the growing tendency of consumers to avoid foods prepared with preservatives of chemical origin (Rusu et al, 2018). Indeed, several studies have been recently carried out, intended to discover natural alternatives, like food industry by-products, rich in bioactive compounds with functional properties for human health (Soccol et al., 2017; Zhang et al., 2016). The inedible portions of food might be characterized by a higher nutritional content than their corresponding edible part. Indeed, these inedible sections contain a great amount of bioactive compounds with significant antioxidant activities and present phytochemical profiles distinct from other parts (Gondim et al., 2005; Remorini et al., 2008).

In this respect, phenolic compounds are of great importance in the nutritional, organoleptic and commercial characteristics of fruit. Recently, much attention has been paid recently not only to the edible parts, but also to the bioactive compound content of fruit and vegetables processing by-products such as peels, seeds, and skins (Russo et al., 2018). These compounds are renowned for their several beneficial influences on human health, among which antioxidant (Lachman et al., 2013; Rockenbach et al., 2011), cardioprotective (Otero-Pareja et al., 2015), anti-inflammatory (Manca et al., 2016; Rodríguez-Morgado et al., 2015), antimicrobial (Oliveira et al., 2013), antiaging (Xia et al., 2010) and anticancer (Jara-Palacios et al., 2015; Tounsi et al., 2009) activities are the most acknowledged (Pintać at al., 2018). Polyphenols, such as flavonoids and phenolic acids, are among the most active natural antioxidants, which act as natural antioxidants donating electrons or hydrogen atoms to reactive oxygen species (ROS) in order to prevent the degradation of vital molecules or cellular damage and reduce the risk of degenerative diseases (Shahidi & Ambigaipalan, 2015; Oroian & Escriche, 2015; Tepe et al., 2006). In contrast to that, common synthetic antioxidants, like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butyl hydroquinone (THBQ) have been reported to be dangerous for human health and could have potential health risks, as cancer (Valantina & Neelamegam, 2012; Krishnaiah et al, 2011). This problem has led to search alternative antioxidants of natural origin, considered safer than synthetic ones (Mei et al., 2014; Can-Cauich et al., 2017). Antioxidants could be applied as food additives to slow down the oxidation of lipids or other molecules by constraining the beginning or propagation of oxidative chain reactions (Saravanan & Aradhya, 2011). Figure 1 shows the classification of the main phenolic and synthetic antioxidants. Besides the antioxidant activity, various studies (Smeriglio et al., 2017; Do Prado et al., 2014) also confirmed the antimicrobial properties of the phenolic compounds, considering them as excellent substitutes to chemical preservatives (Rusu et al., 2018).

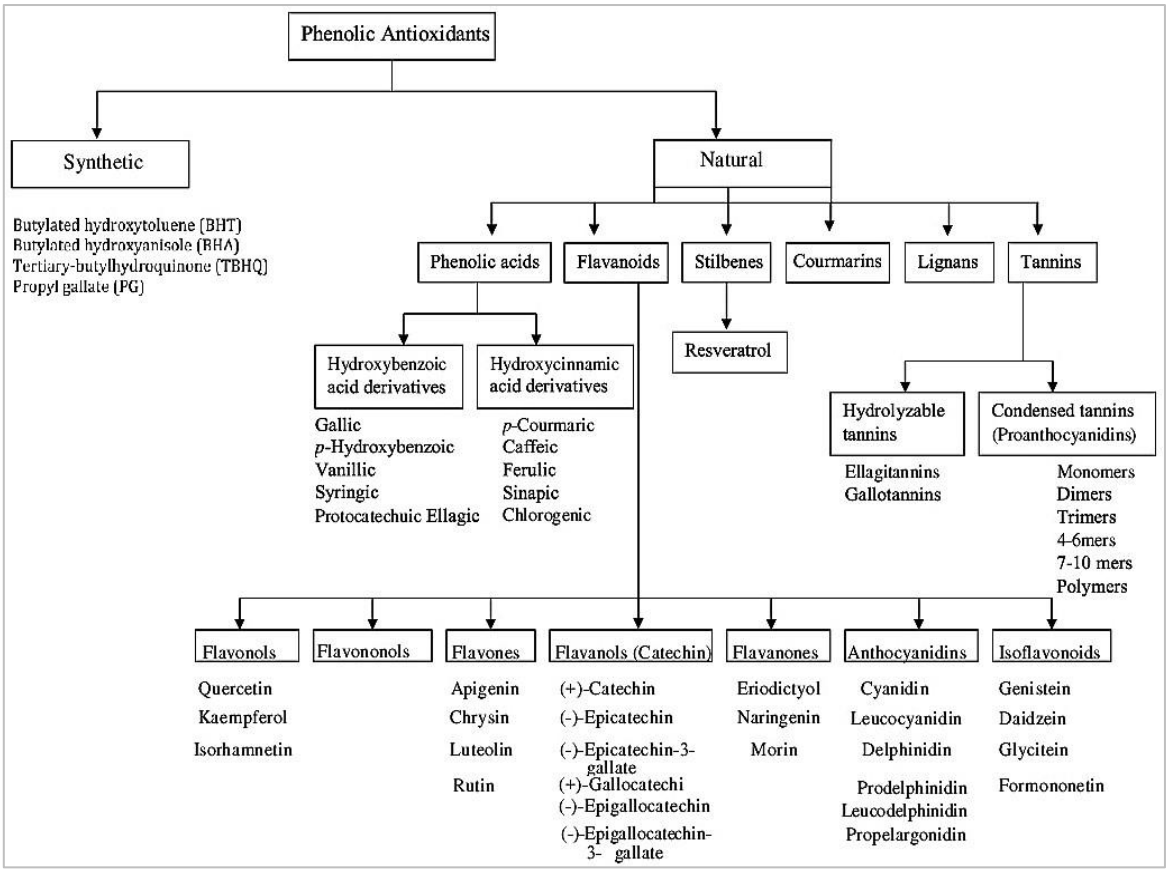

Figure 1: Classification of phenolic antioxidants (Shahidi & Ambigaipalan, 2015).

Furthermore, another implemented strategy for the valorisation of food industry by-products consists in the formation of edible coatings, generally used for the extension of fruits and vegetables shelf life in an environmentally friendly way, which can also be safely eaten as part of the product and without adding undesirable characteristics to the foodstuff (Raghav et al., 2016). Recently, new edible films and coatings formulations have been created with the addition of different and edible antimicrobial compounds to preserve fresh fruits and vegetables (Valencia-Chamorro et al., 2011). They prevent loss of firmness and moisture, control maturation, gas exchanges and respiratory rate, prevent oxidative browning and decrease growth of microorganism, as well as by reducing the physiological disorders in fresh cut fruits and vegetables (Kumar & Bhatnagar, 2014; Dhall, 2013).

As part of the continuing research for new natural ingredients rich in valuable component with potential uses as functional ingredients to improve the functionality and safety of food products, in this PhD research, the processing by-products of two of the main Mediterranean crops were considered for a food grade recovery of bioactive compounds therein and their subsequent use in food production or in the formulation of strategies to extend the shelf life of minimally processed vegetables.

Lemon (Citrus limon L.): once the juice and essential oil have been extracted from lemons, the remaining by-products constitute approximately 50% of the original fruit, which consist of peels (albedo and flavedo), pulp and seed. Normally these are used for animal feed or for pectin extraction (Lario et al., 2004). However, some research studies have highlighted the potentially healthy attributes of lemon by-products, characterized by a high content of antioxidants and polyphenols, like most citrus fruits (O'Shea et al., 2012).

Onion (Allium cepa L., cv. Tropea): around 500.000 tonnes of onion by-products are being produced in the EU every year (Benítez et al., 2011). This 'waste' mainly consists of onion dried skins, two outer fleshy scales and apical and basal trimmings. Unfortunately, onion waste is not ideal for the common vegetable waste disposal, due to its too pungent aromatic characteristics for animal feed and its phytopathogenic agents that prevent its use as a soil fertiliser (Benítez et al., 2011). Thus, researchers are considering its application as food ingredient, thanks to its nutritional properties, nutrients, such as dietary fibre, and the presence of nutraceuticals, like flavonoids and phenolic acids (Griffiths et al., 2002; Ng et al., 2000; Slimestad et al., 2007; Roldán et al., 2008).

The production of the so-called functional foods, which consist in foods playing a significant contribution to the prevention and onset of risk factors for several diseases or improving the physiological functions, have taken on an increasingly important role (Ballard et al., 2009; Panusa et al., 2013; Sgarbossa et al., 2015; Giacomazza et al., 2018).

Positive results from several studies have stated that the production of food products with food industry by-products is possible, reporting encouraging results in terms of content of bioactive compounds in the final products (Ozkan et al., 2007; Özcan & Ayar, 2003; Nadeem et al., 2015; Abid et al., 2017; Azabou et al., 2016; O'Shea 2012). Further research is ongoing in this sector, which is continuing to gain interest, focusing on the design of more efficient systems for enriching food matrices with phenolic compounds and on the development of edible coatings that can ensure greater stability of natural phenolic compounds over time and, consequently, a significant industrial impact.

ORGANISATION

The aim of this Ph.D. research was focused on the valorisation of food industry by-products through various uses in the food sector, such as food production or strategies to extend the shelf life of ready-to-eat products. Indeed, the obtained results are intended to encourage an innovative and more sustainable food production, considering food by-products as natural sources of bioactive compounds.

Specifically, the Ph.D. project was organised into three main steps: the first one consisted in the characterization of food industry by-products, such as lemon peel, pulp and seed and onion solid waste, and the developing of suitable techniques of food grade extractions of bioactive compounds:

Chapter 1: *"Green-sustainable extraction techniques for the recovery of antioxidant compounds from "Citrus limon" by-products".*

Chapter 2: *"Development of suitable food grade extraction techniques of bioactive compounds from onion solid waste".*

Then, the research included the production of functional food using the previously characterized extracts as functional ingredients (Chapter 3: "*Production of functional bakery products*"). In particular, the decision to produce bakery goods, like biscuits (Chapter 3.1: *"Functionalized biscuits with bioactive ingredients obtained by citrus lemon pomace"*) and toast bread (Chapter 3.2: *"Shelf-life study of toast bread enriched with an antioxidant extract obtained from onion processing by-products"*), enriched with bioactive compounds was since these products are widely consumed worldwide but, at the same time, known as high in fat and carbohydrates and not always associated with a healthy lifestyle.

Finally, the last step (Chapter 4: *"Edible coating and their application in fresh-cut vegetables"*), developed with the collaboration of the University of Lleida (Spain) – Department of Food Technology- involved the formulation of edible coatings as strategies to extend the shelf-life of minimally processed vegetables, as radish (Chapter 4.1: *"Shelf-life extension of minimally processed radish (Raphanus sativus L.) by using of citrus by-products"*), carrots (Chapter 4.2: "*Shelf-life extension of minimally processed carrots through the application of a pectin-based coating added with an antioxidant extract obtained from lemon by-products"*) and potatoes (Chapter 4.3: *"Anti-browning effect of edible coating added with onion processing by-products in raw minimally processed potatoes"*), thanks to the presence of bioactive compounds previously extracted from food industry by-products. In this case, beyond the valuable compounds content and the antioxidant activity of the final products, the influence of the various coating formulations on the enzymatic activity of the main enzymes involved in the degradation of ready-to-eat vegetables, such as

polyphenol oxidase (PPO), peroxidase (POD) and pectin methylesterase (PME), was also determined. Figure 2 shows a schematic summary of the Ph.D. project.

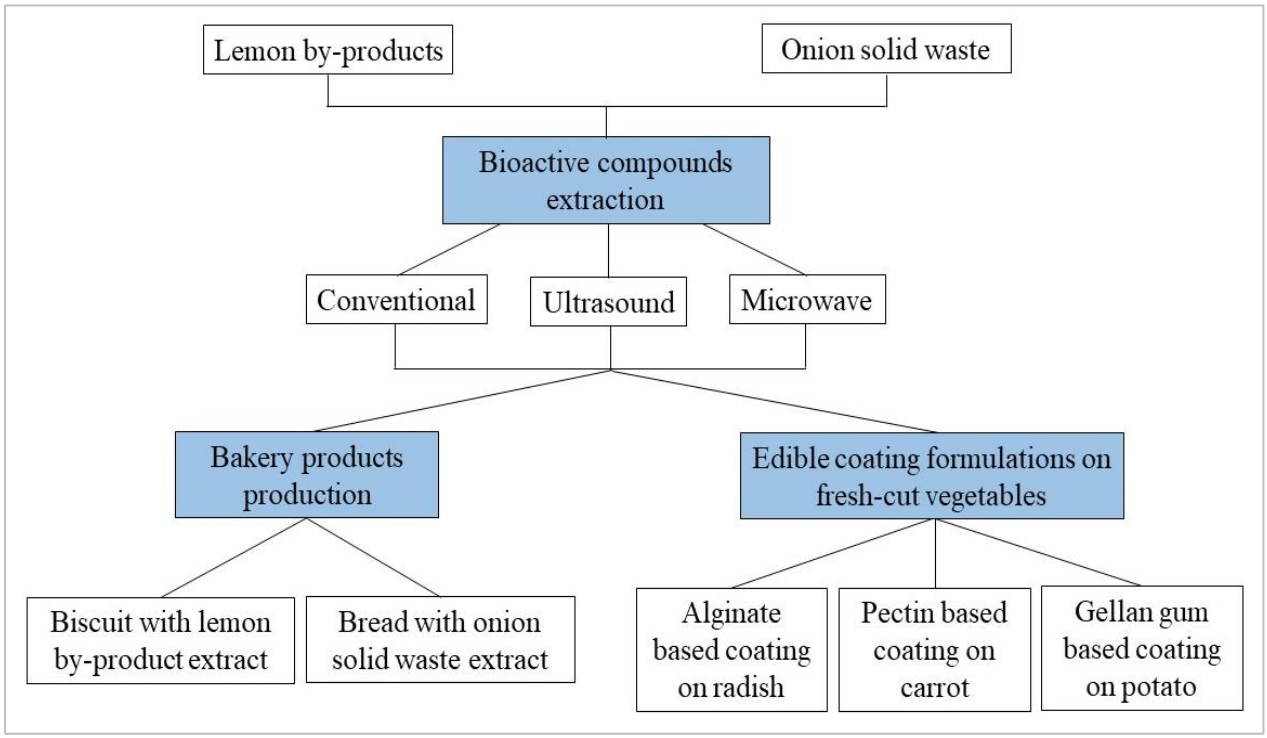

Figure 2: Ph.D. project plan.

1. GREEN-SUSTAINABLE EXTRACTION TECHNIQUES FOR THE RECOVERY OF ANTIOXIDANT COMPOUNDS FROM "CITRUS LIMON" BY-PRODUCTS

1.1. INTRODUCTION

Food industry produces considerable quantity of solid and liquid waste, obtained from the conversion of feedstock into final products, representing a severe environmental issue due to their content of organic substances. On the other hand, interesting perspectives arise from the huge amount of food by-products. Among fruit and vegetables, citruses are one of the world's most abundant fruit crop and the processing of fruits, such as lemon, produces considerable quantities of by-products, often including useful compounds in their peels, pulps, and seeds (Putnik et al., 2017), which could be extracted and utilized as natural antioxidants to avoid oxidation of some foodstuff or may be applied in functional foods formulations (El-ghfar et al., 2016; Garcia-Castello et al., 2015). Among polyphenols, flavonoids are characterized by relevant biological actions, which include antioxidant, anti-inflammatory, anticancer, antiviral, and anti-mutagenic activity (Gabriele et al., 2017).

Extraction represents the first step to get valuable compounds from a food matrix and several optimized techniques can be developed to obtain them from peel wastes, by conventional solvents (Siahpoosh & Javedani, 2016), microwave-assisted extraction (Li et al., 2012; Simic´ et al., 2016), assistance with cyclodextrin (Albahari et al., 2018), enzymes (Ladole et al., 2018), ultrasounds (Chemat et al., 2017) and subcritical water (Lachos-Perez et al., 2018). Among these, microwave-assisted extraction could be an alternative and time saving extraction technique: the water molecules in the plant sample are heated in a short time and this causes a warming up and extension of the cell walls, resulting in their consequent rupture and in a leakage of internal constituents outside of the cells (Rodsamran & Sothornvit, 2019).

In general, for the extraction of polyphenolic compounds from citrus pulp and peel, organic solvents, as ethanol, methanol and their mixture with water are generally used. Although lots of organic solvents, like methanol, are efficient solvents for extraction of polyphenols, ethanol categorized under GRAS (Generally Recognized as Safe) is preferred because of its application in the food system. Water and hydroalcoholic mixture are the most employed solvents in food grade extraction: presence of water in solvent might lead to an increase in the extraction rate since water could be helpful to improve distension of plant material, which allows an increase in the contact surface area between food matrix and solvent (Hayat et al., 2009). Indeed, the yield of phenolic compounds from plants is

correlated to the polarity, solubility, as well as specific extraction parameters such as solvent nature and concentration, temperature, and time (Safdar et al., 2017).

Furthermore, in citrus peels, phenolic acids are often related to several plant components through ester and glucoside bonds, while flavonoids can be either in the free (aglycones) or bound (glycoside) forms, with the former having higher antioxidant properties compared to the latter: for this reason, applying heat on citrus by-products during the extraction process may boost the liberation of polyphenols by breaking down both ester and glucoside bonds, which tend to be very stable at room temperature (Papoutsis et al., 2018a). The extraction rate depends not only on the nature of the applied solvents and the solvent: sample ratio, but also on the extraction time and temperature and the chemical composition and physical characteristics of the matrices (Sharma et al., 2019). As reported by De Bruno et al. (2018), a longer extraction time does not always correspond to a higher extraction yield.

Therefore, one of the main purposes of this investigation was focused on the effect of the working conditions (solvent, time, and temperature) on the lemon by-products extraction in order to obtain extracts with a high quantity of phenolic compounds that, consequently, express an equally high antioxidant activity. Moreover, this study aimed to develop environmentally friendly and food grade techniques (conventional, ultrasound and microwave) having higher extraction efficiency.

1.2. MATERIALS AND METHODS

1.2.1. Material

Lemon by-products samples (*Citrus limon*), consisting of peels, pulp and seeds, were supplied by Agrumaria Reggina company located in Reggio Calabria (Italy) after the extraction of lemon juice and essential oils. Lemon by-products were initially dried at a temperature of 50°C up to a final moisture content of 12% and stored in polyethylene bags under vacuum to avoid rehydration until subsequent extraction procedures of the bioactive compounds.

1.2.2. Chemicals

Standards of gallic acid, *p*-coumaric acid, ferulic acid, eriocitrin, narirutin, hesperidin, neohesperidin, naringin were purchased from Merck (Darmstadt, Germany). Apigenin, caffeic acid and rutin were purchased from Extrasynthèse (France). The solvents used for chromatographic analysis (methanol, water, and acetonitrile) were ultra-high-performance liquid chromatography- mass spectrometry (UHPLC)-MS grade (Carlo Erba, Italy). 2,2′- Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2- diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu's phenol reagent, and Trolox were purchased from Sigma Chemical Co. (USA).

1.2.3. Experimental procedure

The recovery of antioxidant compounds has been carried out through three different extraction techniques: conventional solid-liquid extraction, ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE). The extractions were performed using two different food grade extraction solvents, water (H_2O) and ethanol: water mixture (EtOH:H_2O, 50:50). Three temperatures (25, 40 and 70°C) were tested. The extraction times for MAE were 5 and 15 minutes, and those for conventional and UAE were: 30, 60 and 120 minutes. Figure 3 shows the experimental scheme.

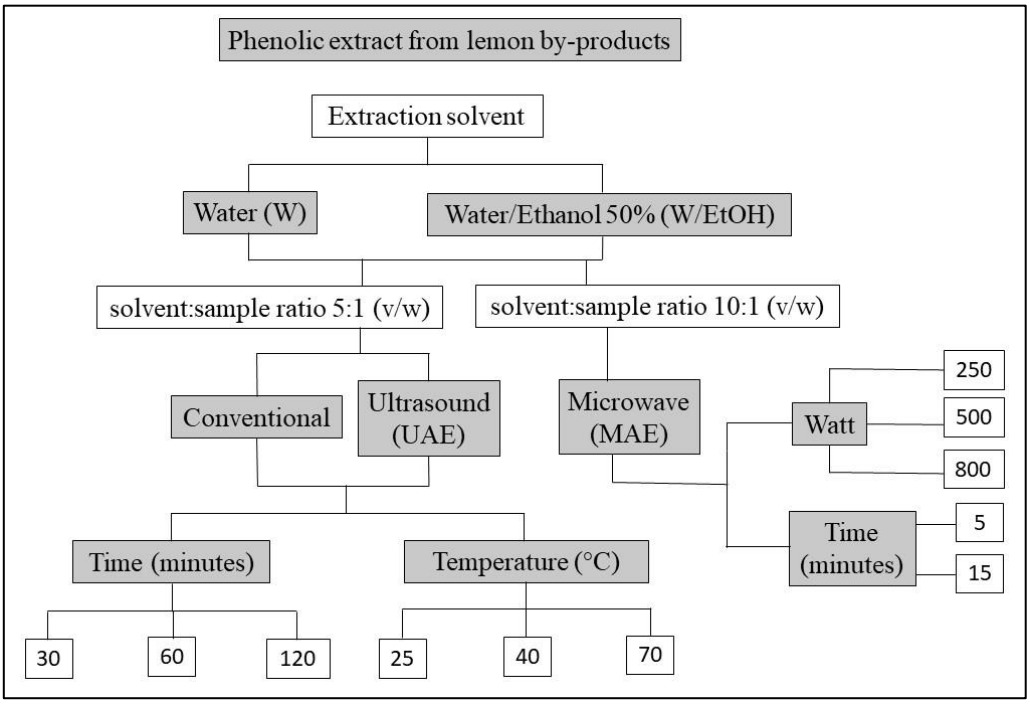

Figure 3: Simplified outline of the experimental procedures of lemon by-products extractions.

- **Conventional solid-liquid extraction**

The extraction was performed according to Papoutsis et al. (2018b) with some modifications.
Briefly, 50 mL of solvent were added to 10 g of dried and ground lemon by-productss and placed on a heating magnetic stirrer for the desired times and temperatures, monitored by a digital thermometer. Subsequently, samples were centrifuged (NF 1200R, Nüve, Ankara, Turkey) at 5000 rpm for 5 min at 4°C, filtered through a Büchner apparatus with 0.45 µm filter paper and the resulting extracts were diluted with the respective extraction solvent to 50 mL. The extracts were then filtered with 0.45 µm nylon filters and stored at -20°C until subsequent analyses.

- **Ultrasound-assisted extraction**

Ultrasound-assisted extraction (UAE) was performed using a Sonoplus Ultrasonic homogeniser, Series 2000.2, HD 2200.2 (BANDELIN, Ultraschall seit 1955), composed of an inox jug with a

capacity from 20 to 900 mL, a VS 70 T probe of 13 mm and a maximum permitted amplitude setting of 100%. The ultrasonic generator converts the mains energy input (mains frequency 50 or 60 Hz) into high-frequency energy (frequency of 20 kHz), guaranteeing the reproducibility of the process parameters and the validation of the extraction procedure.

In the ultrasonic homogenisers used for the extraction, the desired temperature conditions have been achieved through the control and regulation of radiation rate over time, in terms of radiation amplitude (expressed as percentage) and radiation rate per second, monitoring the temperature trend throughout the extraction time with a digital thermometer. It was found that by applying a specific radiation amplitude (ω) and pulsation time, in relation to the frequency of 20 kHz ± 500 Hz, it was possible to obtain a corresponding temperature for the entire extraction process:

- 30 minutes at 25°C (ω = 10%, pulsation time on 1s off 15s), 40°C (ω = 20%, pulsation time on 1s off 5s), 70°C (ω = 50%, pulsation time on 1s off 1s);
- 60 minutes at 25°C (ω = 10%, pulsation time on 1s off 15s), 40°C (ω = 25%, pulsation time on 1s off 5s), 70°C (ω = 50%, pulsation time on 1s off 1s);
- 120 minutes at 25°C (ω = 10%, pulsation time on 1s off 30s), 40°C (ω = 20%, pulsation time on 1s off 10s), 70°C (ω = 50%, pulsation time on 1s off 1s).

Subsequently, each sample was treated as described above for conventional extraction.

- **Microwave-assisted extraction**

The extraction was performed according to Li et al. (2012), with some modifications. In the Microwave Digestion System (ETHOS EASY, Millestone, Bergamo, Italy) used for the extraction, the thermal conditions have been achieved through control (by easyTEMP thermal sensor - ATC-CE) and regulation of the applied microwave power (Watt), acquiring for each applied power the corresponding temperature. Specifically, it was found that:

- 250 W corresponds to 25°C;
- 500 W corresponds to 40°C;
- 800 W corresponds to 70°C.

In brief, 2.5 g of dried lemon by-productss were ground and dissolved in 25 mL of solvent extraction, homogenized with ultra-turrax apparatus (IKA T 25, Staufen, Germany) and transferred into PTFE-TFM vessels of 100 mL (SK-15 easyTEMP, high-pressure rotor). The vessels were placed at the centre of the microwave apparatus, heated to temperature in 3 minutes and held at temperature for a certain extraction time (5 or 15 minutes), according to the experimental design. After microwave heating, the mixtures in the extraction vessels have been left to cool down to room temperature in 10 minutes. Subsequently, each sample was treated as described above for other extraction techniques.

1.2.4. Analytical methods

1.2.4.1. Total phenolic content (TPC)

TPC was determined according to the method reported by González-Molina et al. (2009), with appropriate modifications. 0.2 mL of extract were placed inside a 25 mL flask and mixed with 5 mL of deionized water and 1 mL of Folin-Ciocalteu reagent. After 8 minutes, 10 mL of saturated sodium carbonate solution (Na_2CO_3) at 20% (w/v) was added and made up to volume with deionized water. At the same time, the solution used as a blank was prepared. The mixtures were incubated for two hours at room temperature and in the dark.

The absorbance of the samples was measured at 765 nm against a blank using a double-beam ultraviolet-visible spectrophotometer (Perkin-Elmer UV- Vis λ2, Waltham, Massachusetts, U.S.) and comparing with a gallic acid calibration curve (concentration between 1 and 10 mg L^{-1}). The results were expressed as mg of gallic acid g^{-1} (mg GAE g^{-1} d.w.) of lemon by-products dry weight.

1.2.4.2. Total flavonoid content (TF)

The total flavonoid content was quantified on the obtained extracts by the method described by Papoutsis et al. (2018b), with some modifications. In brief, 0.2 mL of extract, 1 mL of deionised water and 0.15 mL of 5% (w/v) $NaNO_2$ (Sodium nitrite) were placed in a 5 mL flask and incubated at room temperature for 6 minutes. Subsequently, 0.15 mL of 10% (w/v) $AlCl_3$ (Aluminium chloride) were added and incubated at room temperature for 6 minutes. Then, 2 mL of 4% (w/v) NaOH (Sodium hydroxide) and 0.7 mL of deionised water were added and finally the mixture was made up to volume with deionised water. At the same time, a solution used as a blank was prepared. The mixture was incubated in the dark for 15 min. The absorbance was measured at 510 nm against a blank using a double-beam ultraviolet-visible spectrophotometer (Perkin-Elmer UV- Vis λ2, Waltham, Massachusetts, U.S.) and comparing with a catechin calibration curve (concentration between 1 and 50 mg L^{-1}). The results were expressed as mg of catechin g^{-1} (mg CE g^{-1} d.w.) of lemon by-products dry weight.

1.2.4.3. Antioxidant activity determination

It was tested by two assays, based on $DPPH^{\cdot}$ and $ABTS^{+}$ extinction, which often do not give the same results because of the two different action mechanisms and the two distinct radicals involved. This is the reason why it could be appropriate to perform both antiradical tests (De Bruno et al., 2018).

- **DPPH assay**

The DPPH assay was carried out as reported by Brand-Williams et al. (1995), consisting of the reaction between the DPPH· (2,2-diphenyl-1-picrylhydrazyl) and the antioxidants compounds in the samples, resulting in discoloration of the reaction solution due to the disappearance of the radical. In a cuvette, 50 µL of the phenolic extract (aqueous and hydroalcoholic) properly diluted was added to 2950 µL of a 6 x 10^{-5} M of methanol solution of DPPH· and left in darkness for 30 min at room temperature. The absorbance was measured at 515 nm, against methanol as blank, using a double-beam ultraviolet-visible spectrophotometer (Perkin-Elmer UV- Vis λ2, Waltham, Massachusetts, U.S.). The results were expressed as µM Trolox equivalents g^{-1} of lemon by-products dry weight (µM TE g^{-1} d.w.), comparing with a Trolox calibration curve (from 3 to 18 µM).

- **ABTS assay**

The antioxidant activity of the extracts was determined by ABTS (2,2'-azino-bis acid (3-ethylbenzothiazolin-6-sulfonic acid) assay, a spectrophotometric discoloration method (De Bruno et al., 2018). The working solution was prepared by mixing two stock solutions of 7 mM ABTS solution and 2.4 mM potassium persulphate ($K_2S_2O_8$) solution and was incubated at room temperature for 12 hours in the dark to achieve a stable value of absorbance: the reaction between $ABTS^+$ and potassium persulphate determines the direct production of a blue-green chromogen. The resulting $ABTS^+$ solution was diluted with ethanol and showed an absorbance of 0.70 (±0.02) at 734 nm.

The reaction mixture was prepared by mixing 25 µL of aqueous extract and 2975 µL of the ethanol solution of $ABTS^+$; the hydroalcoholic extracts required different reaction ratios, such as 10 µL of extract and 2990 µL of $ABTS^+$ solution. The absorbance was measured after 6 minutes in the dark at 734 nm using a double-beam ultraviolet-visible spectrophotometer (Perkin-Elmer UV- Vis λ2, Waltham, Massachusetts, U.S.). The results were expressed as µM Trolox equivalents g^{-1} of lemon by-products dry weight (µM TE g^{-1} d.w.), comparing with a Trolox calibration curve (from 3 to 18 µM).

1.2.4.4. Identification and quantification of antioxidant compounds

Identification and quantification of antioxidant compounds was performed in each extract following Romeo et al. (2019), with some modifications. Chromatographic system consisted in UHPLC PLATINblue (Knauer, Berlin, Germany) provided with a binary pump system, Knauer blue orchid C18 column (1.8 µm, 100 x 2 mm) coupled with a PDA–1 (Photo Diode Array Detector) PLATINblue (Knauer, Berlin, Germany) and Clarity 6.2 software.

Extracts were filtered through a 0.22 μm nylon syringe filters (diameter 13 mm) and then 5 μL were injected in the system. The mobile phases used were (A) water acidified with acetic acid (pH 3.10) and (B) acetonitrile; the gradient elution program consisted in 0–3 min, 5% B; 3–15 min, 5%–40% B; 15–15.5 min, 40%–100% B. Ultimately, restoration of the initial conditions was reached during analysis maintaining the column at 30°C. For the quantification of each compound, external standards (concentration between 1 and 100 mg kg^{-1}) were used, and the results were expressed as mg kg^{-1} of lemon by-products dry weight (mg kg^{-1} d.w.).

1.2.4.5. Antimicrobial activity of extracts

The three foodborne pathogens *Listeria monocytogenes* (ATCC 13932 strain), *Escherichia coli* (ATCC 8739 strain) and *Salmonella enterica* (wild strain) were used as test organisms to determine antimicrobial activity of the selected extracts via the agar diffusion method based on the inhibition zones. 10 μL of each extract were inoculated onto Petri plates on the surface of solid soft agar with the test organism (TSA, Tryptic Soy Agar, for *L. monocytogenes*; MH, Mueller Hinton Agar, for *E. coli* and *S. enterica*) and incubated at 37°C for 24 h. EtOH:H_2O 50% mixture was used as control. Tests were repeated in duplicate. The results were expressed as mm of the halo diameter resulting from the inhibition zone taken as a measure of the antimicrobial activity of the extract.

1.2.5. Statistical analysis

All the experimental results were expressed as mean value (n=4) ± standard deviation. SPSS Software (Version 15.0, SPSS Inc., Chicago, IL, USA) was used for data statistical elaboration. Multivariate and One-way analysis of variance (MAVOVA and ANOVA) with Tukey's post hoc test at $p<0.05$. Pearson's correlation test was employed for the determination of correlation coefficients (r) among TPC, TF and antioxidant assays.

1.3. RESULTS AND DISCUSSION

1.3.1. Conventional solid-liquid extraction

Multivariate data analysis evidenced a significant difference ($p<0.01$) between the effect of water and hydroalcoholic mixture when considered jointly on the dependent variables, whereas the applied times affected only TF. Consequently, a separate ANOVA was conducted for each dependent variable, with each ANOVA evaluated at an alpha level of 0.05.

In this regard, a different TPC extraction yield was highlighted between the two types of used extraction solvents, particularly, hydroalcoholic one promoted a higher efficiency for TPC than water

(Table 1). These results are confirmed by De Bruno et al. (2018) who that the mixture water/ethanol increases the phenolic recovery from agricultural by-products.

Using W solvent, after 30 and 60 extraction minutes a decrease of TPC was observed with the increasing extraction temperature involved in the aqueous extracts obtained. Then the temperature of 25°C was considered more useful. The reduction could be explained by the possible degradation of phenolic compounds at any considered temperature as the extraction time increases, due to the stabilizing effect of extraction and bioactive degradation (Amendola et al., 2010). After 120 minutes of extraction with W solvent, the recovery was the lowest without thermal differences. The maximum recovery of total phenolics in the water (W) extracts was 2.96±0.11 mg GAE g^{-1} d.w. (30 min, 25°C), with higher results than some data reported in literature (Li et al., 2006).

Table 1: Total polyphenol content (TPC), Total flavonoid content (TF) and expression of antioxidant activity (DPPH and ABTS assays) of extracts (W and W/EtOH) obtained by conventional solid-liquid extraction.

		TPC (mg GAE g^{-1} d.w.)			TF (mg CE g^{-1} d.w.)		
Minutes	**°C**	**W**	**W/EtOH**	**Sign.**	**W**	**W/EtOH**	**Sign.**
30	**25**	2.96±0.11^{a}	3.41±0.10^{c}	**	0.94±0.04^{ab}	0.61±0.04^{c}	**
	40	2.52±0.28^{b}	5.42±0.17^{b}	**	0.89±0.03^{b}	1.43±0.04^{b}	**
	70	2.69±0.02^{ab}	6.37±0.22^{a}	**	1.07±0.06^{a}	1.84±0.10^{a}	**
	Sign.	*	**		**	**	
60	**25**	2.24±0,06^{a}	4.92±0.09^{c}	**	0.31±0,06^{b}	1.11±0.05^{c}	**
	40	1.09±0.21^{b}	6.23±0.24^{b}	**	0.49±0.02^{a}	1.58±0.09^{b}	**
	70	1.35±0.58^{b}	6.75±0.25^{a}	**	0.30±0.00^{b}	1.85±0.07^{a}	**
	Sign.	**	**		**	**	
120	**25**	1.23±0.06	5.01±0.13^{b}	**	0.25±0.03 ^{b}	1.12±0.04^{c}	**
	40	1.48±0.48	6.86±0.17^{a}	**	0.38±0.05^{ab}	1.85±0.10^{b}	**
	70	1.93±0.60	7.03±0.48^{a}	**	0.58±0.24 ^{a}	2.22±0.29^{a}	**
	Sign.	ns	**		*	**	
		ABTS (µM TE g^{-1} d.w.)			**DPPH (µM TE g^{-1} d.w.)**		
Minutes	**°C**	**W**	**W/EtOH**	**Sign.**	**W**	**W/EtOH**	**Sign.**
30	**25**	7.65±0.74	14.13±0.79^{b}	**	4.46±0.85	5.13±0.30^{b}	ns
	40	6.72±0.63	20.72±1.82^{a}	**	4.48±0.21	6.75±0.09^{a}	**
	70	7.52±0.57	19.43±0.95^{a}	**	4.22±0.11	6.96±0.24^{a}	**
	Sign.	ns	**		ns	**	
60	**25**	7.28±1.66	17.02±0.77^{b}	**	4.06±0.46	6.77±0.43	**
	40	6.78±2.32	19.11±0.60^{a}	**	3.61±1.30	7.23±0.11	**
	70	5.54±1.57	19.47±0.70^{a}	**	3.25±1.24	7.08±0.18	**
	Sign.	ns	**		ns	ns	
120	**25**	5.96±0.24	17.46±0.19^{c}	**	3.30±0.67	6.80±0.22^{b}	**
	40	6.03±0.74	21.50±0.76^{a}	**	3.31±0.73	7.76 ±0.51^{a}	**
	70	7.45±1.34	19.33±0.89^{b}	**	3.82±0.69	8.27±0.13^{a}	**
	Sign.	ns	**		ns	**	

The data are presented as means ± SD (n=4). Means within a column with different letters 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.

Hydroalcoholic extraction showed different behaviour. The obtained extraction yield is in accordance with literature data related to the phenolic extraction from lemon by-products (Casquete et al., 2015). Our results showed that the recovery of phenolic compounds linearly increased with the increase of extraction temperature at all the considered extraction times (Table 1). Despite the similar ($p>0.05$) TPC yield after 120 minutes at both 40°C and 70 °C, the multivariate analysis evidenced no time effect on TPC values regardless the extraction temperature, as well as on the showed antioxidant activity. As reported by Barrales et al. (2018), the polyphenols content in ethanol increases as the temperature increases, probably because of the surface tension decrease, which represents a factor that regulates the penetration of the solvent into the solid matrix. Moreover, higher temperature increases the diffusivity of phenolic compounds into the solvent boosting their transport, even if at 70°C we should approach the boiling point of the W/EtOH azeotropic (78.4°C), which composition is highly dependent on temperature and pressure.

About TF, highly significant differences ($p<0.01$) were found among samples in both the solvents. It was noticeable in the hydroalcoholic one, with a maximum total amount of 2.22±0.29 mg CE g^{-1} d.w. in extract obtained at 70°C for 120 minutes. Such value was consistent with that found by Papoutsis et al. (2018b) by extraction on freeze dried lemon peel.

In this study, TPC and TF showed similar patterns of extractability, with significant variation in the interaction effects by the independent variables ($p<0.05$): as the temperature increases, the extraction rate in ethanol 50% increases significantly at the same time.

Regarding the antioxidant activity of the extracts, the aqueous extracts did not show a significant difference in the expression of antioxidant activity for both assays performed, even if the correlation between polyphenols and flavonoids compounds and the DPPH assay was higher, $r=0.92$ and $r=0.83$, respectively. This is possible since a high phenolic content is not necessary characterized by a high antioxidant capacity, which also depends on the structure and interaction among the extracted phenolic compounds.

For the conventional solid-liquid extraction, the hydroalcoholic mixture of W/EtOH (50%) and the highest temperature (70°C), were considered as the most appropriate parameters for the extraction of antioxidant compounds from dried lemon by-products, at any time among those considered, in terms of the highest content in TPC and TF (Table 1).

1.3.2. Ultrasound-assisted extraction

The multivariate statistical analysis shows that in ultrasound-assisted extraction all individual independent variables and the interaction of them significantly affected ($p<0.05$) the TPC and TF content, as well as the expression of the antioxidant activity performed by ABTS and DPPH assays.

The lack of significance of the DPPH assay suggests the absence of interactions among the independent variables in the studied range. Consequently, a separate ANOVA was conducted for each dependent variable, with each ANOVA evaluated at an alpha level of 0.05.

Regarding the aqueous extracts (W), there was a highly significant difference ($p<0.05$) in the extraction yield of TPC and TF content, which decreased with the extension of ultrasonic extraction time and temperature (Table 2). The application of high ultrasonic intensity may result in degradation effects. Indeed, the increment in amplitude of ultrasonic radiations had both positive and negative effects on the extraction yield. More precisely, the highest TPC and TF values (5.91±0.20 mg GAE g^{-1} and 2.30±0.08 mg CE g^{-1}, respectively), were obtained with temperature of 25 °C and extraction time of 60 minutes. As described by Papoutsis et al. (2018b), extraction temperature could have a significant negative effect on TPC yields, suggesting that an extraction temperature higher than the optimum leads to a decrease in TPC. The degradation of cell walls may expand as the temperature increases, follow up on release of both phenolic compounds and enzymes involved in polyphenol oxidation (i.e., peroxidase and polyphenol oxidase). Indeed, our results refer that the extraction yield was decreased by a further increment of the process intensity level, up to 70°C for 120 minutes, leading to the lowest TPC and TF values, respectively of 1.31±0.04 mg GAE g^{-1} and 0.25±0.05 mg CE g^{-1} of lemon by-products.

Table 2: Total polyphenol content (TPC), Total flavonoid content (TF) and expression of antioxidant activity (DPPH and ABTS assays) of extracts (W and W/EtOH) obtained by ultrasound-assisted extraction.

		TPC (mg GAE g^{-1} d.w.)			TF (mg CE g^{-1} d.w.)		
Minutes	**°C**	**W**	**W/EtOH**	**Sign.**	**W**	**W/EtOH**	**Sign.**
30	**25**	5.13±0.10^{b}	4.64±0.13^{c}	**	1.47±0.09^{a}	1.17±0.04^{c}	**
	40	5.38±0.06^{a}	5.82±0.26^{b}	*	1.55±0.15^{a}	1.77±0.11^{b}	**ns**
	70	4.14±0.08^{c}	6.93±0.32^{a}	**	1.21±0.04^{b}	2.07±0.11^{a}	**
	Sign.	**	**		**	**	
60	**25**	5.91±0.20^{a}	6.75±0.34	**	2.30±0.08^{a}	2.04±0.09^{c}	**
	40	3.93±0.19^{b}	6.85±0.21	**	1.59±0.07^{b}	2.23±0.04^{b}	**
	70	2.61±0.06^{c}	6.73±0.21	**	1.03±0.02^{c}	2.40 0.09^{a}	**
	Sign.	**	**ns**		**	**	
120	**25**	4.27±0.12^{a}	6.30±0.15^{b}	**	0.95±0.05^{a}	1.97±0.07	**
	40	4.39±0.08^{a}	6.90±0.28^{a}	**	1.01±0.05^{a}	1.98±0.10	**
	70	1.31±0.04^{b}	6.93±0.15^{a}	**	0.25±0.05^{b}	2.13±0.12	**
	Sign.	**	**		**	**ns**	
		ABTS (µM TE g^{-1} d.w.)			**DPPH (µM TE g^{-1} d.w.)**		
Minutes	**°C**	**W**	**W/EtOH**	**Sign.**	**W**	**W/EtOH**	**Sign.**
30	**25**	18.67±1.10	14.06±0.02^{c}	**	6.38±0.22^{a}	6.40±0.16^{c}	**ns**
	40	17.73±1.02	16.37±0.83^{b}	**ns**	6.16±0.15^{a}	7.13±0.13^{b}	**
	70	16.24±2.12	18.36±0.86^{a}	**ns**	5.03±0.58^{b}	7.43±0.09^{a}	**
	Sign.	**ns**	**		**	**	
60	**25**	10.24±0.94^{a}	19.42±0.63	**	6.26±0.28^{a}	8.25±0.24	**
	40	9.16±0.40^{a}	19.77±0.67	**	5.22±0.62^{b}	7.90±0.94	**
	70	7.03±0.67^{b}	19.62±1.06	**	3.86±0.31^{c}	7.95±0.95	**
	Sign.	**	**ns**		**	**ns**	
120	**25**	9.00±0.53^{a}	19.60±0.46^{b}	**	3.58±0.09^{a}	7.14±0.45	**
	40	5.91±0.34^{b}	20.41±0.55^{ab}	**	3.43±0.16^{a}	7.61±0.24	**
	70	3.07±0.44^{c}	21.28±0.29^{a}	**	2.08±0.21^{b}	7.59±0.20	**
	Sign.	**	**		**	**ns**	

The data are presented as means ± SD (n=4). Means within a column with different letters are significantly different by Tukey's post hoc test. Abbreviation: Sign., significance; ns, not significant. **Significance at $p < 0.01$; *Significance at $p<0.05$.

In addition, this aspect could be attributed to the simultaneous effects of the high radiation amplitude and the extended extraction time, producing several temporary hot spots through the collapse of the cavitation bubbles and an increase in temperature and pressure, which leads to the destruction of polyphenols in the UAE process (Kazemi et al., 2016; Nipornram et al., 2018).

On the antioxidant activity showed by the W extracts, there was a high positive linear correlation between the polyphenols (r=0.84) and flavonoids (r=0.88) compounds and the DPPH assay. Even if

statistical analysis revealed a lower correlation between TPC and ABTS assay (r=0.67), the antioxidant activity of plant extracts not only depend on their composition but also on the assay's conditions. Indeed, $ABTS^{+}$ reacts not only with antioxidant compounds but also with any hydroxylated aromatic elements despite of their antioxidant potential (Romeo et al., 2019).

Contrary to what previously described, in W/EtOH extracts, the yield increased with increasing of temperature and time. Looking at Table 2, there was a clear temperature effect on extractions for 30 minutes, whose increase led to an increment in TPC. Equally, it was noted that the increase of the extraction time beyond 30 minutes at the same temperature led to an increase in the extraction yield of total polyphenols. Extraction temperature and time had a significant positive linear effect on the extraction process ($p<0.05$), implying that higher yields of TPC can be achieved by increasing ultrasonic radiation amplitude and/or extraction time in agreement with Khan et al. (2010). In comparison to water, ethanol is characterized by a higher heating efficiency when applied in an aqueous mixture and it is preferred thanks to its better capacity in solving the phenolic compounds (Rafiee et al., 2011). Additionally, high positive correlations were found ($r >0.80$) between TPC and TF content and both antiradical assays. Indeed, high antioxidant capacity value corresponds to a high phenolic content, with a maximum of 21.28±0.29 μM TE g^{-1} d.w. and 8.25±0.24 μM TE g^{-1} d.w. of W/EtOH extracts for ABTS and DPPH assays.

The greater efficiency of UAE than conventional extraction agrees with results obtained by several authors in the extraction of polyphenols from different matrices (Safdar et al.,2017; Saini et al., 2019a).

1.3.3. Microwave-assisted extraction

From multivariate data analysis, among the independent variables taken into consideration in this research, the extraction solvent and power showed a highly significant influence on the extraction process. Indeed, the MANOVA (Multivariate analysis of variance) shows that a different solvent (W or W/EtOH) and the combination of this variable with the extraction power significantly affected ($p<0.05$) the extraction yield of TPC and TF and the expression of the antioxidant activity (ABTS and DPPH assays). Extraction power also had a significant influence on the dependent variables considered in this study, with the only exception of the total flavonoid content. Contrary to solvent and power variables, the MAVOVA test suggests a lack of significant influence by time, especially on TPC and ABTS variables, and by the interaction of solvent and extraction time on the extraction process in the studied range. Consequently, a separate ANOVA was conducted for each dependent variable, with each ANOVA evaluated at an alpha level of 0.05.

Looking at the aqueous extracts (W) obtained by five minutes extraction (Table 3), there was a high significance difference ($p<0.01$) between the values within each dependent variable considered, which confirms the influence of microwave power on the extraction process. As reported in Table 3, a similar content of TPC and TF was observed for the extractions conducted at 250 and 500 Watt for 5 minutes, as well as for the expression of antioxidant activity by DPPH assay ($p>0.05$). However, a reversal was noted when the applied microwave power was increased up to 800 Watt, observing a significant decline in all the studied parameters.

Table 3: Total polyphenol content (TPC), Total flavonoid content (TF) and expression of antioxidant activity (DPPH and ABTS assays) of extracts (W and W/EtOH) obtained by microwave-assisted extraction.

		TPC (mg GAE g^{-1} d.w.)			TF (mg CE g^{-1} d.w.)		
Minutes	**Watt**	**W**	**W/EtOH**	**Sign.**	**W**	**W/EtOH**	**Sign.**
5	**250**	2.94±0.11^{a}	6.74±0.22^{a}	**	0.90±0.04^{a}	2.02±0.13^{a}	**
	500	3.28±0.27^{a}	5.56±0.30^{b}	**	0.98±0.09^{a}	1.62±0.11^{b}	**
	800	2.48±0.09^{b}	5.56±0.27^{b}	**	0.69±0.07^{b}	1.75±0.13^{b}	**
	Sign.	**	**		**	**	
15	**250**	3.00±0.10	5.62±0.49	**	0.50±0.05^{b}	1.59±0.28	**
	500	3.14±0.54	5.87±0.22	**	0,75±0.08^{a}	1.74±0.12	**
	800	2.56±0.11	5.63±0.19	**	0,89±0.10^{a}	1.69±0.08	**
	Sign.	**ns**	**ns**		**	**ns**	
		ABTS (µM TE g^{-1} d.w.)			**DPPH (µM TE g^{-1} d.w.)**		
Minutes	**Watt**	**W**	**W/EtOH**	**Sign.**	**W**	**W/EtOH**	**Sign.**
5	**250**	7.45±0.50^{b}	24.08±1.01^{a}	**	5.33±0.46^{a}	12.01±0,30^{a}	**
	500	8.61±0.51^{a}	20.03±0.94^{b}	**	5.44±0.37^{a}	10.55±0.40^{b}	**
	800	6.87±0.66^{b}	21.35±1.09^{b}	**	4.37±0.22^{b}	10.72±0.19^{b}	**
	Sign.	**	**		**	**	
15	**250**	7.96±0.33^{a}	21.56±0.81	**	5.05±0.27	10.81±0.36	**
	500	7.91±0.66^{a}	21.73±1.94	**	5.06±0.75	10.82±0.31	**
	800	6.37±0.29^{b}	21.99±2.12	**	4.74±0.70	10.41±0.12	**
	Sign.	**	**ns**		**ns**	**ns**	

The data are presented as means ± SD (n=4). Means within a column with different letters are significantly different by Tukey's post hoc test. Abbreviation: Sign., significance; ns, not significant. **Significance at $p < 0.01$; *Significance at $p < .05$.

Increasing the microwave power over 500 Watt for 5 minutes extraction, the recovery of polyphenols and flavonoids decreased: these results agree with those reported by Shao et al. (2012). A different trend was found for the 15 minutes extractions, among which there was no significant difference in terms of TPC regardless of the power applied, as well as the results of DPPH assay. In contrast to this, a highly significant difference ($p<0.01$) was determined with regards to the extraction rate of

flavonoid compounds and the relative expression of antioxidant activity by ABTS assay, even if without a positive correlation (r=-0.78). In this case, although water is a highly polar solvent for the extraction of bioactive compounds, the main inconvenience of its use is the difficulty of determining the high content of water-soluble impurities which interfere with the identification and quantification of target compounds (Simic´ et al., 2016).

Despite a certain correlation between increasing of time and extraction yield was found, few minutes up to 500 Watt of microwave treatment seemed to show the best extraction yield in polyphenols and flavonoids: an overexposure in the microwave encouraged in fact the degradation of the thermolabile compounds, as reported by Nayak et al. (2015). According to Rafiee et al. (2011), the increase in irradiation time up to 15 minutes did not result in improvement in the extraction performance, but sometimes might lead to a decrease in the concentration yield.

About the hydroalcoholic extracts (W/EtOH), by increasing the microwave power from 250 to 500 Watt with extraction time fixed at 5 minutes, the TPC decreased significantly from 6.74±0.22 to 5.56±0.30 mg GAE g^{-1} d.w. The reduction in TPC was noticed when the optimal conditions determined within the range of variables considered in this study have been exceeded (beyond 250 Watt and 5 minutes). This result was due to the increment in direct effect of microwave energy on the extraction system by the dipolar rotation, which resulted in temperature increase of the system and produced the deterioration of the bioactive substances (Nayak et al., 2015).

Results indicated that the content of phenolic and flavonoid compounds decreased significantly with power during microwave treatment ($p<0.05$) and extraction time of 15 minutes did not show any obvious positive effect on extraction yield. In fact, when lemon by-products were treated at 250 Watt for 15 min there was a sudden decrease of value, compared with the extraction at 250 Watt for 5 minutes, which indicated that longer irradiation time was harmful to the TPC. This suggests that a shorter treatment time is ideal for the release of TPC under microwave irradiation. The highest content of total phenolic content and total flavonoids content was obtained when dried lemon by-products were treated at 250 Watt for 5 minutes, with aqueous-ethanol mixture as extraction solvent. The results suggested that the interaction between the extraction solvent (W or W/EtOH) and microwave power on the extraction efficiency of TPC was highly significant ($p<0.01$). Water content, being the most common absorbing phase for microwave energy, shows a crucial role during microwave extraction process (Hayat et al., 2010). Phenolic compounds are polar molecules and the presence of water boosts the polarity index of organic solvents compared with pure solvents, as ethanol, contributing to get higher values for the TPC and antioxidant activity. Moreover, the addition of water not only increases the dielectric constant of the solvent, but also intensifies absorption of microwave energy provoking a higher temperature inside the samples, which causes the fracture of cells and a

quick outflow of antioxidant compounds (Rodsamran & Sothornvit, 2019). However, the applied prolonged extraction time and a high microwave power led to lower TPC values, as a result of the almost certainly damage of phenolic acids by microwave treatment (Hayat et al., 2010; Dahmoune et al., 2013).

For the antioxidant capacity of aqueous extracts, the same trend of the valuable compounds previously discussed was observed, increasing slightly as microwave power increases up to 500 Watt, even if there is no significant difference between the values: after 5 minutes of extraction the antioxidant activity of aqueous extracts performed by DPPH and ABTS assays was 5.44±0.37 and 8.61±0.51 µM TE g^{-1} d.w., respectively.

The highest antioxidant capacity of hydroalcoholic extracts (W/EtOH), at both DPPH and ABTS assays, was found after a microwave treatment of 250 Watt for 5 minutes. The expression of antioxidant activity, through both assays, perfectly reflects the values of the reported TPC and TF: correlation coefficients, in each case, were above 0.86, which meant that the increase in the antioxidant capacities of the extracts was due at least in part to the increase of the extracted valuable compounds. In general, the higher radical scavenging activity of MAE extracts could be explained by the fact that microwave treatment might affect cellular structure due to the sudden and swift increase in temperature and internal pressure, provoking a direct effect of microwaves on molecules, which results in rapid rise of the temperature and fast completion of a reaction (Nayak et al., 2015). In parallel, it may be related to the synergistic effect of the polyphenolic compounds and others available in the extract, which act as free radical scavengers (Xu et al., 2017).

It can be observed that microwave extractive technique has proved to be more efficient in terms of time saving, with an extraction rate over six times faster than conventional and ultrasound assisted extraction. Indeed, MAE is characterised by physical and chemical phenomena that are basically distinct from those of the other two applied extraction techniques. The developed microwave system suggests that this kind of extraction offers clear advantages, such as less time required for the extraction process, as well as being environmentally safe. In agreement with other authors, MAE could be identified as a fast and reliable method for bioactive compounds extraction from citrus wastes and by-products (Putnik et al., 2017; Dahmoune et al., 2013).

1.3.4. Comparison of conventional, ultrasound- (UAE) and microwave-assisted extraction (MAE)

As discussed above, for all studied extraction techniques, the ethanol: water mixture (EtOH:H_2O, 50:50) was found to be the best extraction solvent. In this regard, in Figure 4, the most considerable extracts obtained from each extraction technique were compared according to their antioxidant

properties. Among them, the best extracts obtained were selected, not only with reference to the total content of bioactive compounds and related antioxidant activity, but also from the point of view of the cost-effectiveness of the extraction process, in terms of time saving. Indeed, choice of an extraction method would mostly depend on the advantages and disadvantages of the processes such as extraction yield, complexity, production cost, time saving, environmental friendliness and safety (Dahmoune et al., 2013).

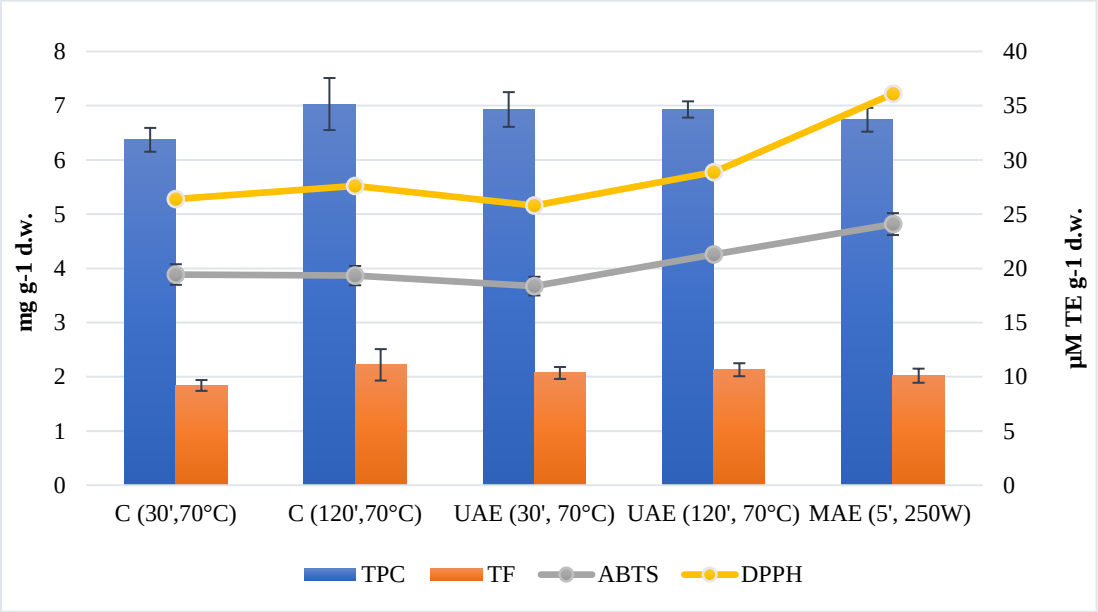

Figure 4: Main antioxidant results of the best extracts. The data are presented as means ± SD (n=4).

In this study, for each kind of extraction, the following optimal conditions were identified: 30 minutes at 70°C (conventional), 30 minutes at 70°C (UAE) and 5 minutes at 250 Watt (MAE). Phenolic characterisation of the selected extracts obtained under optimal extraction conditions was performed by UHPLC system (Table 4).

Table 4: Phenolic compounds identified in selected hydroalcoholic lemon by-products extracts (mg kg^{-1} d.w.)

Compounds	Conventional	UAE	MAE	Sign.
Gallic acid	72.92±0.14^{b}	103.97±0.28^{a}	2.08±0.14^{c}	**
p-Coumaric acid	59.25±0.23^{a}	58.17±0.29^{b}	57.81±0.19^{b}	**
Ferulic acid	134.86±0.24^{a}	134.48±0.32^{a}	85.73±0.19^{b}	**
Eriocitrin	1129.06±0.22^{b}	1167.28±0.25^{a}	1001.34±0.24^{c}	**
Narirutin	58.50±0.17^{c}	140.89±0.24^{a}	77.21±0.25^{b}	**
Hesperidin	1636.26±0.26^{b}	1694.98±0.36^{a}	780.78±0.04^{c}	**
Neohesperidin	6.11±0.24^{b}	12.45±0.19^{a}	1.87±0.19^{c}	**
Naringin	33.39±0.22^{b}	44.14±0.22^{a}	19.84±0.21^{c}	**
Apigenin	3.43±0.26^{b}	4.29±0.08^{a}	n.d.	**
Caffeic acid	87.80±0.21^{a}	87.46±0.21^{a}	65.29±0.18^{b}	**
Rutin	70.71±0.21^{b}	76.96±0.13^{a}	32.76±0.17^{c}	**

The data are presented as means ± SD (n=3). Means within a row with different letters are significantly different by Tukey's post hoc test. Abbreviation: Sign., significance; ns, not significant; n.d., not detected; **Significance at $p < 0.01$; *Significance at $p < 0.05$.

Eleven phenolic compounds were identified: specifically, in UAE extract the chromatographic profile showed different concentrations of individual phenolic compounds, with hesperidin (1694.98±0.36 mg kg^{-1} dw) and eriocitrin (1167.28±0.25 mg kg^{-1} dw) as the most abundant flavonoids followed far away by narirutin and ferulic acid. Neohesperidin, naringin and rutin were found in very low amounts when compared to the other flavonoids. Apigenin was the least quantified phenolic compound. Compared to conventional extraction, UAE allowed a better and more efficient extraction of these bioactive compounds. Indeed, as reported in Figure 1, it can be observed that a 30-minute UAE at 70°C extracted an equal amount of TPC and TF as a conventional 120-minute extraction at the same temperature.

As shown in Table 4, among all, UAE proved to be the best method that resulted in higher extraction yield of phenolic compounds from lemon by-products, which was significantly higher than those of conventional and MAE. Even if UAE extraction showed the best effect on the qualitative and quantitative characteristics of extracted bioactive compounds, it is a time-consuming method compared to the shortest process time (5 minutes) of microwave assisted extraction. MAE proved to be instead an interesting alternative extraction technique for the recovery of phenolic compounds from lemon by-products through an environmentally green approach. The extraction time required for optimal recovery of antioxidant compounds was in fact significantly less than that needed by the other applied techniques, showing to be a more rapid method of extraction.

In addition, the best selected extracts were subjected to antimicrobial activity analyses. Conventional and UAE ones showed an evident antimicrobial activity against some of foodborne pathogens compared to the MAE extract (Table 5). The results of antibacterial activities are often highly correlated with their total phenolic content. The results suggest that the bacteria species tested did not show an extremely sensitivity to the lemon by-products extracts, especially *S. enterica*, showing a halo diameter less or slightly more than 6 mm (Bai et al., 2021).

Table 5: Antimicrobial activity of selected hydroalcoholic lemon by-products extracts. Data are expressed as mm of the halo diameter resulting from the antimicrobial activity of the extract.

	L. monocytogenes	*E. coli*	*S. enterica*
	ATCC 13932	**ATCC 8739**	**wild**
Conventional	7.1±0.1	0.0	4.0± 0.0
UAE	7.0±0.0	6.6±0.14	4.0± 0.0
MAE	0.0	0.0	0.0
Control	0.0	0.0	0.0

The data are presented as means ± SD (n=2).

The antimicrobial properties of plant extracts are due to the presence of secondary metabolites, such as tannins, flavonoids and phenolic compounds, which are the most relevant active elements against bacteria (Sielicka-Różyńska & Gwiazdowska, 2020). The absence of antimicrobial activity in the MAE extract might be due to the lack of some valuable compounds, such as apigenin. Indeed, as reported by Budiati et al. (2021), apigenin has the capability to inhibit the growth of *L. monocytogenes*, thanks to its intense antibacterial activity by deactivating microbial adhesion, enzymes and cell transport proteins. A similar effect was detected by Ivasenko et al. (2021), who reported the contribution of gallic acid in the expression of antimicrobial activity against *E. coli*. As described by the author, the method of biologically active substances extraction is relevant to the final composition of the extract. In general, the UAE was found to be the best in terms of phenolic characterisation and antimicrobial activity.

1.4. CONCLUSIONS

In this study, the optimal extraction yield and antioxidant activity values were obtained when the extraction process was performed with the hydroalcoholic solvent (W/EtOH, 50%) for all the techniques applied. The comparative studies revealed that the recovery of phenolic compounds in the extracts from lemon by-products using UAE at 70°C for 30 minutes was significantly higher than those of conventional and microwave extraction, as well as antimicrobial activity against food

pathogens. Anyway, results obtained by MAE revealed it can be considered a novel time-consuming and high-efficient method in the extraction of many bioactive compounds from various natural matrices. The studied extraction procedures involved an environmentally green approach and it could be generalized to other by-products to obtain antioxidant extracts for the future utilization as ingredients in functional food preparations.

2. DEVELOPMENT OF SUITABLE FOOD GRADE EXTRACTION TECHNIQUES OF BIOACTIVE COMPOUNDS FROM ONION SOLID WASTE

2.1. INTRODUCTION

Red onion skins are a rich source of natural bioactive compounds with marked antioxidant properties, such as flavonoids and anthocyanins (Albishi et al., 2013). Significant differences in total flavonoid content were noted among the red and white onion varieties. The concentration of flavonoids is substantially higher in red onions, which are suggested for their most important health benefits (Perez-Gregorio et al. 2010). As reported in literature, flavonoids are mainly concentrated in the skin of onions rather than in the edible part (Price et al., 1997; Schieber et al., 2001) High content of quercetin was found in dry red onion skin, approximately 32-fold higher than the flesh layers of the onion (Nuutila et al., 2003). Moreover, Albishi et al. (2013) reported that the HPLC analysis revealed that onion skin is rich in quercetin and kaempferol and has numerous antioxidative functions. Dry onion skin also has a distinct concentration of quercetin derivatives compared to the edible part (Wiczkowski et al., 2003). In fact, as reported by Perez-Gregorio et al. 2010, levels of flavonols decreased from the outer to the inner scales and from the top to the base.

Contrary to flavonols, no changes were observed in anthocyanin concentration from outer to inner scales. Total anthocyanin levels also showed a non-significant decrease from the top to the bottom of the bulb (Perez-Gregorio et al. 2010). Anthocyanins represent about 10% of the total flavonoid content of red onions, which are mostly concentrated in the skin and in the outer fleshy layers, whereas in the edible tissue they are confined to a single layer of cells in the epidermal tissue (Rhodes & Price, 1996). Gennaro et al. (2002) also reported that the dry skin of onions is characterized by a great amount of anthocyanins and flavonols, with high content of glycone forms that corresponds to 2% of the total weight in the non-edible part. Therefore, 63% of total red onion anthocyanins are present in the dry skin and outer fleshy layers, which are commonly discarded. Because onions are especially rich in these phytochemicals (Griffiths et al., 2002), the exploitation of onion solid waste for the recovery of these compounds is an important issue, in relation with both lowering the environmental impact from food waste disposal and utilisation of high added-value substances with beneficial aspects (Kiassos et al., 2009). In this regard, it has been discussed that solvents such as methanol or ethanol, with a significantly lower polarity than water, encourage the solubilisation of polyphenols (Tsakona et al., 2012), most of which are poorly soluble in water. Thus, ethanol could reasonably be expected to lower the polarity of water, allowing the solubilisation of higher amounts of polyphenols. This agrees with results supporting the fact that polyphenols can be easily solubilised

in polar protic media, such as hydroalcoholic mixture (Katsampa et al., 2015). In addition, heat treatments contribute to provoke thermal destruction of cell walls and sub cellular compartments during the extraction process, which promote the release of internal compounds (Juàniz et al., 2016). The utilization of waste from pigmented onions and the importance of factors such as temperature, could make onion wastes as a great source of anthocyanins as water-soluble pigments and other precious molecules, useful for the elaboration of value-added products (Makris, 2010).

For these reasons, this study was focused on the extraction of valuable compounds from onion solid waste, through sustainable extraction techniques (conventional, ultrasound- and microwave-assisted extraction) and food grade extraction solvent, such as water and an hydroalcoholic mixture. In addition, the effects of extraction solvents, time, and temperature were studied to valorise a production waste by developing environmentally friendly and food grade techniques characterized by a higher extraction efficiency.

2.2. MATERIALS AND METHODS

2.2.1. Material

Red onions (*Allium cepa L., cv. Tropea*) were supplied by a local producer in the province of Reggio Calabria (Italy). The bulbs were transported to the laboratory and the outer dry and semi-dry layers were separated, as well as the apical and basal trimmings, which were considered as onion solid waste (OSW) and used in the extraction processes. The OSW were initially dried at a temperature of 50°C up to a moisture content of 17%, then pulverised in a domestic blender and stored in polyethylene bags under vacuum to avoid rehydration until subsequent extraction procedures of the bioactive compounds.

2.2.2. Experimental procedures

The recovery of antioxidant compounds has been carried out through three different extraction techniques: conventional solid-liquid extraction, ultrasound-assisted extraction (UAE), and microwave extraction (MAE). Figure 5 shows the experimental scheme.

The extractions were performed using two different food grade extraction solvents, water (H_2O) and ethanol: water mixture (EtOH:H_2O, 50:50). Three temperatures (25, 40 and 70°C) were tested. The extraction times for MAE were 5 and 15 minutes, and those for conventional and UAE were 30, 60 and 120 minutes.

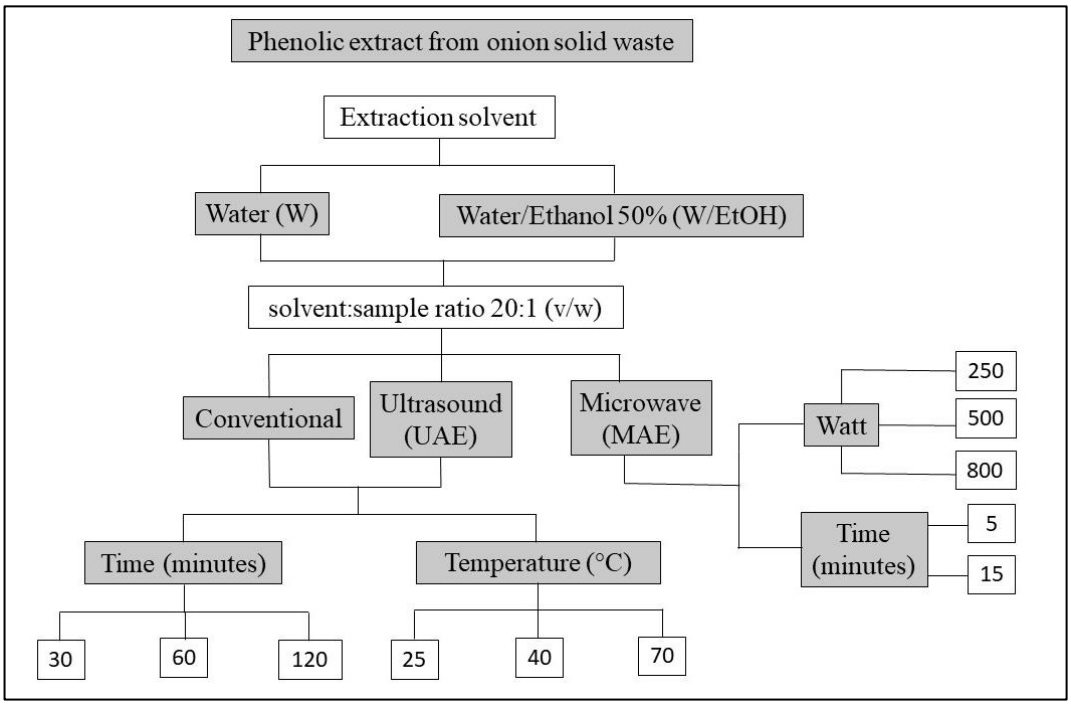

Figure 5: Simplified outline of the experimental procedures of onion solid waste extractions.

- **Conventional solid-liquid extraction**

The extraction was performed according to Mi Jang et al. (2013), with some modifications.

Briefly, 50 mL of solvent were added to 2.5 g of dried and ground OSW and placed on a heating magnetic stirrer for the desired times and temperatures, monitored by a digital thermometer. Subsequently, samples were centrifuged (NF 1200R, Nüve, Ankara, Turkey) at 5000 rpm for 5 min at 4°C, filtered through a Büchner apparatus with 0.45 µm filter paper and the resulting extracts were made up to volume of 50 mL with the respective extraction solvent. The extracts were then filtered with 0.45 µm nylon filters and stored at -20°C until subsequent analyses.

- **Ultrasound-assisted extraction**

Ultrasound-assisted extraction (UAE) was performed using a Sonoplus Ultrasonic homogeniser, Series 2000.2, HD 2200.2 (BANDELIN, Ultraschall seit 1955), as reported in Section 1.2.3. (Ultrasound-assisted extraction), considering the solvent:sample ratio reported in Figure 5. After the extraction time, each sample was treated as described above for conventional extraction.

- **Microwave-assisted extraction**

The extraction was performed as reported in Section 1.2.3. (Microwave-assisted extraction), considering the solvent:sample ratio reported in Figure 5. Subsequently, each sample was treated as described above for other extraction techniques.

2.2.3. Analytical methods

2.2.3.1. Total flavonoid content (TF)

Total flavonoid content (TF) was quantified on the obtained extracts applying the method described by Munir et al. (2018), with some modifications.

In brief, 0.5 mL of extract, 2 mL of deionised water and 0.15 mL of 5% (w/v) $NaNO_2$ were placed in a 5 mL flask and incubated at room temperature for 5 minutes. Subsequently, 0.15 mL of 10% (w/v) $AlCl_3$ were added and incubated at room temperature for 6 minutes. Then, 2 mL of 4% NaOH (w/v) and finally the mixture was made up to volume with deionised water. At the same time, a solution used as a blank was prepared with the same amounts of reagents but without the addition of the sample. The mixture was incubated in the dark for 15 min. The absorbance measured at 510 nm against a blank using a double-beam ultraviolet-visible spectrophotometer (Perkin-Elmer UV- Vis λ2, Waltham, Massachusetts, U.S.) and comparing with a quercetin calibration curve (concentration between 20 and 50 mg L^{-1}). The results were expressed as mg of quercetin g^{-1} of onion solid waste dry weight (mg QE g^{-1} d.w.).

2.2.3.2. Determination of anthocyanin content (TAC)

Total anthocyanin content (TAC) was determined spectrophotometrically according to the AOAC method (2005.02) on the onion waste extracts diluted (D = 1:5, v:v) with pH 1.0 buffer (potassium chloride, 0.025 M) and pH 4.5 buffer (sodium acetate, 0.4M), and the absorbance was determined against the blank (distilled water) at both 520 and 700 nm. Anthocyanin pigment concentration was expressed as mg of cyaniding 3-glucoside g^{-1} of onion solid waste dry weight (mg c-3-gluc g^{-1} d.w.) and was calculated as follows:

$$\frac{A * MW * DF * 10^3}{\varepsilon * 1}$$

Where:

A= (A_{520nm}-A_{700nm}) pH 1.0 - (A_{520nm}-A_{700nm}) pH 4.5; MW (molecular weight) = 449.2 g mol^{-1} for cyaniding 3-glucoside (cyd-3-glu); DF = dilution factor established in D; 1=pathlength in cm; ε=26900 molar extinction coefficient, in L*mol^{-1}*cm^{-1}, for cyd-3-glu; 10^3=factor for conversion from g to mg.

2.2.3.3. Antioxidant activity determination

- **DPPH assay**

The DPPH assay was performed as reported in Section 1.2.4.3. In a cuvette, 50 µL of aqueous extract were added to 2950 µL of a 6 x 10^{-5} M of methanol solution of DPPH· and left under darkness for 15

min at room temperature; the hydroalcoholic extracts required different reaction ratios, such as 25 µL of extract and 2975 µL of DPPH· solution. The results were expressed as µM Trolox equivalents g^{-1} of onion solid waste dry weight (µM TE g^{-1} d.w.), comparing with a Trolox calibration curve (from 6 to 21 µM).

- **ABTS assay**

The antioxidant activity of the extracts was determined by ABTS assay as reported in Section 1.2.4.3. The reaction mixture was prepared by mixing 25 µL of aqueous extract and 2975 µL of the ethanol solution of $ABTS^+$; the hydroalcoholic extracts required different reaction ratios, such as 10 µL of extract and 2990 µL of $ABTS^+$ solution. The quenching of initial absorbance was plotted against the Trolox concentration (from 3 to 18 µM) and the results were expressed as µM Trolox equivalents g^{-1} of onion solid waste dry weight (µM TE g^{-1} d.w.).

2.2.3.4. Identification and quantification of antioxidant compounds

Identification and quantification of antioxidant compounds was performed in each extract following what reported in Section 1.2.4.4. For the quantification of each compound, external standards (concentration between 1 and 100 mg kg^{-1}) were used and the results were expressed as mg kg^{-1} of onion solid waste dry weight (mg kg^{-1} d.w.).

2.2.4 Statistical analysis

All the experimental results were expressed as mean value (n=4) ± standard deviation. SPSS Software (Version 15.0, SPSS Inc., Chicago, IL, USA) was used for data statistical elaboration. Multivariate and One-way analysis of variance (MAVOVA and ANOVA) with Tukey's post hoc test at $p<0.05$. Pearson's correlation test was employed for the determination of correlation coefficients (r) among TF, TAC and antioxidant assays.

2.3. RESULTS AND DISCUSSION

2.3.1. Conventional solid-liquid extraction

As reported in Table 6, among the independent variables studied in this research, the extraction solvent showed a highly significant influence on the extraction process ($p<0.01$). In fact, the MANOVA confirmed that a different solvent (W or W/EtOH) and its combination with other process variables, as the extraction temperature and time, significantly influenced the extraction yield of TF and TAC and the expression of the antioxidant activity (ABTS and DPPH assays). Extraction temperature and time also showed a significant influence on the dependent variables considered, with

the only exception of TF and DPPH, respectively. Therefore, a separate ANOVA was performed for each dependent variable, with each ANOVA valued at an alpha level of 0.05.

Table 6: Multivariate statistical analysis of conventional onion solid waste extracts.

	TF	TAC	ABTS	DPPH
Solvent	**	**	**	**
Temperature	ns	**	**	**
Time	**	**	**	ns
Solvent*Temperature	**	**	ns	*
Solvent*Time	**	ns	ns	**
Temperature*Time	**	**	**	**
Solvent*Temperature*Time	**	**	**	*

Note: The relevance of the symbols is given by the statistical analysis (ANOVA), by the Tukey test with $p < 0.05$. **Significance at $p < .01$; *Significance at $p < .05$. Abbreviation: ns, not significant.

The aqueous extracts (W) showed a highly significant lower extraction yield compared with their respective hydroalcoholic extracts (W/EtOH), as well as antioxidant activity values (Table 7), confirming the extraction trend already reported in other works in literature (Vojvodić et al., 2014; Munir et al., 2018). In fact, as described by Makris (2010) and Nile et al. (2017), solvents composition strongly influences its properties, for example density and dynamic viscosity, which influence the extraction amount, determining phenolic content and thus antioxidant activity of the final extracts.

Among all the aqueous extracts, the best in terms of TF appeared to be the extract obtained at 70°C for 30 minutes, with 14.32±0.71 mg QE g^{-1} d.w. of onion solid waste. At the same time, these extraction conditions were not found to be the best in terms of TAC, ABTS and DPPH activity, for which the best parameters were 40°C and 60 minutes, supported by the lower correlation coefficient between TF and ABTS (r=0.49) and DPPH (r=0.66) than TAC (r=0.87 and r=0.89 for ABTS and DPPH, respectively). The fact that a higher flavonoids content does not always correspond to a higher antioxidant activity of the extracts could be explained by the circumstance that only one method or assay is not adequate to establish in a meticulous and complete way the antioxidant activities of several phytochemicals. So, it is necessary to make use of various approaches to quantify the antioxidant activities of flavanols recovered from OSW (Nile at el., 2018).

The values investigated in this study were comparable to those stated by Benítez et al. (2011) for TF in onion outer scales and brown skin, who found that a decline of total phenolics and flavonoids was detected from the outer to the inner layers of the bulb.

Concerning the extracts obtained with the W/EtOH mixture, the best extraction conditions were found to be like the previous ones, 40°C and 60 minutes, with a TF of 25.64±1.40 mg QE g^{-1} d.w. of onion

solid waste. The only exception was noticed for the DPPH values, among which the highest one was detected at 25°C and 120 minutes. This aspect could be clarified by the lower correlation coefficient between TF and DPPH (r=0.39) than between TF and ABTS (r=0.93), as well as for the TAC. Antioxidant activity in foodstuffs depends on numerous factors, such as polarity, solubility, metal-chelating capacity and the method applied for its valuation (Albishi et al., 2013). Commonly, it is considered that onion varieties rich in polyphenols (yellow, red, purple) show higher antioxidant activity (Benkeblia, 2005), but the connection has been shown to be stronger with the flavonoid portion rather than total phenols (Sellappan & Akoh, 2002). In agreement with literature (Rodrigues et al., 2010), it is obvious that the amounts of anthocyanins in OSW samples considered in this study are much lower than those of flavonoids.

The data obtained in this study was comparable to the one stated by Makris & Kefalas (2015), who stated that the greatest extraction amount was obtained at 40°C, while either at 20°, 60 °C or beyond, its values were lower, demonstrating the influence of temperature on the extraction process. Indeed, temperature of the extraction process influences the molecules stability due to chemical and enzymic degradation and/or losses by thermal decomposition; these have been indicated to be the leading aspect producing the drop in polyphenol content (Moure et al., 2001). In addition to thermal decomposition, extraction time becomes crucial, since larger extraction periods might provoke more evident polyphenol losses. The severe influence that extraction time may exert could indicate that extraction might be protracted until maximum yield is achieved (Kiassos et al., 2009).

Even if the best extraction conditions of both kinds of extracts considered in this study are similar, the greatest extraction rate of bioactive compounds and expression of antioxidant activity in the hydroalcoholic ones confirms the highly significant influence of the extraction solvent on the process, as observed previously by multivariate analysis (Table 6).

Table 7: Total flavonoid content (TF), Total anthocyanin content (TAC) and expression of antioxidant activity (DPPH and ABTS assays) of extracts (W and W/EtOH) obtained by conventional solid-liquid extraction.

		TF (mg QE g^{-1} d.w.)			TAC (mg C-3-GLUC g^{-1} d.w.)		
Minutes	**°C**	**W**	**W/EtOH**	**Sign.**	**W**	**W/EtOH**	**Sign.**
30	**25**	9.85 ± 0.93^{b}	21.30 ± 0.49^{a}	**	0.18 ± 0.01^{a}	0.65 ± 0.03^{a}	**
	40	4.84 ± 0.19^{c}	16.66 ± 1.32^{b}	**	0.15 ± 0.04^{a}	0.32 ± 0.07^{b}	**
	70	14.32 ± 0.71^{a}	19.47 ± 2.16^{ab}	**	0.08 ± 0.01^{b}	0.37 ± 0.08^{b}	**
	Sign.	**	**		**	**	
60	**25**	3.88 ± 0.13^{c}	18.09 ± 0.83^{b}	**	0.08 ± 0.03^{b}	0.39 ± 0.05^{b}	**
	40	10.33 ± 0.91^{a}	25.64 ± 1.40^{a}	**	0.32 ± 0.05^{a}	0.78 ± 0.01^{a}	**
	70	6.74 ± 0.40^{b}	19.59 ± 2.29^{b}	**	0.15 ± 0.03^{b}	0.28 ± 0.06^{c}	**
	Sign.	**	**		**	**	
120	**25**	5.22 ± 0.46^{a}	20.61 ± 2.03^{a}	**	0.14 ± 0.05^{a}	0.47 ± 0.01^{a}	**
	40	3.98 ± 0.25^{b}	17.17 ± 0.65^{b}	**	0.12 ± 0.03^{a}	0.34 ± 0.02^{b}	**
	70	3.81 ± 0.59^{b}	18.47 ± 1.55^{ab}	**	0.03 ± 0.00^{b}	0.31 ± 0.05^{b}	**
	Sign.	**	*		**	**	
		ABTS (µM TE g^{-1}d.w.)			**DPPH (µM TE g^{-1}d.w.)**		
Minutes	**°C**	**W**	**W/EtOH**	**Sign.**	**W**	**W/EtOH**	**Sign.**
30	**25**	26.55 ± 2.67^{a}	76.27 ± 5.92^{a}	**	8.50 ± 0.45^{a}	29.42 ± 1.31^{a}	**
	40	20.01 ± 0.99^{b}	60.49 ± 9.79^{b}	**	6.44 ± 0.75^{b}	25.93 ± 2.04^{b}	**
	70	16.34 ± 1.06^{c}	66.97 ± 7.34^{ab}	**	7.25 ± 0.39^{b}	30.44 ± 1.50^{a}	**
	Sign.	**	*		**	**	
60	**25**	16.88 ± 2.44^{b}	62.42 ± 3.59^{b}	**	5.26 ± 0.53^{b}	27.06 ± 2.14^{a}	**
	40	31.03 ± 1.48^{a}	94.11 ± 3.31^{a}	**	10.63 ± 1.10^{a}	30.68 ± 0.85^{a}	**
	70	19.98 ± 0.26^{b}	56.78 ± 9.80^{b}	**	6.45 ± 0.57^{b}	21.88 ± 3.18^{b}	**
	Sign.	**	**		**	**	
120	**25**	18.08 ± 0.90^{a}	69.13 ± 3.61^{a}	**	6.57 ± 0.38^{a}	37.95 ± 5.27^{a}	**
	40	10.72 ± 0.30^{b}	57.47 ± 6.36^{b}	**	5.68 ± 0.77^{a}	26.13 ± 4.72^{b}	**
	70	12.46 ± 1.45^{b}	64.56 ± 3.98^{ab}	**	2.39 ± 0.17^{b}	30.02 ± 3.33^{ab}	**
	Sign.	**	*		**	*	

The data are presented as means ± SD (n=4). Means within a column with different letters 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.

2.3.2. Ultrasound-assisted extraction

The multivariate statistical analysis (Table 8) showed that in ultrasound-assisted extraction all individual independent variables considered in this study and the interaction of them affected in a highly significant way (p<0.01) the total flavonoids (TF) and anthocyanins contents (TAC), as well as the expression of the antioxidant activity performed by ABTS and DPPH assays. Subsequently, a

separate ANOVA was carried out for each dependent variable, with each ANOVA valued at an alpha level of 0.05.

Table 8: Multivariate statistical analysis of UAE onion solid waste extracts.

	TF	TAC	ABTS	DPPH
Solvent	**	**	**	**
Temperature	**	**	**	**
Time	**	**	**	**
Solvent*Temperature	**	**	**	**
Solvent*Time	**	**	**	**
Temperature*Time	**	**	**	**
Solvent*Temperature*Time	**	**	**	**

Note: The relevance of the symbols is given by the statistical analysis (ANOVA), by the Tukey test with $p < 0.05$. **Significance at $p < .01$; *Significance at $p < .05$. Abbreviation: ns, not significant.

Looking at the W extracts (Table 9), the extraction performed at 70°C for 30 minutes was the best one in term of all dependent variables considered. There seems to be an increasing trend in the extraction rate as the temperature increases for 30-minute extractions, until a TF and TAC values of 6.52±0.03 mg QE g^{-1} d.w. and 0.07±0.00 mg C-3-GLUC g^{-1} d.w. of onion solid waste, respectively. As previously discussed for the conventional extraction, also in UAE an obvious influence by the extraction solvent can be seen, with W/EtOH being the best one. As reported by Vojvodić et al. (2014), for onion peel, ethanol has been demonstrated to be better than water. Considering the 30-minutes extractions in both W and W/EtOH, a significant upward trend was noted with increasing temperature both in terms of extraction yield of TF and TAC and antioxidant activity, with the maximum value at 70°C. A similar trend is noticeable with the W/EtOH extractions performed at 25° and 40°C for all times considered, with an increasingly better performance as the extraction time increases and an optimum at 120 minutes. Total flavonoids and anthocyanins extraction content from onion solid waste showed to decrease at temperatures higher than 40°C, in agreement with previous studies (Makris, 2016; Khiari et al., 2009). Indeed, temperature cannot be risen above specific levels, as this has been proven damaging to anthocyanins, provoking their thermal degradation (Cacace & Mazza, 2002; Monrad et al., 2010). These aspects confirm the high significance influence of time and temperature on the studied extraction process. Although the influence of high temperature on the extraction yield of phenolic compounds has been proven to be positive in numerous situations (Aliakbarian et al., 2012; Brahim et al., 2014; Qu et al., 2010), because of the promoted polyphenol diffusion and their improved solubility (Boussetta et al., 2011; Galanakis et al., 2013), in this research TF and TAC of onion solid waste decreased at temperatures higher than 40°C for 60- and 120-minutes extractions. Indeed, contrary to previous reports, temperature cannot be increased beyond certain

limits, as this has been proven detrimental to anthocyanins (Cacace & Mazza, 2002; Jing & Giusti, 2007; Monrad et al., 2010). In this regard, the highest TF was detected for the extraction at 25° and 40°C for 120 minutes, showing a high correlation (r >0.90) to the other variables considered. The results stated by Albishi et al., 2013 are comparable to those of the present research for total flavonoid and anthocyanins content in red onion peel. Moreover, results obtained from the current work confirmed what reported by Lee et al. (2014) that the DPPH radical scavenging activity of onion peel extract was higher in ethanol compared to hot water. Velioglu et al. (1998) and Shahidi & Naczk (2004) reported that the antioxidant activity of food products depends on the chemical nature of its components and not always on their quantities, because their efficiencies vary significantly.

Table 9: Total flavonoid content (TF), Total anthocyanin content (TAC) and expression of antioxidant activity (DPPH and ABTS assays) of extracts (W and W/EtOH) obtained by ultrasound-assisted extraction.

		TF (mg QE g^{-1} d.w.)			TAC (mg C-3-GLUC g^{-1} d.w.)		
Minutes	**°C**	**W**	**W/EtOH**	**Sign.**	**W**	**W/EtOH**	**Sign.**
30	**25**	1.55 ± 0.07^{c}	10.94 ± 0.22^{c}	**	0.05 ± 0.00^{b}	0.19 ± 0.01^{b}	****
	40	3.58 ± 0.17^{b}	13.58 ± 1.13^{b}	**	0.04 ± 0.00^{c}	0.26 ± 0.02^{a}	****
	70	6.52 ± 0.03^{a}	16.22 ± 0.33^{a}	**	0.07 ± 0.00^{a}	0.29 ± 0.02^{a}	****
	Sign.	**	**		**	**	
60	**25**	2.59 ± 0.13^{b}	13.28 ± 0.49^{b}	**	0.02 ± 0.00^{b}	0.29 ± 0.02^{b}	****
	40	1.18 ± 0.06^{c}	20.79 ± 0.56^{a}	**	0.03 ± 0.00^{a}	0.50 ± 0.01^{a}	****
	70	4.15 ± 0.11^{a}	13.54 ± 0.38^{b}	**	0.04 ± 0.00^{a}	0.25 ± 0.02^{c}	****
	Sign.	**	**		**	**	
120	**25**	2.37 ± 0.10^{c}	22.71 ± 0.63^{a}	**	0.03 ± 0.00^{b}	0.59 ± 0.02^{a}	****
	40	3.50 ± 0.11^{a}	23.12 ± 0.52^{a}	**	0.05 ± 0.00^{a}	0.48 ± 0.02^{b}	****
	70	2.67 ± 0.16^{b}	15.92 ± 0.17^{b}	**	0.03 ± 0.00^{b}	0.29 ± 0.01^{c}	****
	Sign.	****	****		****	****	
		ABTS (µM TE g^{-1}d.w.)			**DPPH (µM TE g^{-1}d.w.)**		
Minutes	**°C**	**W**	**W/EtOH**	**Sign.**	**W**	**W/EtOH**	**Sign.**
30	**25**	16.00 ± 1.21^{ab}	36.16 ± 0.43^{b}	**	0.38 ± 0.13^{b}	11.84 ± 1.43^{c}	**
	40	14.74 ± 1.90^{b}	47.61 ± 0.67^{a}	**	1.45 ± 0.46^{a}	14.52 ± 0.39^{b}	**
	70	19.03 ± 2.79^{a}	46.53 ± 2.69^{a}	**	1.34 ± 0.38^{a}	17.27 ± 0.50^{a}	**
	Sign.	*	**		**	**	
60	**25**	15.77 ± 1.28	47.36 ± 1.97^{b}	**	0.14 ± 0.04^{b}	15.75 ± 1.34^{b}	**
	40	15.62 ± 0.68	57.18 ± 0.61^{a}	**	0.18 ± 0.08^{b}	23.43 ± 1.29^{a}	**
	70	16.09 ± 2.03	47.04 ± 3.73^{b}	**	0.52 ± 0.19^{a}	14.69 ± 1.00^{b}	**
	Sign.	ns	**		**	**	
120	**25**	21.44 ± 2.08^{a}	66.30 ± 1.95^{a}	**	0.15 ± 0.03^{b}	23.06 ± 0.33^{b}	**
	40	16.77 ± 1.27^{b}	59.18 ± 1.97^{b}	**	0.79 ± 0.29^{a}	25.17 ± 0.40^{a}	**
	70	15.03 ± 2.76$^{\mathbf{b}}$	45.80 ± 4.01$^{\mathbf{c}}$	****	0.12 ± 0.06$^{\mathbf{b}}$	15.59 ± 0.69$^{\mathbf{c}}$	****
	Sign.	****	****		****	****	

The data are presented as means ± SD (n=4). Means within a row with different letters 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.

2.3.3. Microwave-assisted extraction

From multivariate data analysis reported in Table 10, among the independent variables examined in this research, the extraction solvent and power revealed a highly significant influence on the extraction process. In fact, the MANOVA pointed out that a different solvent (W or W/EtOH) significantly affected ($p<0.01$) the extraction yield of TF and TAC and ABTS and DPPH results. Extraction power and its interaction with time also showed a significant influence, with the only exception of TF. Contrary to solvent and power variables, the multivariate data analysis reported a lack of significant influence by time on TAC and DPPH variables. A separate ANOVA was performed for each dependent variable, with each ANOVA valued at an alpha level of 0.05.

Table 10: Multivariate statistical analysis of MAE onion solid waste extracts.

	TF	TAC	ABTS	DPPH
Solvent	**	**	**	**
Power	**	*	**	**
Time	**	ns	**	ns
Solvent*Power	**	ns	*	**
Solvent*Time	ns	ns	**	ns
Power*Time	**	**	**	**
Solvent*Power*Time	**	ns	**	**

Note: The relevance of the symbols is given by the statistical analysis (ANOVA), by the Tukey test with $p < 0.05$.
**Significance at $p < .01$; *Significance at $p < .05$.
Abbreviation: ns, not significant.

Looking at the 5-minutes aqueous extracts (Table 11), there was no significant difference among the values of all the dependent variables considered, which suggests that in this case there was no influence at all by the power on the extraction process. Contrary to this, the 15-minutes extractions showed a high significance difference among them, with the 250- and 500W-extractions being the best ones. Indeed, it seems that over this power there was a drop in both extraction yield of valuable compounds and expression of antioxidant activity. As determined by multivariate analysis, there is no influence of extraction time on the TAC and DPPH variables, contrary to what can be found for TF and ABTS. In fact, for extractions at 250 and 500W there is a significant increase in total flavonoids content and antioxidant activity values passing from 5 to 15 minutes of extraction, whereas a drop of values can be seen for extractions carried out at 800°C, which indicated that a higher TF could be linked to a proportional antioxidant activity, in accordance with what reported by Juàniz et al. (2016). However, the proportionality between the polyphenolic content and the antioxidant activity is not a general trend, as suggested by previous studies (Mylonaki et al., 2008; Karvela et al., 2009; Khiari et al.,2009). Although higher polyphenols levels are frequently associated with a higher

reducing power, increased extraction temperatures might negatively affect radical scavenging activity (Qu et al., 2010). On the other hand, the W/EtOH extracts showed a different trend, as both 5- and 15-minutes extracts presented significant differences (Table 11). Specifically, they showed an inverse extraction trend depending on whether the extraction was conducted for a short or longer time: in the first case, 800W was the best power when combined with a 5-minutes extraction time, with a maximum TF value of 18.04±0.83 mg QE g^{-1} d.w. of onion solid waste, in contrast to 15-minute extractions where the best power was 250W (19.09±0.45 mg QE g^{-1} d.w. of onion solid waste). Thus, the time requirement in MAE is greatly reduced compared to the conventional techniques and UAE (Pal et al., 2018). In accordance with other literature studies (Gorinstein et al., 2008; Albishi et al., 2013), red onion skins are the richest in terms of total anthocyanin content if compared with other onion varieties. The TAC values obtained in this study are higher than those reported by Gorinstein et al., 2008, in which the highest content of anthocyanins in red onion skin was 10.04±0.90 mg C-3-gluc 100 g^{-1} skin, and by Lauro & Francis (2000), who reported that the total anthocyanins in red onions was 7–21 mg 100 g^{-1} sample.

Table 11: Total flavonoid content (TF), Total anthocyanin content (TAC) and expression of antioxidant activity (DPPH and ABTS assays) of extracts (W and W/EtOH) obtained by microwave assisted extraction.

		TF (mg QE g^{-1} d.w.)			TAC (mg C-3-GLUC g^{-1} d.w.)		
Minutes	**Watt**	**H_2O**	**W/EtOH**	**Sign.**	**H_2O**	**W/EtOH**	**Sign.**
5	**250**	5.83 ± 1.41	13.49 ± 0.94^{b}	**	0.22 ± 0.04	0.43 ± 0.02^{b}	**
	500	4.58 ± 1.09	13.99 ± 0.06^{b}	**	0.30 ± 0.01	0.50 ± 0.02^{a}	**
	800	6.26 ± 0.99	18.04 ± 0.83^{a}	**	0.27 ± 0.06	0.52 ± 0.01^{a}	**
	Sign.	**ns**	****		**ns**	****	
15	**250**	9.58 ± 1.36^{a}	19.09 ± 0.45^{a}	**	0.31 ± 0.01^{a}	0.55 ± 0.05^{a}	**
	500	9.19 ± 2.84^{a}	15.23 ± 0.50^{b}	**	0.26 ± 0.05^{a}	0.47 ± 0.02^{b}	**
	800	3.89 ± 0.58^{b}	13.27 ± 0.74^{c}	**	0.17 ± 0.01^{b}	0.44 ± 0.03^{b}	**
	Sign.	****	****		****	****	
		ABTS (µM TE g^{-1}d.w.)			**DPPH (µM TE g^{-1}d.w.)**		
Minutes	**Watt**	**H_2O**	**W/EtOH**	**Sign.**	**H_2O**	**W/EtOH**	**Sign.**
5	**250**	21.20 ± 3.31	41.24 ± 3.70^{b}	**	8.81 ± 1.40	9.21 ± 0.37^{b}	**ns**
	500	20.75 ± 2.20	50.08 ± 2.66^{a}	**	7.94 ± 1.28	12.68 ± 0.52^{a}	**
	800	21.17 ± 2.41	55.18 ± 6.16^{a}	**	7.25 ± 1.04	12.36 ± 0.82^{a}	**
	Sign.	**ns**	****		**ns**	****	
15	**250**	24.99 ± 0.94^{a}	59.29 ± 3.49^{a}	**	10.38 ± 0.51 ^{a}	13.00 ± 0.25^{a}	**
	500	27.22 ± 3.26^{a}	60.29 ± 1.66^{a}	**	7.54 ± 0.32^{b}	9.50 ± 0.38^{c}	**
	800	16.59 ± 1.22^{b}	48.34 ± 1.39^{b}	**	5.31 ± 0.99^{c}	11.60 ± 0.46^{b}	**
	Sign.	****	****		****	****	

The data are presented as means ± SD (n=4). Means within a row with different letters 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.

2.3.4. Comparison of conventional, ultrasound (UAE) and microwave assisted extraction (MAE)

The best extracts from each of the tested extraction techniques were selected not only with reference to the total content of bioactive compounds and related antioxidant activity, but also from the point of view of the cost-effectiveness of the extraction process and in terms of time saving. For all methods considered, the hydroalcoholic mixture (EtOH 50%) was the best extraction solvent. In this study, for each kind of extraction, the following optimal conditions were identified: 60 minutes at 40°C (conventional), 120 minutes at 25°C (UAE) and 5 minutes at 800 Watt (MAE). All major flavonoids of the selected extracts were identified and quantified by UHPLC system (Table 12).

Isorhamnetin-3-O-glucoside, quercetin and quercetin 3,4'-diglucoside were among the main flavonols determined and among the minor ones were isorhamnetin and quercetin 3-glucoside. According to literature, one of the main flavonol in onion outer scales was the quercetin 3,4'-diglucoside (Benítez et al., 2011; Lombard et a., 2005). Protocatechuic acid have been demonstrated to derive from quercetin through oxidative cleavage by onion peroxidase (Osman et al., 2008). Indeed, the OSW include a specific range of polyphenols, many of them derived from quercetin and quercetin glucosides, by peroxidase action (Ly et al., 2005; Ramos et al., 2006).

Table 12: Phenolic compounds identified in selected hydroalcoholic onion solid waste extracts (mg kg^{-1} d.w.).

Compounds	Conventional	UAE	MAE	Sign.
Rutin	44.79 ± 0.30^{c}	125.03 ± 0.05^{a}	98.43 ± 0.13^{b}	**
Isorhamnetin	114.01 ± 0.02^{a}	62.37 ± 0.02^{b}	60.04 ± 0.03^{c}	**
Quercetin	5322.61 ± 0.32^{a}	179.21 ± 0.17^{b}	148.95 ± 0.05^{c}	**
Kaempferol	256.27 ± 0.13^{a}	29.03 ± 0.01^{b}	27.20 ± 0.07^{c}	**
Quercetin-3-O-glucoside	69.54 ± 0.01^{c}	85.02 ± 0.10^{a}	84.09 ± 0.12^{b}	**
Quercetin-3-4-diglucoside	454.55 ± 0.18^{c}	1023.80 ± 0.34^{a}	916.08 ± 0.04^{b}	**
Apigenina-7-O-glucoside	9.82 ± 0.03	n.d.	n.d.	**

The data are presented as means ± SD (n=3). Means within a row with different letters are significantly different by Tukey's post hoc test. Abbreviation: Sign., significance; ns, not significant; n.d., not detected; **Significance at $p<0.01$; *Significance at $p<0.05$.

The obtained results confirmed what stated in literature, as quercetin 3,4-diglucoside was identified among the predominant class of flavonoids present in onion and quercetin 3-glucoside was reported to be one of the minors (Rodrigues et al., 2010). In accordance with Albishi et al., 2013, quercetin 3,4-diglucoside and quercetin were among the main phenolics in the selected onion solid waste extracts, thanks to their higher thermal stability than other flavonoids, such as kaempferol (Juàniz et al., 2016). In addition, both losses and gains in quercetin derivates content in onions was reported

depending on the heat treatment circumstances (Price et al., 1997; Lombard et al., 2005; Crozier et al., 1997; Ewald et al. 1999; Rodrigues et al., 2009). Indeed, the softening effect due to heat-induced wall and cells fractures could also impact on phenolic extractability (Harris et al., 2015; Palermo et al., 2014). The greatest amount of quercetin (5322.61±0.32 mg kg^{-1} d.w.) was recovered from onion solid waste by conventional method. This result confirms the highest radical scavenging activity detected by DPPH assay previously reported (Table 2). Indeed, as reported by Nuutila et al. (2003), among the onion flavonoids, quercetin showed the most efficient DPPH radical scavenging activity and it also reacted more quickly than the other flavonoids, such as rutin and kaempferol, and is one of the major flavonoids detected (Beesk et al., 2010). Some minor flavonoids, such as luteolin and myricetin derivates, quantified in onion by other authors were not identified in the studied extracts, probably due to differences in onion cultivar or agricultural practices adopted (Sellappan & Akoh, 2002; Simin et al., 2013; Benítez et al., 2011; Perner et al., 2008). The influence of field treatment on flavonoid levels was already described by Rodrigues et al. (2009), as well as the effect of post-harvest practices (Rodrigues et al., 2010).

However, the results obtained in this study are comparable with what described by Albishi et al., 2013, who reported that quercetin, quercetin 3,4-diglucoside and kaempferol were prevalent in all onion samples considered. In addition, Sellappan & Akoh (2002) observed that the kaempferol in onions was observed to be in minor amounts in comparison to quercetin.

2.4. CONCLUSIONS

In conclusion, it was observed that the solvent system composed of W/EtOH 50% can efficiently extract flavonoids and anthocyanins from onion solid waste, through conventional, ultrasound and microwave extraction processes. Looking at the comparative data discussed previously (Table 7), it can be stated that the W/EtOH 50% and conventional extraction (for 60 minutes at 40°C) could be applied successfully as a food grade alternative technique for recovering valuable phenolic compounds from onion solid waste, representing a crucial point in the valorisation of food waste as functional ingredients. In fact, the implementation of similar extraction techniques by food industries would build the basis for the growth and expansion of green processes, to enhance the value of food waste and the sustainable production of new value-added products in the food, pharmaceutical and cosmetic sectors.

3. PRODUCTION OF FUNCTIONAL BAKERY PRODUCTS

The research in paragraph 3.1 is reported as an article published in the journal "Foods" (*Foods* 2021, 10, 2460.https://doi.org/10.3390/foods10102460).

3.1. FUNCTIONALIZED BISCUITS WITH BIOACTIVE INGREDIENTS OBTAINED BY CITRUS LEMON POMACE

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Abstract: In this study, functionalized biscuits were prepared through the enrichment of dough with natural antioxidants extracted from lemon pomace. Lemon pomace extract (LP_E) was analyzed before being applied for food aims and a known concentration equal to 50 mg kg^{-1} of total phenolic compounds was used for the formulation of enriched biscuits. Three different samples typologies were compared with a control biscuit, without the addition of functional ingredients. The main physico-chemical, microbiological and sensory aspects of doughs and biscuits enriched with LP_E were investigated. As results, the enriched biscuits showed higher phenolic content and antioxidant activity than the control one and a longer Induction Period (IP), which means that the enriched products had a higher intrinsic resistance to lipid oxidation, thanks to the antioxidant effect exerted by the added fresh lemon peel and the LP_E. Furthermore, from a sensorial point of view, they showed a good acceptability, in terms of appearance, flavor and aromatic attributes. Thus, the results indicated that the incorporation of lemon processing by-products allowed the production of functional enriched biscuits with improved antioxidant properties.

Keywords: Antioxidant, biscuits, lemon pomace, phenolic compounds, oxidative stability, UHPLC.

3.1.1. INTRODUCTION

Wastes are widely generated by food production, of whom fruits and vegetables have the highest waste rates of any food. Although these by-products still contain nutrients and bioactive compounds, they are considered a problem for their environmental footprint (de Castro et al., 2020). For this

reason, the demand for more sustainable practices towards the application of a circular economy in the food system represents a key strategy for the future. Consequently, most studies are focused on improving the possibility to extract nutrients from by-products and using parts of them in the production of new functional foods (Curutchet et al., 2019; Sullivan et al., 2013; Saini et al., 2019; Romeo et al., 2020). In this context, citrus is one of the most important fruit crops in the world with an annual production exceeding 122.5 million tons and one-third of the crop is processed, e.g., to produce juices, jams, or the extraction of essential oils. Worldwide industrial citrus residues represent about 50% of the whole fruit mass: during citrus processing, peel residues are the primary waste fraction, amounting to almost half of the total weight of the fruit (Wang et al., 2015).

Particularly, lemon (*Citrus limon (L.) Osbeck*) pomace, a by-products of juice and essential oil processing industries, and are a good source of sugars, minerals, organic acids, dietary fibers and phenolic compounds, such as phenolic acids (ferulic, p-coumaric and sinapic acids) and flavonoids (flavanones, flavonols, flavones) with salubrious characteristics, such as antioxidants, anti-inflammatory and antimicrobial properties (Nair et al., 2019; Mathew et al., 2013; Al-Qassabi et al., 2018).

In response to consumers' requests for healthier foods, the use of new functional ingredients recovered from food by-products could be a valid alternative for the formulation of high added value products (Pasqualone et al., 2014). Among bakery goods, biscuits could represent a viable and acceptable candidate for the addition of functional ingredients (Ismail et al., 2014), even if their consumption is usually associated with an unhealthy lifestyle because of high levels of fat and sugar. They are the most consumed bakery products in many parts of the world thanks to their ready to eat nature, economic cost, interesting nutritional qualities, differentiation of tastes and longer shelf life (Ajila et al., 2008). Much research has focused on the production of biscuits with functional ingredients from by-products (Ismail et al., 2014; Egea et al., 2018; Larrea et al., 2005; de Abreu et al., 2019). At the same time, sensory quality is a fundamental aspect of most food products since it relates to their different taste perceptions, stability and nutritional properties. Sensory evaluation is a scientific topic used to evoke, measure, analyze and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch and hearing (Arifin et al., 2010).

The aim of this research consists in preparing biscuits enriched with functional ingredients obtained from lemon by-products, characterizing their physico-chemical aspects, antioxidant profile and sensory quality in comparison with the control ones, so that a consumer food product with a beneficial impact on health can be produced.

3.1.2. MATERIALS AND METHODS

3.1.2.1. Raw material

Citrus lemon pomace (*Citrus limon (L.) Osbeck*) were supplied by a company located in the province of Reggio Calabria (Italy) that produce juice and essential oils and after, were transported to the Food Technology laboratory of the Mediterranea University of Reggio Calabria. Lemon by-products samples were immediately freeze-dried (-70°C) in a VirTis lyophilizer (Gardiner, NY, USA) and then vacuum-stored in polyethylene bags until subsequent extraction procedures of the bioactive compounds. The other ingredients used for the biscuit's preparation, particularly: wheat flour, corn seed oil, white sugar, cow skimmed milk and chemical baking powder (disodium diphosphate, sodium hydrogen carbonate, cornstarch), were purchased into a supermarket.

3.1.2.2. Preparation of Lemon pomace extract (LP_E)

The extraction of antioxidant compounds from freeze-dried lemon pomace (LP) has been carried out according to Papoutsis et al. (2018b). 200 mL of ethanol:water (1:1, v:v) solution were mixed to 20g ground LP in a Sonoplus Ultrasonic homogenizers, Series 2000.2, HD 2200.2 (BANDELIN, Ultraschall seit 1955), for 40 minutes at 40°C. Subsequently, the sample was centrifuged (5000 rpm, 5 min, 4°C, in a refrigerated centrifuge, (NF 1200R, Nüve, Ankara, Turkey)), filtered (0.45 µm filter paper) and the resulting extract was diluted to 200 mL with the extraction solvent (Food-Grade ethanol:water, 1:1, v:v), which is reported to be suitable for the production of extracts to apply in the food system (Hidalgo & Almajano, 2017; D'Alessandro et al., 2012; Rodrigues et al., 2015; Reddy et al., 2005). Before being used for the enrichment of biscuits, the extract thus obtained (LP_E) was subjected to qualitative and quantitative analysis.

3.1.2.3. Formulation of enriched biscuits with bioactive compounds

All the biscuit dough formulations and denominations are reported in Tables 13. Samples were prepared to offer three variations compared to a control sample (A). The modifications to this formulation (A) were made to produce biscuits with the adding of: fresh lemon peel (B); fresh lemon peel and LP_E (C) and with only LP_E (D), by skimmed milk substitution. Concerning the preparation, corn seed oil and white sugar were firstly mixed (3200 rpm min^{-1}, 1 min) in an electric lab-scale mixer (Bimby TM31, Vorwerk, Wuppertal, Germany); then, the skimmed milk was added and mixed for two minutes (1800 rpm min^{-1}). Finally, the wheat flour and chemical baking powder were added and mixed (1100 rpm min^{-1}) for two more minutes. The obtained dough was rolled out with a calibrated

rolling pin and shaped using a cutter for biscuits to assure the same thickness of approximately 3.5 mm for all the samples. The dough shapes were placed on a perforated rectangular tray and baked in a preheated industrial oven (Angelo Po Combistar FX, Carpi, Modena, Italy) for 7 minutes at 180°C (Fig.6, example of final products). Subsequent, product characterization analyses were carried out on both doughs and final products.

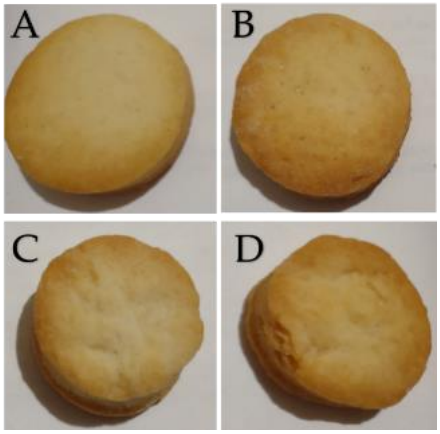


Figure 6: Image of final products (biscuits A, B, C, D).

Table 13: Samples denomination.

A	Control			
B	Dough with fresh lemon peel			
C	Dough with fresh lemon peel and LP_E			
D	Dough with LP_E			
Ingredients	**A**	**B**	**C**	**D**
Wheat flour (g)	400	400	400	400
Corn seed oil (mL)	80	80	80	80
White sugar (g)	120	120	120	120
Skimmed milk (mL)	120	120	70	70
Baking powder (g)	8	8	8	8
Fresh lemon peel (g)	0	10	10	0
Lemon pomace extract (mL)	0	0	50	50

3.1.2.4. Characterization of physico-chemical properties of LP_E, Doughs and biscuits

3.1.2.4.1. Physico-chemical evaluation

The moisture content in both doughs and baked biscuits was determined using a Sartorius Moisture Analyzer MA37 thermal balance, by the gravimetric method at 105°C until constant weight. The results were expressed as a percentage of moisture (%).

Water activity (a_w) of doughs and biscuits was conducted using an Aqualab LITE hygrometer (Decagon) and the pH of LP_E doughs and biscuits in a Crison pH-meter, basic model 20, according to the AOAC International Method (AOAC, 1980).

The color analysis was performed using an automatic Minolta CR 300 tristimulus colorimeter. The CIE L*a*b* system was used as reference. The color parameters were L*, brightness (from 0 black to 100 white), a* (from –50 green to 50 red), b* (from -50 blue to 50 yellow), Chroma (C*), represents

the degree of saturation/ fullness of color, was calculated as $(a^2 + b^2)^{1/2}$ and hue angle (H) which describes the amounts of redness and yellowness (from 90 yellow to 180 green) was calculated as "arctan (b*/a*)". The measurement was carried out on 15 mL of LP_E in ten different points, placing the sample in an optical glass, and directly on doughs and biscuits, performing the readings at different points.

3.1.2.5. Microbiological analysis

10 g of each dough and biscuit was homogenized for 3 minutes with 10 mL of Ringer solution using a Stomacher BagMixer 400 (Interscience, 30 Ch.Bois Arpents F.78860 St. Nom - France). Series dilutions of the homogenates were poured into the Petri plates in specific agar for total bacterial count (Plant Count Agar, Oxoid, at 25 ± 2°C for 48h), and for yeasts and molds count into the DRBC (Dichloran Rose Bengal Chloramphenicol) agar base plates (at 28 ± 2 °C for 48 h). At the end of the incubation period, the microbial colonies obtained were counted and expressed in Log10 CFU g^{-1} of biscuit.

3.1.2.6. Evaluation of Total phenol content and antioxidant activity

The phenolic extraction method for dough and biscuit samples was carried out following Miskiewicz et al. (2018), with appropriate modifications. 5 g of sample were ground in an electric grinder and mixed with 20 mL of methanol, 2.5 mL of distilled water and 0.250 mL of concentrated hydrochloric acid (HCl). The mixtures were placed in an ultrasonic bath for 60 minutes at 30°C and then centrifuged for 10 minutes at 6000 rpm at 4°C. The supernatant of the respective formulations was recovered, filtered, and made up to volume in a 25 mL flask with a 1:10 methanol: water mixture.

- **Total phenolic compounds (TPC)**

TPC was determined according to the method reported by González - Molina et al. (2009), with appropriate modifications. For the reaction, different concentrations of the extract were used, or rather: 0.2 mL of LP_E and 1 mL of doughs and biscuits extracts, that were placed inside a 25 mL flask and mixed with 5 mL of deionized water and 1 mL of Folin-Ciocalteu reagent. After 8 minutes, 10 mL of saturated sodium carbonate solution (Na_2CO_3, 20%) was added and diluted to volume with deionized water. The mixtures were incubated for two hours at room temperature and in the dark. The absorbance was measured at 765 nm against a blank using a double-beam ultraviolet-visible spectrophotometer (Perkin-Elmer UV- Vis λ2, Waltham, Massachusetts, U.S.) and comparing with a gallic acid calibration curve (concentration between 1 and 10 mg kg^{-1}). The results were expressed as mg of gallic acid equivalents g^{-1} dry weight of lemon pomace (mg GA g^{-1} d.w.) and as mg of gallic acid equivalents 100 g^{-1} dry weight of dough and biscuit (mg GA 100 g^{-1} d.w).

- **Total flavonoid content (TF)**

The total flavonoid content (TF) was quantified following the method described by Papoutsis et al. (2018b), with some modifications.

In brief, 0.5 mL of LP_E, 1 mL of deionized water and 0.15 mL of $NaNO_2$ (5%, w/v) were placed in a 5 mL flask and incubated at room temperature for 6 minutes. Subsequently, 0.15 mL of $AlCl_3$ (10%, w/v) were added and incubated at room temperature for 6 minutes. Then, 2 mL of NaOH (4%, w/v) and 0.7 mL of deionized water were added and, finally, the mixture was made up to volume with deionized water. At the same time, a solution used as a blank was prepared with the same amounts of reagents but without the addition of the sample. The mixture was incubated in the dark for 15 min. The absorbance was measured at 510 nm against a blank using a double-beam ultraviolet-visible spectrophotometer (Perkin-Elmer UV- Vis λ2, Waltham, Massachusetts, U.S.) and comparing with a catechin calibration curve (concentration between 1 and 50 mg kg^{-1}). The results were expressed as mg of catechin equivalents g^{-1} dry weight of lemon pomace (mg CE g^{-1} d.w.).

- **DPPH assay**

The DPPH assay was performed as reported by Brand-Williams et al. (1995), based on the reaction between the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical and antioxidant compounds of the samples, resulting in discoloration of the reaction solution due to the extinction of the radical. In a cuvette, after appropriate dilution, 15 µL of sample (LP_E, dough and biscuit extracts) and 2985 µL of a 6 x 10^{-5} M of methanol solution of DPPH, were allowed to react under darkness for 30 min at room temperature. The absorbance was measured at 515 nm, against methanol as blank, using a double-beam ultraviolet-visible spectrophotometer (Perkin-Elmer UV- Vis λ2, Waltham, Massachusetts, U.S.) and comparing with a Trolox calibration curve (from 3 to 18 µM). The results were expressed as µM Trolox g^{-1} dry weight of lemon pomace for LP_E (µM TE g^{-1} d.w.) and as µmol Trolox 100 g^{-1} dry weight of dough and biscuit (µmol TE 100 g^{-1} d.w.).

- **ABTS assay**

The antioxidant activity of LP_E was determined by ABTS (2,2'-azino-bis acid (3- ethylbenzothiazolin-6-sulfonic acid) assay, a spectrophotometric discoloration method (Re et al., 1999). The working solution was prepared by mixing two stock solutions of 7 mM ABTS solution and 2.4 mM potassium persulphate ($K_2S_2O_8$) solution and was incubated at room temperature for 12 hours in the dark to achieve a stable value of absorbance: the reaction between $ABTS^+$ and potassium persulphate determines the direct production of a blue/green chromogen. The resulting $ABTS^+$ solution was diluted with ethanol, showing an absorbance of 0.70 (±0.02) at 734 nm.

The reaction mixture was prepared by mixing 15 µL of LP_E and 2985 µL of the ethanol solution of $ABTS^+$. The absorbance was measured after 6 minutes in the dark using a double-beam ultraviolet-visible spectrophotometer (Perkin-Elmer UV- Vis λ2, Waltham, Massachusetts, U.S.). The quenching of initial absorbance was plotted against the Trolox concentration (from 3 to 18 µM) and the results were expressed as µmol Trolox g^{-1} dry weight of lemon pomace (µmol TE g^{-1} d.w.).

3.1.2.7. Identification and quantification of antioxidant compounds

The individual antioxidant compounds were identified and quantified in LP_E, dough and biscuit following the methods reported by Romeo et al. (2019), making the appropriate modifications. The chromatographic system comprised of an UHPLC PLATINblue (Knauer, Berlin, Germany) provided with a binary pump system, which utilizes a Knauer blue orchid column C18 (1.8 µm, 100 x 2 mm) coupled with a PDA–1 (Photo Diode Array Detector) PLATINblue (Knauer, Berlin, Germany). The Clarity 6.2 software was employed.

Extracts were filtered through a 0.22 µm nylon syringe filters (diameter 13 mm) and then 5 µL were injected in the system. The mobile phases used were water acidified with acetic acid (pH 3.10) (A) and acetonitrile (B); the gradient elution program consisted in 0–3 min, 95% A and 5% B; 3–15 min, 95%–60% A and 5%–40% B; 15–15.5 min, 60%–0% A and 40%–100% B. Ultimately, restoration of the initial conditions was reached during analysis maintaining the column at 30°C and the injection volume at 5 µL. Peaks were revealed at 280 nm. For the identification and quantification of each compound, external standards (concentration between 1 and 100 mg kg^{-1}) were used, and the results were expressed as mg 100 g^{-1} dry weight of lemon pomace and mg 100 g^{-1} dry weight of dough or biscuit.

3.1.2.8. Oxidative stability study

The Oxitest (Oxidation Test Reactor, VELP Scientifica) method consists of monitoring the oxygen uptake by reactive components in the tested food samples, allowing evaluation of oxidative stability under accelerated oxidation conditions (Romeo et al., 2020).

In this study, the analysis was performed on 30 g of minced biscuits distributed homogenously in a hermetically sealed titanium chamber and pressurized until 6 bars at 90°C. Each accelerated oxidation test was repeated using a single reactor, for a total of two analytical replies. The OXITEST result is the induction period (IP), expressed as "stability time" before fat oxidation and corresponding to a decrease of O_2 pressure due to the consumption of oxygen by the sample (Caruso et al., 2016). OXISoft™ Software (Version 10002948 Usmate Velate, MB, Italy) automatically calculates the IP (h).

3.1.2.9. Sensory analysis

The evaluation of sensory analysis was conducted to assess whether there were differences among the different samples, through a preference test useful to allow comparisons between the control and the functionalized samples. The biscuits were scored compared to several qualitative factors, such as: appearance attributes (superficial brown color, firmness, crunchiness, friability, and fragrance), aromatic aspects (lemon, bergamot, caramel and ethanol) and flavor (bitter and sweet). The test was performed by a panel of 10 judges (males and females) from 25 to 60 years old, recruited among researchers and technicians of the Food Science and Technology Unit of Reggio Calabria University with previous experience in sensory analysis. The judges were trained before the sessions to identify the gustatory attributes to be evaluated. Sensory data were elaborated by calculating the median of results.

3.1.2.10. Statistical analysis

In this study, each analysis was conducted in triplicate (n=3) and all the experimental results were expressed as mean value ± standard deviation. The significant differences ($p<0.05$) among mean values were determined by one-way ANOVA analysis, applying SPSS Software (Version 15.0, SPSS Inc., Chicago, IL, USA). A series of multiple comparisons, with Tukey's post hoc test, was performed to determine individual significant differences ($p<0.05$). The Pearson's correlation test was employed for the determination of correlation coefficients (r) among the extracted polyphenolic compounds and antioxidant assays.

3.1.3. RESULTS AND DISCUSSION

3.1.3.1. Characterisation of Lemon Pomace Extract (LP_E)

Results of LP_E characterization are reported in Table 14. TPC and TF determinations denoted mean values of 0.59±0.01 mg GA g^{-1} d.w. and 0.16±0.00 mg CE g^{-1} d.w. respectively, with the predominant compounds represented by eriocitrin and hesperidin, as confirmed by literature (Sharma et al., 2017). The showed antioxidant activity was of 36.29±4.62 µmol TE g^{-1} d.w. The obtained results agreed with the ranges reported in literature (Casquete et al., 2015; Mcharek et al., 2017).

Table 14: Physico-chemical characterisation of LP_E.

pH	3.93 ± 0.01
Colour:	L*: 42.33 ± 0.14
	a*: 1.22 ± 0.04
	b*: 7.12 ± 0.09
	C*: 7.22 ± 0.08
	H: 80,30 ± 0.38
TPC (mg GAE g^{-1} d.w.)	0.59 ± 0.01
TF (mg CE g^{-1} d.w.)	0.16 ± 0.00
DPPH (µmol TE g^{-1} d.w.)	1.86 ± 0.28
ABTS (µmol TE g^{-1} d.w.)	36.29 ± 4.62
Eriocitrin (mg 100 g^{-1} d.w.)	190.15 ± 0.04
Hesperidin (mg 100 g^{-1} d.w.)	231.60 ± 0.13

GAE: Gallic acid equivalent; TPC: Total phenolic compounds; LPE (lemon peel extract); TF: Total Flavonoids; DPPH and ABTS: Total antioxidant activity assays.

3.1.3.2. Physico-chemical analysis of doughs and biscuits

Although the water activity (a_w) in the doughs was quite similar in all samples without any significant difference (Table 15), it was found that the a_w detected in shortcrust biscuit samples decreases significantly ($p<0.01$). Shortcrust biscuits including only LP_E (sample D) had the lowest water activity value (a_w= 0.23±0.01) than the other samples. This agrees with Miskiewicz et al. (2018) who state that the water-binding capacity of the dough ingredients improved in the presence of the extract, reducing water evaporation in the process of baking.

The moisture percentages quantified in dough samples were similar among samples with the only exception of dough D, which showed the lowest value ($p<0.05$) of moisture (14%) (Table 15). This result might be due to the different formulation that involved the only use of LP_E diluted in hydroalcoholic solution for the enrichment where ethanol possesses a higher volatility than water. After the cooking, no significant differences in moisture were observed among samples.

In both dough and shortcrust biscuit samples, the pH value was significantly lower ($p<0.01$) in the products C and D, at 6.91±0.11 and 6.87±0.02 for enriched dough and 6.79±0.02 and 6.72±0.03 in biscuits, respectively (Table 15). This also suggests the possible influence of the LP_E, for the presence of acidic components.

Table 15: aw, moisture and pH values of dough and biscuit samples.

DOUGH	aw	Moisture (%)	pH	BISCUIT	aw	Moisture (%)	pH
A	0.86±0.00	19.28±0.63^{a}	7.13±0.01^{a}	**A**	0.28±0.01^{a}	4.69±0.18	6.87±0.03^{a}
B	0.89±0.00	18.70±1.88^{a}	7.15±0.02^{a}	**B**	0.28±0.00^{a}	4.76±0.05	6.88±0.00^{a}
C	0.89±0.00	18.25±0.28^{a}	6.91±0.11^{b}	**C**	0.28±0.00^{a}	5.16±0.52	6.79±0.02^{b}
D	0.89±0.00	14.14±1.34^{b}	6.87±0.02^{b}	**D**	0.23±0.01^{b}	4.60±0.30	6.72±0.03^{c}
Sign.	**ns**	****	****		****	**ns**	****

The data are presented as means ± SD (n=3). Means within a column with different letters are significantly different by Tukey's post hoc test. Abbreviation: ns, not significant. **Significance at p<0.01; *Significance at p<0.05.

Concerning the colour evaluation of dough samples (Table 16), colour brightness (L*) did not vary significantly whereas bigger variations ($p<0.01$) were observed for the other chromatic components, especially for b* that increased in B and C, linked to the presence of fresh lemon peel in the formulation.

In bakery products the final colour is a result of chemical reactions, such as Maillard reaction, which consists in non-enzymatic browning reactions affected by the quantity of reducing sugars in the foodstuff and by baking temperature (Pedreschi et al., 2006; Friedman, 2015) and caramelization (Purlis & Salvadori, 2007). As expected, comparing the colorimetric parameters measured for the dough and biscuit samples, a reduction in the brightness (L*) and a parallel increase in the values of components a* and b* were detected, being influenced by Maillard and caramelization reactions that occur during baking process (Pasqualone et al., 2014).

As found by Borrelli et al. (2003) the reaction occurring between proteins and carbohydrates may be the cause of the final brown colour: in this context, the superficial colour is an important quality element, influencing the acceptability of baked goods formulated using wheat (de Abreu et al., 2019). Even if baking parameters might affect baked foods colour because of also added ingredients (Yilmaz & Karaman, 2017), in this experimentation no significant colour difference was found among bisciut samples, denoting no visual influence by the LP_E.

Table 16: Colour parameters of dough and biscuit samples.

	Samples	A	B	C	D	Sign.
DOUGH	L*	72.35±2.08	72.09±2.32	73.69±2.39	70.98±2.27	ns
	a*	2.50±0.11^{ab}	2.35±0.17^{bc}	2.24±0.16^{c}	2.57±0.21^{a}	**
	b*	18.90±0.64^{b}	19.74±0.93^{ab}	20.43±0.92^{a}	18.97±0.38^{b}	**
	C*	19.06±0.64^{b}	19.88±0.94^{ab}	20.55±0.92^{a}	19.15±0.37^{b}	**
	H	82.52±0.36^{b}	83.25±0.46^{a}	83.79±0.44^{a}	82.33±0.65^{b}	**
BISCUIT	L*	65.39±7.88	66.94±5.91	68.99±6.00	69.73±3.32	ns
	a*	8.78±3.71	8.96±2.69	8.04±3.31	8.15±1.78	ns
	b*	27.56±1.88	28.58±1.41	27.28±1.92	28.66±1.24	ns
	C*	29.13±2.03	30.07±1.22	28.58±2.45	29.83±1.57	ns
	H	72.59±0.13	72.63±0.09	73.97±0.10	74.27±0.05	ns

The data are presented as means ± SD (n=10). Means within a row with different letters 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.

3.1.3.3. Microbiological analysis

Results of microbiological analysis denoted a microbial growth only in dough samples, as showed in Table 17. The total bacterial count was similar among samples, with 2.3 Log10 CFU g^{-1}, except for D in which a lower count was enumerated (1.95 Log10 CFU g^{-1}). This result might be due to the possible influence of the LP_E to reduce the bacterial growth, as confirmed by Ahmed & Noor (2020), for Citrus lemon extract, and by Schieber et al. (2001) for the antimicrobial and antioxidant activity of eriocitrin and hesperidin present in LP_E.

The determination of mould and yeast colonies denoted quite similar counts among dough samples around 1.5 Log10 CFU g^{-1}. After the baking process, no total microbial, mould and yeast counts were observed in the biscuits.

Table 17: Total bacterial count (TBC) and mould (M) and yeast (Y) growth values for doughs.

DOUGH	TBC (Log_{10} CFU g^{-1})	M and Y (Log_{10} CFU g^{-1})
A	2.22±0.01^{a}	1.76±0.12
B	2.13±0.12^{a}	1.62±0.03
C	2.10±0.03^{a}	1.45±0.21
D	1.95±0.02^{b}	1.53±0.24
Sign.	**	ns

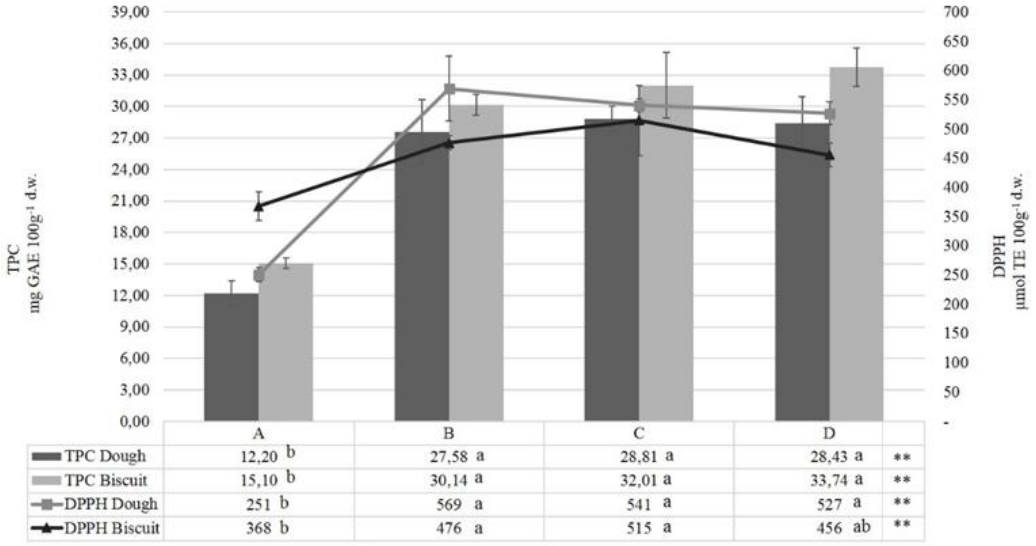
The data are presented as means ± SD (n=3). Means within a column with different letters are significantly different by Tukey's post hoc test. Abbreviation: Sign., significance; ns, not significant. **Significance at p<0.01; *Significance at p<0.05.

3.1.3.4. Total polyphenol content and antioxidant activity

Total polyphenol content (TPC) and antioxidant activity detected in dough and biscuit samples are reported in Figure 7.

The TPC values tended to double after the enrichment both in dough and biscuit samples (about 28 and 30 mg GA g^{-1} d.w. of dough and biscuit, respectively) compared to the control ones (about 12 and 15 mg GAE 100 g^{-1} d.w. of dough and biscuit, respectively). The addition of functional compounds to biscuit samples (B, C and D) besides increasing the antioxidant properties in doughs allows to protect the samples during the thermal treatment of cooking. Indeed, some of the valuable compounds initially added to the doughs were well preserved until the end of the baking process ($p<0.05$). No significant differences were observed among B, C and D samples ($p>0.05$).

In this regard, as reported by Pasqualone et al. (2014), the literature is not univocal about the effects of baking on phenolics that seem to depend on several factors, such as the type and structure of food ingredients and food matrix, source and nature of bioactive compounds, recipe, and thermal processing (Gélinas & McKinnon, 2006; Holtekjølen et al., 2008; Menga et al., 2009; Abdel-Aal & Rabalski, 2013). Moreover, the increase of TPC of the biscuits may be due to the formation of neo-antioxidants compounds during baking process because of the Maillard reaction (Zieliński et al., 2010). As discussed for TPC, the enriched samples showed also significantly higher values of antioxidant activity by DPPH assay compared to the control ones. A positive correlation was observed between antioxidant activity and TPC both for doughs and biscuits, with Pearson's correlation coefficients of 0.98 and 0.88, respectively. The antioxidant activity observed in control biscuits might be due to the presence of phenolic acids which are the main antioxidants in wheat and to their ability to scavenge free radicals (Abdel-Aal et al., 2012).



	A	B	C	D	
TPC Dough	12,20 b	27,58 a	28,81 a	28,43 a	**
TPC Biscuit	15,10 b	30,14 a	32,01 a	33,74 a	**
DPPH Dough	251 b	569 a	541 a	527 a	**
DPPH Biscuit	368 b	476 a	515 a	456 ab	**

Figure 7: TPC values and antioxidant activity of dough and biscuit samples. Means within a row with different letters are significantly different by Tukey's post hoc test.
Abbreviation: ns, not significant. **Significance at $p<0.01$; *Significance at $p<0.05$.

3.1.3.5. Oxidative stability results

The oxidation phenomenon is particularly important in foods such as biscuits for the lipid content in their formulation (Reddy et al., 2005). The presence of antioxidants compounds in formulation preserves bakery goods for a longer time, resulting in exceptional antioxidant effect (Bassiouny et al., 1990). Specifically, literature reports the lemon antioxidant activity, protecting the fatty component of the matrix during reactions of oxidation and influencing the formation of primary compounds, such as peroxides (Caruso et al., 2016).

The indication of biscuits oxidation degree, represented by the Induction Period (IP), was obtained by the point of intersection between the line passing through the inflection of the curve and the inflection itself (Figure 8). This point corresponded to the end of the product's intrinsic resistance to lipid oxidation and the beginning of the accelerated absorption of oxygen by the food (appearance of olfactory rancidity).

The results suggested that the enriched biscuits showed a higher resistance to lipid oxidation compared to the control biscuit A (IP of 47 h). In particular, the highest IP (57 h) was recorded for sample C: it was reported that the synergistic antioxidant effect exerted by the added fresh lemon peel and the hydroalcoholic extract (LP_E) contributed to preserve biscuit from the oxidation.

Indeed, as reported by Palombini et al. (2018), the correlation between the presence of antioxidant compounds and the result of the accelerated oxidation test observed in enriched cookies could justify the connection between the detected antioxidant compounds in fresh lemon peel and extract and their possible action in protecting lipids against the oxidation process.

The same ingredients were also singularly active in cookies B and D, with a lightly lower and similar IP (51 h). It was interesting to note that significantly higher values of TPC and antioxidant activity of biscuit C (Figure 7) were matched by the longest induction period (IP) observed in Figure 8.

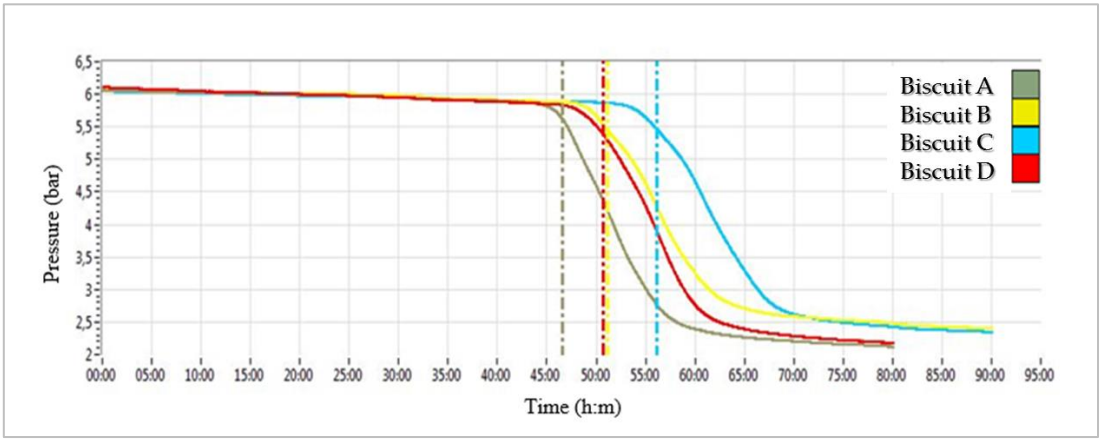


Figure 8: Oxidation curves and IP values of biscuit samples.

3.1.3.6. UHPLC Phenolic profile

The principal phenolic compounds identified and quantified in the enriched samples by UHPLC analysis are reported in Table 18. C and B doughs possessed the highest amount of eriocitrin among the enriched samples, but only C dough showed the highest hesperidin value, as well as for both identified flavonoids in the enriched biscuits. From dough to biscuit the specific amount tended to decrease significantly for eriocitrin in sample C and for hesperidin in sample D, probably due to the applied thermal process and this effect was particularly evident in B biscuit for the absence of eriocitrin and hesperidin.

Comparing the amount of eriocitrin and hesperidin in baked biscuits, also in this case it is evident that the richest is the one with fresh lemon peel and extract inside (C), followed by biscuit enriched only with the extract (D). At the same time, the biscuit formulated only with fresh lemon peel (B) did not show a determinable quantity of the two flavonoids identified in the other samples. This confirms the potentiality of enriched products to boost the day-to-day consumption of valuable compounds since cookies are frequently consumed foods. The samples after the thermal treatment exhibited a significantly lower antioxidant activity ($p<0.05$) than what was found in corresponding dough samples.

Table 18: Phenolic characterization of dough and biscuit samples. Data are expressed as mg 100 g^{-1} of product.

	Dough				Biscuit			
	B	**C**	**D**	**Sign.**	**B**	**C**	**D**	**Sign.**
Eriocitrin	3.54 ± 0.07^{a}	3.40 ± 0.13^{a}	1.62 ± 0.1^{b}	**	n.d.	2.75 ± 0.16^{a}	1.32 ± 0.20^{b}	**
Hesperidin	2.32 ± 0.25^{b}	2.67 ± 0.06^{a}	2.41 ± 0.03^{ab}	*	n.d.	2.84 ± 0.30^{a}	2.22 ± 0.08^{b}	**

The data are presented as means ± SD (n=3). Means within a row with different letters are significantly different by Tukey's post hoc test. Abbreviation: Sign., significance; ns, not significant; n.d., not detected. **Significance at $p < .01$; *Significance at $p < 0.05$.

3.1.3.7. Sensory studies

The shortcrust biscuits were also evaluated in terms of their sensory attributes, which are presented in Figure 9.

In all examined biscuit samples, the crust colour was from pale to brownish yellow. The superficial brown colour of the biscuits had lower scores with the addition of fresh lemon peel and LP_E (sample C) , probably due to the lower amount of skimmed milk, thus less proteins and lactose, thus less Maillard reaction and caramelization. Indeed, colour and external aspect of biscuits are affected by reducing sugars, which caramelise during baking process producing brown colour (Ingle et al., 2017). The fragrance of the products was quite similar among biscuits, except for sample D. Moreover, bisciut D prepared by adding the LP_E and with the lowest a_w value (Table 15) was the crunchiest, confirming what discussed by Arimi et al. (2010) about the water activity value, whose increase is associated with the plasticisation of the matrix due to the higher amount of water, causing the product

to lose its crispness, leading to softening of the matrix. The other appearance attributes are not significantly different between the control cookie and the enriched ones. The biscuit texture was quite similar among the samples.

The flavour attributes scores were quite different referred to bitter and sweet components. In this kind of food, sugar is one of the most important components, playing a key role in the structural and textural characteristics in dough arrangement and subsequent cooking, giving a distinctive shape and texture to the final product (Biguzzi et al., 2014) After the enrichment, the biscuits took on a more bitter taste, which means that the presence of these ingredients might have influenced the taste of the final products due to the presence of bitter-tasting compounds typically found in fibrous component of the lemon peel.

Finally, regarding the aromatic attributes, the enriched samples clearly differed from the control one, especially about the perception of the lemon scent. This suggests that the aromatic components in the fresh lemon peel and LP_E were preserved in the cooking process, enhancing the aroma in the final product. In addition, panelists did not detect the ethanol aroma in any biscuits.

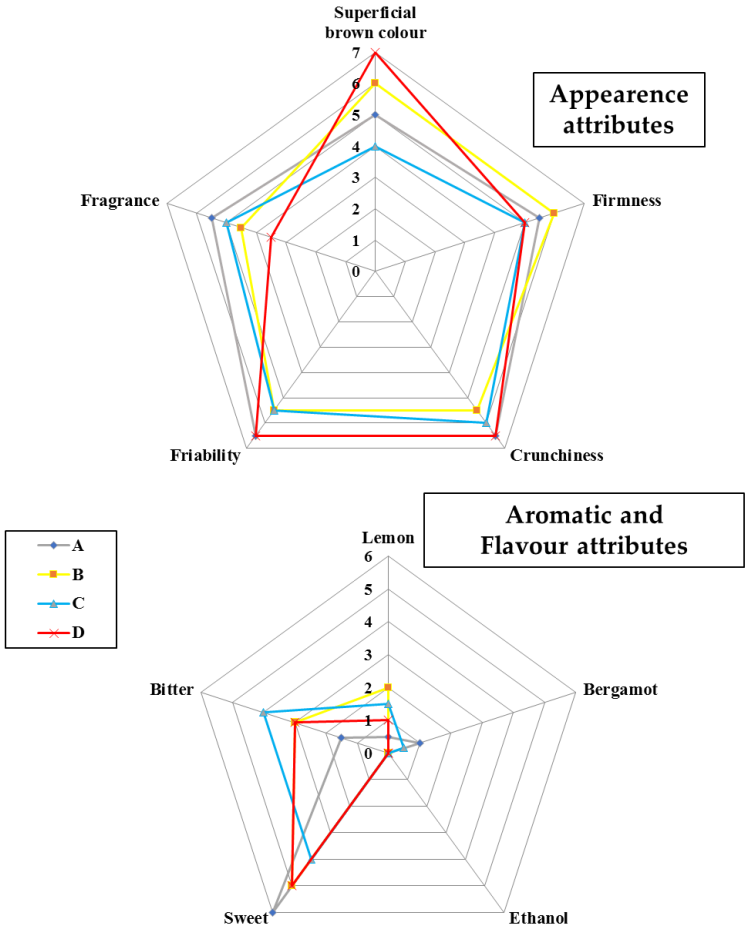


Figure 9: Median values of biscuits sensory evaluation.

3.1.4. CONCLUSIONS

The chemical composition of LP_E showed that it was a good source of polyphenols and the obtained results remarked the positive effects of using lemon peel and its extract in biscuit-making.

Enriched biscuits showed higher polyphenol content and antioxidant activity than the control one, with positive results in terms of sensory acceptability of the product as well.

It may be concluded that lemon peel and LP_E could be incorporated in the formulation of biscuits, increasing their bioactive compounds content without affecting their overall appearance, resulting in acceptable products and with improved functional and nutraceutical properties. In this view, innovative uses of food wastes, rich in phenolics, can lead to increase the production of food with good nutritional characteristics and to satisfy customers' expectative for healthy functional foods.

3.2. SHELF-LIFE STUDY OF TOAST BREAD ENRICHED WITH AN ANTIOXIDANT EXTRACT OBTAINED FROM ONION PROCESSING BY-PRODUCTS

3.2.1. INTRODUCTION

Food and agricultural industries produce significant amounts of waste rich in bioactive compounds, which could represent as a natural source of antioxidants in food production. In this regard, phytochemicals are considered the main bioactive compounds from fruits, vegetables and grains with health advantages (Mateo Anson et al., 2010; Roldan et al., 2008, Gawlik-Dziki et al. 2013). In this contest, onion is one of the most cultivated crops in the world, with a production of approximately 98 million tons every year, which produce a huge amount of by-products, counting different portions such as outer dry and semi-dry layers as well as the apical and basal trimmings (Nile et al., 2017). The by-products produced by industrially processed onions contain a substantial amount of phenolic compounds, mainly quercetin and its derivatives, mostly represented by glucosides (Benítez et al., 2011), with well-known anti-inflammatory, antioxidant and anticancer properties (Gawlik-Dziki et al. 2013; Wiczkowski et al., 2008; Almeida et al., 2018; Lesjak et al., 2018). For these reasons, consumers are increasingly preferring healthier and functional foods that can help prevent diseases. Indeed, industry and researchers are interested in boosting food making technology to increase the quality, taste, functionality and bioavailability of food products, such as bakery goods. Besides herbs and spices, used as ingredients in bakery products formulations to improve their nutraceutical potential (Balestra et al., 2011), many studies reported a lot of evidence that proposes onion peel as a good source of quercetin and dietary fibre which can be added to wheat bread (Gawlik-Dziki et al., 2013; Prokopov et al., 2018), showing an increase in antioxidant activity compared to the control and fortifying bread with bioaccessible lipid oxidation inhibitors and compounds with reducing and chelating capacities (Gawlik-Dziki et al., 2011).

So, this study explores the possibility of making use of onion solid waste as functional ingredient in bread production, evaluating their effects on bread sensory quality and improving antioxidant properties and polyphenols content of the final product.

3.2.2. MATERIALS AND METHODS

3.2.2.1. Raw material

Red onions (*Allium cepa L., cv. Tropea*) were supplied by a local company located in the province of Reggio Calabria, (Italy). The bulbs were transported to the laboratory and peeled immediately. The

outer dry and semi-dry layers as well as the apical and basal trimmings (onion solid waste, OSW), were used in the extraction process. Before extraction, the OSW were initially dried at 50°C up to a moisture content of 17%, then ground using a high-speed blender and stored in polyethylene bags under vacuum to avoid rehydration until subsequent extraction procedure of the bioactive compounds.

3.2.2.2. Preparation of onion solid waste extract (OSW_E)

For the recovery of bioactive compounds, a Sonoplus Ultrasonic homogeniser, Series 2000.2, HD 2200.2 (BANDELIN, Ultraschall seit 1955) was used, as described by M. Khan (2010), with appropriate modifications. To 2.5 g of dried and ground onion solid waste were added 50 ml of water (1:20 w/v ratio). Extraction was conducted for 30 min at 70°C. Subsequently, the sample was centrifuged (NF 1200R, Nüve, Ankara, Turkey) at 5000 rpm for 5 min at 4°C, filtered through a Büchner apparatus with 0.45 µm filter paper and the resulting extract was made up to volume of 200 mL with water. Before being used for the enrichment of bread, the extract (OSW_E) was subjected to qualitative and quantitative analysis.

3.2.2.3. Formulation of enriched bread with onion solid waste extract (B_{OSW})

The bread samples (type toast bread) were prepared in the Laboratory of Food Science and Technology of the "Mediterranea" University of Reggio Calabria, according to the following recipe: 250 g wheat flour, 250 g Manitoba flour, 200 mL water, 50 g milk, 8 g yeast, 50 g sunflower oil, 5 g sugar, 12.5 g salt. The enriched bread was prepared using the ingredients listed above, replacing 100 mL of water with 100 mL of OSW_E obtained as previously described (Section 3.2.2.2.).

The ingredients were mixed using a spiral planetary mixer (Sigma Srl) until a well-blended and elastic dough was obtained, which was then subjected to two leavening phases. The first one took place in leavening cells for 90 minutes at a constant temperature of 25°C and relative humidity of 75%. The two leavening phases were interspersed with the manual operations of dough breaking and shaping, which were necessary to give the product the correct final shape and a structure as homogeneous as possible, favouring a more appropriate distribution of the cavities during the baking process.

At the end of the second leavening phase, which took place under the same conditions of the first one as described above, doughs were baked in the oven (180°C for 35 minutes, humidity 40%), which represents a crucial step for the acquisition of the appropriate structural, sensory and nutritional characteristics of the final products. The final products were allowed to cool down at room temperature, portioned into slices of about 2 cm thickness and packed in polypropylene bags.

Table 19: Samples denomination.

B	Control
B_{OSW}	Bread with onion solid waste extract
Ingredients	**B**	**B_{OSW}**
Wheat flour (g)	250	250
Manitoba flour (g)	250	250
Sunflower oil (g)	50	50
White sugar (g)	5	5
Skimmed milk (g)	50	50
Yeast (g)	8	8
Salt (g)	12.5	12.5
Water (mL)	200	100
OSW_E (mL)	0	100

3.2.2.4. Characterization of physico-chemical properties of OSW_E and bread samples

The physico-chemical characterisation was carried out on both B and B_{OSW} samples, at the initial time (time 0), after three days (time 3) and seven days (time 7) of storage.

The pH of OSW_E was performed with a Crison pH-meter, basic model 20.

The moisture content of bread samples (expressed as a percentage of moisture %) was determined using a Sartorius Moisture Analyzer MA37 thermal balance, by the gravimetric method at 105°C until constant weight.

Water activity (a_w) was performed using an Aqualab LITE hygrometer (Decagon) and the colour analysis was conducted using an automatic Minolta CR 300 tristimulus colorimeter.

The CIE L*a*b* system was used as reference. The measurement was carried out on 15 mL of OSW_E, placing the sample in an optical glass, and directly on both crumb and crust of bread slices in ten different points.

3.2.2.5. Texture analysis

A TA-TX Plus texture analyser with a 50 kg load cell (Stable Micro System) was used to determine the structural profile of bread slices over time. The force required to compress the bread slice (2 cm thick) by 25%, expressed as g s^{-1}, was measured with a P/3 cylindrical probe (3 mm diameter), at a rate of 3 mm/s. The changes in resistance to compression were detected in three zones of the slice bread (centre, near the bottom crust and near the top crust). The compression test was carried out after 2 h of cooling and after 3 and 7 days of storage. Three replicates were performed for each sample.

3.2.2.6. Microbiological analysis

The total aerobic bacterial count, the presence of lactic acid bacteria and the determination of yeasts and moulds were performed for each monitoring time (t0, t3, t7) and for both bread samples. The analysis was carried out in duplicate for each sample and the mean values are reported.

Under sterile conditions, 10 g of sample were homogenised for 2 minutes with 10 mL of Ringer solution using a Stomacher BagMixer 400 (Interscience, 30 Ch.Bois Arpents F.78860 St. Nom, France). Series dilutions of homogenate were poured into Petri plates in specific agar for total bacterial count (Plant Count Agar, Oxoid, at 25 ± 2°C for 48h), for lactic acid bacteria count (MRS Agar, Liofilchem, at 37°C for 48h in anaerobiosis) and in DRBC agar specific Petri plates for yeast and mould count (Dichloran Rose Bengal Chloramphenicol, 28 ± 2°C for 4-5 days). After the incubation period, the microbial load was determined and expressed in Log10 colonies forming units (CFU) g^{-1} of bread.

3.2.2.7. Evaluation of total phenol content and antioxidant activity of OSW_E and bread samples

The phenolic extraction method for both B and B_{OSW} was performed as reported by Zielinski et al., (2008), with a few modifications. The bread slices were dried in an oven at 40°C for 24 hours and then were ground in an electric grinder. Subsequently, 5 g of ground bread sample were mixed with 50 mL of methanol 80% and homogenized for 2 h at 37°C. Then, the mixture was centrifuged (9000 rpm for 15 minutes at 10°C) and the supernatant was recovered. The resulting extract was filtered through a 0.45 µm nylon filter before analysis. The determination of total phenolic content and antioxidant activity was carried out on both B and B_{OSW} samples at the initial time (time 0), after three days (time 3) and after seven days (time 7) of storage.

- **Total phenolic compounds of OSW_E (TPC_E)**

TPC_E determination was carried out as reported by Imeneo et al., (2021), The results were expressed as mg of gallic acid equivalents g^{-1} dry weight of onion solid waste (mg GAE g^{-1} d.w.).

- **Total phenolic compounds of B and B_{OSW} (TPC)**

TPC was determined according to the method described by Ibrahim et al. (2018), with appropriate modifications. 1 mL of B or B_{OSW} extract was mixed with 5 mL of deionized water and 1 mL of Folin-Ciocalteu reagent in a 25 mL flask. After 8 minutes, 10 mL of saturated sodium carbonate solution (Na_2CO_3, 7.5%) were added and made up to volume with deionized water. The mixture was incubated for two hours at room temperature in the dark. The absorbance was measured at 750 nm against a blank using a double-beam ultraviolet-visible spectrophotometer (Perkin-Elmer UV- Vis λ2, Waltham, Massachusetts, U.S.) and comparing with a gallic acid calibration curve (concentration

between 1 and 7 mg Kg^{-1}). The results were expressed as mg of gallic acid equivalents Kg^{-1} dry weight of bread (mg GAE Kg^{-1} d.w.).

- **Total flavonoid content of OSW_E (TF)**

TF was quantified according to Munir et al. (2018), as reported in Section 2.2.3.1. The results were expressed as mg of quercetin g^{-1} dry weight of onion solid waste (mg QE g^{-1} d.w.).

- **Total anthocyanin content of OSW_E (TAC)**

TAC was determined spectrophotometrically according to the AOAC method (2005.02), as described in Section 2.2.3.2. Anthocyanin pigment concentration was expressed as mg of cyanidine- 3-glucoside g^{-1} dry weight of onion solid waste (mg C-3-GLU g^{-1} d.w.).

- **DPPH assay**

The DPPH assay was performed as reported in Section 1.2.4.3. For the reaction, different concentrations of OSW_E and bread extracts were used: in a cuvette, 50 µL of OSW_E or 150 µL of B and B_{OSW} extracts were mixed with 2950 µL or 2850µL of a 6 x 10^{-5} M of methanol solution of DPPH, respectively, and were allowed to react under darkness for 30 min at room temperature. The absorbance was measured at 515 nm, against methanol as blank, using a double-beam ultraviolet-visible spectrophotometer (Perkin-Elmer UV- Vis λ2, Waltham, Massachusetts, U.S.) and comparing with a Trolox calibration curve (from 3 to 18 µM). The results were expressed as µM Trolox g^{-1} dry weight of onion solid waste for OSW_E (µM TE g^{-1} d.w.) and as µM Trolox kg^{-1} dry weight of bread for B and B_{OSW} (µM TE kg^{-1} d.w.).

- **ABTS assay**

The antioxidant activity of OSW_E and B and B_{OSW} was determined by ABTS as reported in Section 1.2.4.3. For the reaction, different concentrations of the extracts were used: 25 µL of OSW_E or 100µL of B or B_{OSW} extracts were mixed with 2975µL or 2900µL of the ethanol solution of $ABTS^+$, respectively. The absorbance was measured after 6 minutes in the dark using a double-beam ultraviolet-visible spectrophotometer (Perkin-Elmer UV- Vis λ2, Waltham, Massachusetts, U.S.). The quenching of initial absorbance was plotted against the Trolox concentration (from 3 to 18 µM), and the results were expressed as µM Trolox g^{-1} dry weight of onion solid waste for OSW_E (µM TE g^{-1} d.w.) and as µM Trolox kg^{-1} dry weight of bread for B and B_{OSW} (µM TE kg^{-1} d.w.).

3.2.2.8. Sensory analysis

The evaluation of sensory analysis was conducted at time 0 and at the 7th day of storage to assess the bread overall acceptability, through a descriptive analysis based on B and the B_{OSW} appearance,

flavour and texture. The bread samples were scored compared to various qualitative factors related both on crumb and crust, on a 10-point hedonic scale, such as:

- appearance attributes: crumb and crust color (intensity of the characteristic browning), cavity distribution (in terms of homogeneity of distribution on the slice of bread), crust-crumb area (detachment of crust from crumb, considered to be a defect in the product);
- aromatic aspects: fragrance (intensity of the aroma of freshly baked bread), stale bread (intensity of the aroma of old bread), cereal, yeast, onion;
- flavor: salty, onion, stale bread, aftertaste (intensity perceived after finishing eating bread);
- structural aspects: firmness (resistance to chewing assessed in the first 2-3 acts of chewing), compactness (from slightly to very compact), adhesiveness (tendency to stick to the palate or tongue during chewing), crumb cohesiveness (way of deformation/crumbling of the crumb, assessed by breaking the crumb by hand).

The test was performed by a panel of 10 judges (males and females) from 25 to 60 years old, recruited among researchers and technicians of the Food Science and Technology Unit of Reggio Calabria University with previous experience in sensory analysis. The judges were trained before the sessions to identify the gustative attributes to be evaluated. Sensory data were elaborated by calculating the median of results.

3.2.2.9. Statistical analysis

In this study, each analysis was conducted in triplicate (n=3) and all the experimental results were expressed as mean value ± standard deviation. The significant differences ($p<0.05$) among mean values were determined by one-way ANOVA analysis, applying SPSS Software (Version 15.0, SPSS Inc., Chicago, IL, USA). A series of multiple comparisons, with Tukey's post hoc test, was performed to determine individual significant differences ($p<0.05$). The Pearson's correlation test was employed for the determination of correlation coefficients (r) among the extracted polyphenolic compounds and antioxidant assays.

3.2.3. RESULTS AND DISCUSSION

3.2.3.1. Characterisation of onion solid waste extract (OSW_E)

Results of OSW_E characterization are reported in Table 20. TPC and TF determinations denoted mean values of 6.27±0.13 mg GAE g^{-1} d.w. and 5.62±0.15 mg QE g^{-1} d.w., respectively. The showed antioxidant activity was of 19.03±2.78 µmol TE g^{-1} d.w by ABTS assay. The obtained results agreed

with the ranges reported in the literature (Munir et al., 2018; Vojvodić et al., 2014; Albishi et al., 2013).

Table 20: Physico-chemical characterization of OSW_E.

pH	4.20 ± 0.02
Colour:	L*: 33.23 ± 0.12
	a*: 16.1 ± 0.23
	b*: 0.14 ± 0.02
TPC_E (mg GAE g^{-1} d.w.)	6.27 ± 0.13
TF (mg QE g^{-1} d.w.)	5.62 ± 0.15
TAC (mg C-3-GLU g^{-1}d.w.)	0.06 ± 0.00
DPPH (µmol TE g^{-1} d.w.)	1.34 ± 0.38
ABTS (µmol TE g^{-1} d.w.)	19.03 ± 2.78

GAE: Gallic acid equivalent; QE: quercetin equivalent; C-3-GLU: cyanidine-3-glucoside equivalent; TPC_E: Total phenolic compounds of onion solid waste extract; OSW_E: onion solid waste extract; TF: Total flavonoids; TAC: total anthocyanins content; DPPH and ABTS: Total antioxidant activity assays; TE: Trolox equivalent.

3.2.3.2. Physico-chemical analysis of bread samples

In contrast to the bread sample used as control (B), the water activity values (a_w) determined for the enriched bread sample (B_{OSW}) showed a significant reduction ($p<0.05$) over time (Table 21): B_{OSW}, including the onion aqueous extract, was characterized by a lower water activity ($a_w = 0.89\pm0.01$) than B. In this case, the presence of the OSW_E may have had a decisive influence on the determination of this value. a_w participates in the estimation of water transfer during baking as the driving force was commonly considered to be the difference in partial vapor pressure between the product surface and the oven atmosphere. The water activity could also be related to the water loss and its consequences on water availability, stealing kinetics and mechanical properties of the final products (Vanin et el., 2009), as discussed in the later part of this study. Contrary to a_w, the moisture content decreases similarly over time in both samples, probably caused by the staling process, which is ascribed to the equilibrium of moisture between crust and crumb. Indeed, during storage, the crust is inclined to trapping moisture from the crumb, resulting in a dehydration of the crumb and in a rapid staling in the case of bread with a thick crust, as in this research (Le-Bail et al, 2009). Both moisture content and water activity in bread crust and crumb could impacts on the organoleptic characteristics of the final product.

Table 21: a_w and moisture values of bread samples.

	a_w			Moisture (%)		
Time (days)	**B**	**B_{OSW}**	**SIGN.**	**B**	**B_{OSW}**	**SIGN.**
0	0.91±0.00	$0.90±0.00^{ab}$	*	$31.20±0.45^{ab}$	$32.20±0.82^{ab}$	**ns**
3	0.92±0.01	$0.91±0.01^{a}$	**ns**	$32.29±0.38^{a}$	$32.57±0.17^{a}$	**ns**
7	0.92±0.00	$0.89±0.01^{b}$	*	$30.50±0.99^{b}$	$31.29±0.63^{b}$	**ns**
Sign.	**ns**	*		*	*	

Data are presented as means ± SD (n=3). Means within a column with different letters are significantly different by Tukey's post hoc test. Abbreviation: Sign., significance; ns, not significant. *Significance at $p<0.05$.

About the determination of colorimetric parameters (Table 22), significant differences were noted comparing the two samples, both on bread crumb and crust. In fact, the B_{OSW} crumb showed lower values of parameter L* and higher values of a* and b*, which highlights the significant influence of OSW_E on the final product. The addition of OSW_E caused a significant decrease in crumb L* value, giving to the enriched sample a darker colour compared to the control one and with a relevant contribution of red component, with the a* value of 2.06±0.33 at the day of production. Similar results were also found by Bedrníček et al. (2020) after the addition of wastes of a red variety of onion. Unlike what was described for the crumb, a different situation was found on the crust of the two bread samples. In this case, L* and a* values did not show any significant differences most of the time, as also noted by Altamirano-Fortoul et al. (2012). On the 7^{th} day a clear reduction of a* value was detected in B_{OSW}. The obvious variation of parameter a* at the end of the monitoring period could be explained by the presence of the extract and the possible degradation of anthocyanin compounds over time, especially after cooking. Even if the crust of bread samples was similar in L* and a* values, b* was significantly slightly different ($p<0.05$). The data showed that B_{OSW} was characterized by a darker crumb due to the obvious influence of the extract and a lighter crust (Figure 10, example of final products).

Table 22: Colour parameters of bread samples.

CRUMB									
	L*			a*			b*		
Time (days)	B	B_{OSW}	SIGN.	B	B_{OSW}	SIGN.	B	B_{OSW}	SIGN.
0	75.66 ± 2.01	68.82 ± 2.22	**	0.60 ± 0.30	2.06 ± 0.33^{ab}	**	18.73 ± 1.42	20.67 ± 0.72	**
3	76.11 ± 2.59	69.84 ± 1.11	**	0.56 ± 0.20	1.91 ± 0.22^{b}	**	18.57 ± 1.44	20.87 ± 0.85	**
7	74.96 ± 1.00	68.48 ± 1.99	**	0.53 ± 0.11	2.29 ± 0.26^{a}	**	18.20 ± 0,67	21.42 ± 0.54	**
Sign.	ns	ns		ns	*		ns	ns	
CRUST									
	L*			a*			b*		
Time (days)	B	B_{OSW}	SIGN.	B	B_{OSW}	SIGN.	B	B_{OSW}	SIGN.
0	56.63 ± 10.20	55.06 ± 11.48	ns	15.45 ± 3.37	15.98 ± 2.98^{a}	ns	30.73 ± 2.92	27.41 ± 3.77	ns
3	63.17 ± 8.77	57.71 ± 9.90	ns	13.21 ± 2.96	15.32 ± 2.27^{a}	ns	32.27 ± 3.24	28.66 ± 3.53	*
7	61.81 ± 9.74	61.48 ± 9.73	ns	13.49 ± 2.73	5.30 ± 1.97^{b}	*	31.44 ± 3.85	26.14 ± 4.69	*
Sign.	ns	ns		ns	**		ns	ns	

Data are presented as means ± SD (n=10). Means within a column with different letters 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.

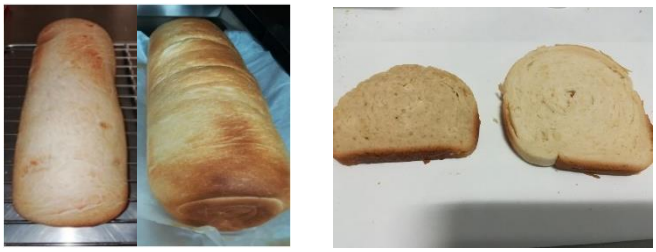


Figure 10: Image of the final products: B_{OSW} (on the left) and B (on the right).

3.2.3.3. Texture analysis

Crumb firmness is one of the most considered parameters to estimate bread staling. In this study the progression of firmness during staling in three different areas of each slice bread, centre, near the crust on the bottom and near the top crust was investigated. Indeed, during baking the increased gas escape due to the proximity of the pan walls results in smaller cells, typical of side crusts (Vanin et al., 2009). For the same reason, the regions which were the most compressed (at the bottom of the mould) tend to present the higher texture due to the presence of small bubbles (Besbes et al., 2013). Looking at the data reported in Figure 11, already at the day of production, B_{OSW} showed a significantly greater resistance to compression than the control B. Several textural analyses reported that the staling rate increased rapidly within the first 24 hours, maybe because of the re-association and re-ordering of amorphous amylose, which is the main cause of short-term starch retrogradation (Sha et al., 2007; Zhu, 2016). Confirming what reported in Table 21 regarding moisture content and

water activity values, B_{OSW} showed a significantly (p<0.01) increasing trend in firmness over time, contrary to what was detected for B, whose texture did not show significant variations. In this case, there was a clear influence of OSW_E on the final product, which probably occurred during the fermentation phase (dough expansion), as can be seen in Figure 9. As reported by Besbes et al. (2013), the differences in textural properties between the two types of bread could be due to the moisture transport properties (effective moisture diffusivity, permeability and sorption), which are influenced by the products microstructure. The morphology of bread product is the result of a successions of aeration taking place during the stage of kneading (formation of bubbles and their entrapment inside the dough), fermentation (increase of bubbles) and baking (extension and breakage) leading to the setting of the final structure (Chiotellis & Campbell, 2003).

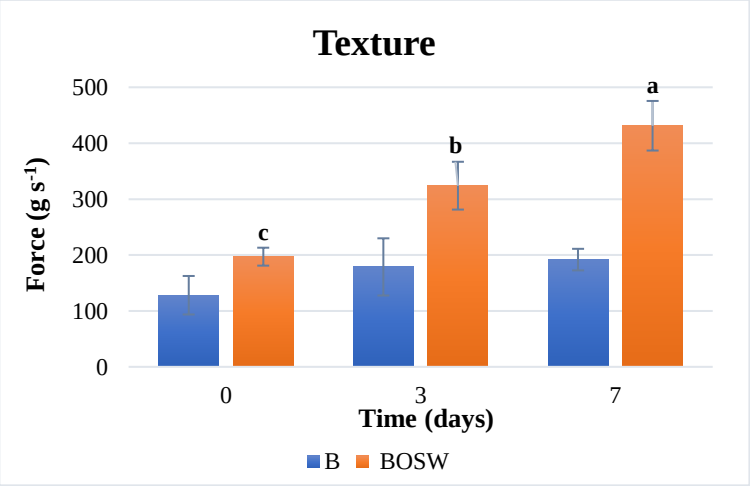


Figure 11: Firmness changes of bread slices.
Data are presented as means ± SD (n=3). Means with different letters are significantly different by Tukey's post hoc test.

3.2.3.4. Microbiological characterization

From the microbiological analysis, both bread samples showed a significant increase of TBC values over time, with similar value the last day of storage (Table 23). A similar trend was detected for the lactic acid bacteria, with the enriched sample characterized by significantly lower values over time compared to the control one. Contrary to that, about mould and yeast content, no relevant values were detected during the monitoring days, except on the 7th day in B_{OSW}. The appearance of mould in the product could be interpreted as the end of shelf life. From microbiological analysis results, the extract did not show any antimicrobial activity, except for lactic acid bacteria.

Table 23: Total bacterial count (TBC), moulds (M) and yeasts (Y) growth and lactic acid bacteria count (LB) on bread samples.

	TBC (Log_{10} CFU g^{-1})			M and Y (Log_{10} CFU g^{-1})			LB (Log_{10} CFU g^{-1})		
Time (days)	**B**	**B_{OSW}**	**Sign.**	**B**	**B_{OSW}**	**Sign.**	**B**	**B_{OSW}**	**Sign.**
0	0.00 ± 0.00^{b}	0.84 ± 0.07^{b}	**	0.00 ± 0.00	0.00 ± 0.00^{b}		1.46 ± 0.04^{c}	0.47 ± 0.05^{c}	**
3	1.46 ± 0.03^{a}	1.22 ± 0.05^{b}	**	0.00 ± 0.00	0.00 ± 0.00^{b}		1.95 ± 0.01^{b}	0.95 ± 0.08^{b}	**
7	2.46 ± 0.70^{a}	2.23 ± 0.53^{a}	ns	0.00 ± 0.00	2.19 ± 0.03^{a}	**	2.04 ± 0.02^{a}	1.80 ± 0.02^{a}	**
SIGN.	**	**			**		**	**	

Data are presented as means ± SD (n=2). Means within a column with different letters are significantly different by Tukey's post hoc test. Abbreviation: Sign., significance; ns, not significant. **Significance at $p<0.01$.

3.2.3.5. Total polyphenols content and antioxidant activity

Total polyphenol content (TPC) and antioxidant activity detected in bread samples are reported in Table 24. The TPC value was significantly higher in B_{OSW} from the first day of monitoring, with a maximum value of 435.20±18.68 mg GAE kg^{-1} d.w., until the 7th day where the TPC of the enriched bread was found to be similar to that of the B sample on the first day of monitoring, of 363.95±24.59 and 333.87±12.67 mg GAE kg^{-1}d.w., respectively. This could be attributed to the presence of OSW_E, even if the detected TPC was lower than those of the added extract, as well as the antioxidant activity (Table 20). In this regard, Han and Koh (2011) demonstrated that phenolics compounds responsible for the antioxidant potential of enriched breads are already strongly bound to the matrix components at the forming and kneading steps of the dough. They noted a decline of phenolic acids content of about 20–30% in breads compared to initial product. The reduction in TPC could be due to the ability of phenolics to precipitate proteins through various mechanisms, such as hydrophobic and ionic interactions, and hydrogen and covalent bindings, thus, their bioavailability might be significantly reduced and, consequently, the expression of the antioxidant activity (Swieca et al. 2013). In model systems, relations between phenolic compounds (mainly flavonoids) and protein were reported also by Sivam et al. (2013), Siebert et al., (1996) and Arts et al. (2002). Researches relating to the effect of these interactions on antioxidant capacity showed that a part of that activity is hidden, depending on both proteins and phenolics used, emphasising a key role of protein–phenolic interactions (Arts et al., 2001; Arts et al., 2002; Han & Koh, 2011; Peng et al., 2010; Sivam et al., 2010; Siebert et al., 1996). Sivam et al. (2013) suggested that a reduced phenolic extraction from enriched breads could be influenced by the stability of these compounds during bread making and their extractability from the bread matrix system, depending on the polyphenol–protein or polyphenol–polysaccharide complexes via hydrogen bonding and/or hydrophobic interactions occurred. In addition, it is known that antioxidant compounds could be damaged or degraded as a consequence of the thermal process during baking, due to the fact that most bioactive compounds become unstable when exposed to heat

and this could be an explanation for the lower than expected antioxidant activity determination (Holtekjølen at al., 2008; Leenhardt et al., 2006; Peng et al., 2010; Swieca et al. 2013). Besides that, antioxidant activity of breads could be influenced also by the activity of oxidative enzymes presented in ingredients used in breads production, or oxidized by environmental oxygen. The addition of water will begin enzyme activities, while a considerable incorporation of oxygen results during the initial kneading (Chlopicka et al., 2012). In this study, in spite of thermal processing during bread preparation, OSW_E addition increased the antioxidant potential of enriched bread. B_{OSW} showed significantly higher values of antioxidant activity on the day of production by ABTS and DPPH assays, even if no linear trend was found between the level of TPC and expression of the antioxidant activity. Similar values of antioxidant activity between the two samples were found on the last day of monitoring. Despite this, B_{OSW} showed greater stability in terms of antioxidant activity expressed by the ABTS assay, showing no significant change in values over time, compared with the control. In accordance with what has just been described, significant improvement of the antioxidant activity of enriched bread has been observed in many studies (Chlopicka et al., 2012; Fan et al., 2006; Gawlik-Dziki et al., 2009; Glei et al., 2006; Lim et al., 2011). The increase in DPPH values at the 7^{th} day could be explained by the presence of Maillard reaction products as antioxidants. This aspect is crucial because phenolics are quite heat unstable and the baking process may have damaged them (Cheynier, 2005; Peng et al., 2010). Indeed, thermally processed foods may be characterized by various levels of Maillard reaction products that have been reported to have antioxidant activity through scavenging oxygen peroxyl, hydroxyl and DPPH radicals, copper and Fe^{2+} chelators (Gawlik-Dziki et al., 2009).

Table 24: Total polyphenol content (TPC) and expression of antioxidant activity (DPPH and ABTS assays) of bread samples.

	TPC (mg GAE Kg^{-1}d.w.)			ABTS (µM TE Kg^{-1}d.w.)			DPPH (µM TE Kg^{-1}d.w.)		
Time (days)	**B**	**Bosw**	**Sign.**	**B**	**Bosw**	**Sign.**	**B**	**Bosw**	**Sign.**
0	333.87 ± 12.67^{B}	435.20 ± 18.68^{A}	**	1227.7 ± 187.2^{B}	2298.3 ± 182.2	**	942.8 ± 44.7 ^{A}	1043.8 ± 16.4^{A}	*
3	379.37 ± 2.77^{A}	404.47 ± 7.32^{AB}	**	1829.7 ± 184.8^{A}	2054.7 ± 175.8	ns	367.7 ± 28.2 ^{C}	484.9 ± 7.4^{C}	**
7	277.77 ± 10.74^{C}	363.95 ± 24.59^{B}	**	1664.8 ± 52.8^{A}	1960.6 ± 363.8	ns	656.6 ± 1.5 ^{B}	671.6 ± 54.6^{B}	ns
SIGN.	**	**		**	ns		**	**	

Data are presented as means ± SD (n=3). Means within a column with different letters are significantly different by Tukey's post hoc test. Abbreviation: Sign., significance; ns, not significant. **Significance at $p<0.01$; *Significance at $p<0.05$.

3.2.3.6. Sensorial characterization

The bread samples were also evaluated in terms of their sensory attributes on the day of production and on the 7th day of storage, as showed in Figures 12 and 13.

In particular, looking at the appearance attributes, B crumb colour used as control showed an increase in terms of superficial brown colour over time, in contrast to the enriched bread, whose crumb colour remained fairly stable over time. Contrary to that, the crust of both samples showed a slight discolouration, confirming what was described previously in the colorimetric analysis for B_{OSW} (Table 22). Also in terms of cavity distribution, B_{OSW} showed greater stability than B. Low crust-crumb area values, which is considered one of the major bread defects, were recorded for both bread samples. Regarding the aromatic aspect, the enriched bread preserved the onion aroma imparted by the addition of the onion solid waste extract, as well as a greater cereal aroma than the control sample, which was found to have a greater yeast sensor. Low water activity values of B_{OSW} and high processing temperatures could accelerated the Maillard reaction, favouring the production of some typical flavours and aromas of bread, such as 2-acetyl-1-pyrroline, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 2,3-butanedione, methional, (E)-2-nonenal and methylpropanal (Charissou et al., 2007; Poinot et al., 2008). As expected, both samples at the end of the monitoring were characterised by a lesser perception of fragrance and a greater hint of stale bread. This type of phenomenon is very common in bread, due to the the staling process which occur over time, due to the progressive loss of moisture and starch retrogradation. As reported by Sullivan et al. (2017), the different starch fractions retrograde at different time and percentage: amylose crystallises first taking from a few minutes to hours whereas the variations of amylopectin happen at a slower rate, in some days. Regarding the flavour attributes, B_{OSW} showed a good level of onion taste perception with a slight decrease at the end of the monitoring period. According to what described for aromatic attributes, both samples showed an increase in stale bread perception. Moreover, significant changes were found in the structure of the two bread samples, which showed a clear decrease in terms of compactness, adhesiveness and crumb cohesiveness. This could also explain the opposite trend in the firmness parameter in both samples: in particular, from the first to the last day of storage, the enriched sample showed a higher firmness than the control one, confirming what was also reported with the texture analysis (Figure 11). The interactions between the gelatinised starch granules and the gluten network in the crumb might have caused an increase in firmness (Swieca et al., 2013). It is evident that there was a significant influence of the added OSW_E on the overall structure of bread.

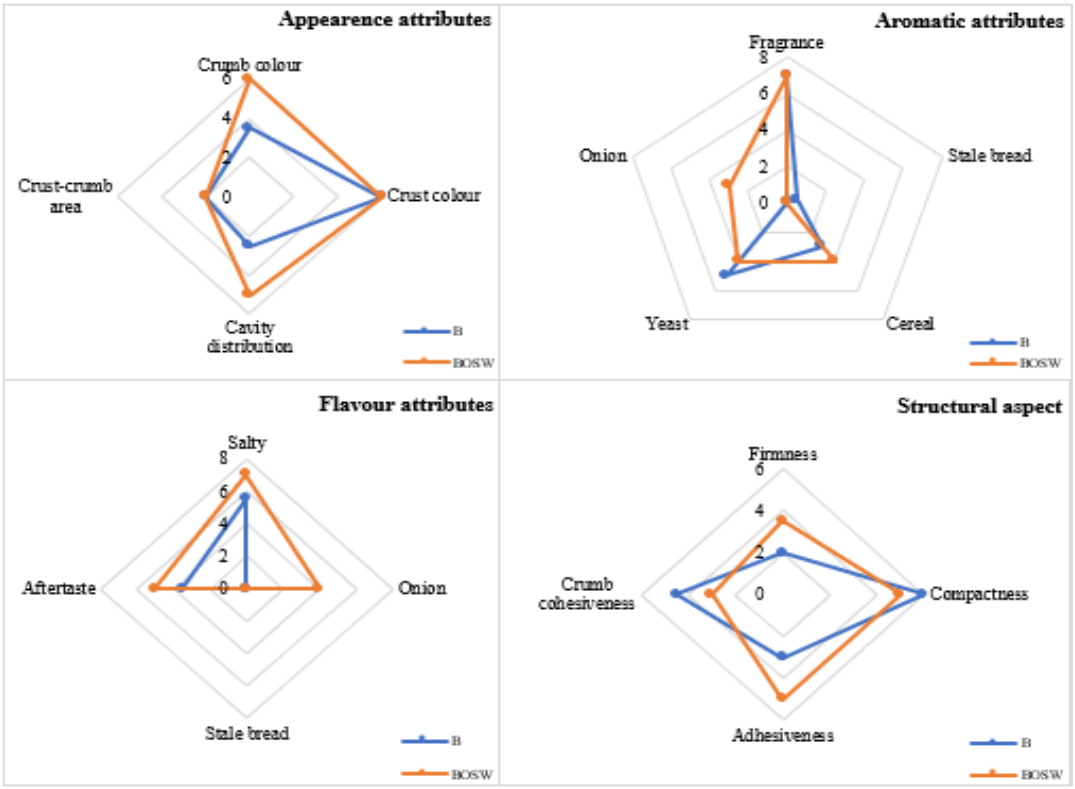


Figure 12: Median value of bread sensorial evaluation at time 0.

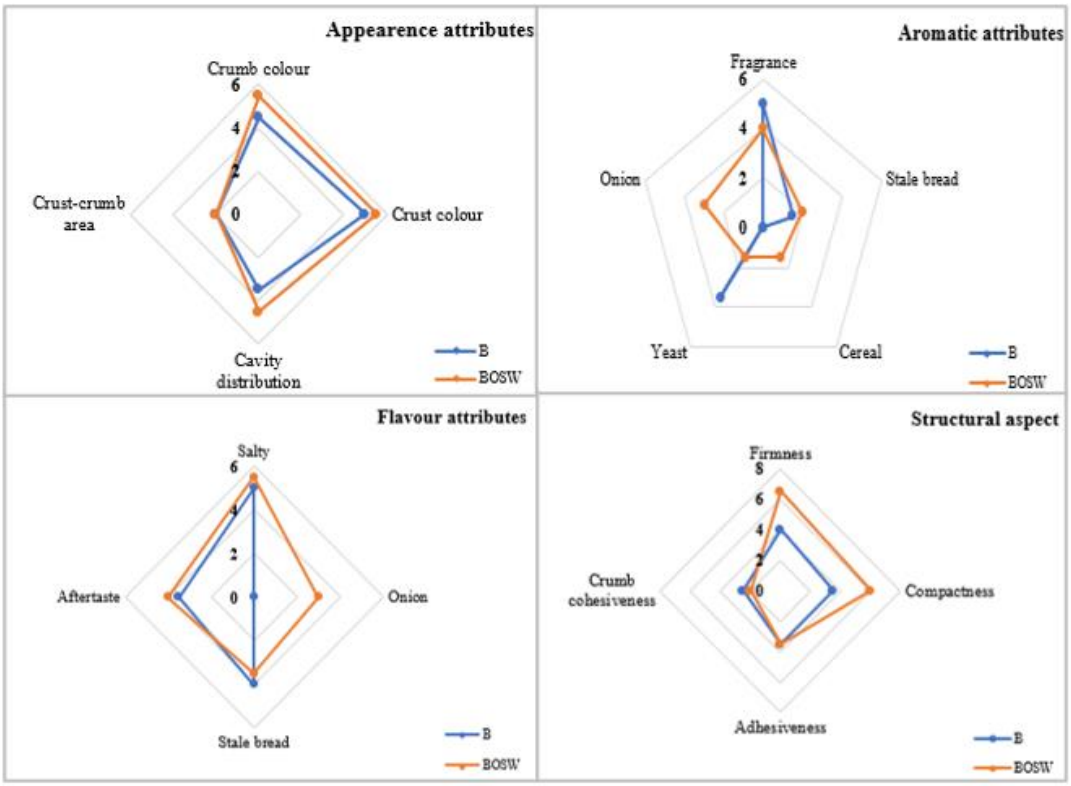


Figure 13: Median value of bread sensorial evaluation at time 7.

3.2.4. CONCLUSIONS

Onion solid waste has been shown to be a good source of bioactive compounds with antioxidant and antimicrobial activity and the results obtained in this study have confirmed the possibility of using them as functional ingredients to produce new food products, such as bread, without compromising its sensory quality. Indeed, B_{OSW} was characterized by a higher level of TPC as well as in vitro antioxidant activity, showing positive results in terms of sensory acceptability. These results confirmed the possibility of producing functional foods by incorporating useful compounds obtained from food industry by-products, reducing their environmental impact and at the same time meeting the growing consumer demand for healthy food. It should be considered that in this kind of enriched products their possible bioactivity could be influenced by many factors and there are no clear procedures for formulating functional products with a definite nutritional and nutraceutical quality. Thus, to obtain healthy and functional baked goods, further studies in this field are necessary as well as the optimization of processing conditions to limit the loss of useful compounds.

4. EDIBLE COATINGS AND THEIR APPLICATION IN FRESH-CUT VEGETABLES

4.1. SHELF-LIFE EXTENSION OF MINIMALLY PROCESSED RADISH (*RAPHANUS SATIVUS L.*) BY USING OF CITRUS BY-PRODUCTS

4.1.1. Introduction

Minimally processed vegetables suggest to consumers high content in bioactive phenolic compounds and nutritional properties (Oms-Oliu et al., 2008). Consumption of vegetables is widespread because it can reduce the incidence of many diseases (Sun et al., 2009).

Radish (*Raphanus sativus L.*) is a worldwide recognized vegetable that contains many bioactive compounds as anthocyanins, flavonoids, phenols, vitamins, pigments that affect its appearance and nutritional quality. Showing antimicrobial, antiviral and antioxidant activity, its leaves and roots have been therefore used in various parts of the world with medical applications (Zhang et al., 2020; Gutiérrez et al., 2004).

Minimal processing operations are known to damage the integrity of vegetables tissues, which triggers wounding and deteriorative processes including oxidative browning, tissue softening, water loss and the production of undesirable flavours and odours (Martìn-Belloso et al., 2007). The greatest losses in quality and quantity of fresh fruits and vegetables occur from harvest to consumption due to the change in the gas balance between the oxygen consumption and carbon dioxide production (Dhall, 2013). The gas transfer rate depends upon internal and external factors, such as cultivar or atmospheric composition, in terms of O_2, CO_2 and ethylene ratio (Kluge et al., 2002). The main objectives of any postharvest technology are quality optimisation and loss reduction in fresh produce (Ghoora and Srividya, 2020). The controlled and modified atmosphere packaging, the dipping with natural additives, like salts or organic acids, have been used for preserving different minimally processed vegetables and for reducing changes in quality and quantity of components (Piscopo et al., 2019; Zappia et al., 2019). Edible coating represents new packaging strategy in the postharvest management of fresh produce, as primary packaging on the surface of vegetables or fruits. It can provide an alternative to modified atmosphere packaging by maintaining the original quality through modification and control of the internal atmosphere of the individual fruit or vegetable. Edible coating can reduce moisture and solute migration, gas exchange, respiration and oxidative reaction rates, have a high potential to carry active ingredients such as antibrowning agents, colorants, flavours, nutrients,

spices and antimicrobial compounds that can extend product shelf life and reduce the risk of microbiological growth (Dhall, 2013).

Edible coatings can be produced from materials with film forming ability. Plasticizers, antimicrobial agents, minerals, vitamins, colours, or flavours can be added in this process (Arvanitoyannis and Gorris, 1999). In the regulation of most countries, chemical substances added as antimicrobials are considered as food additives if the primary purpose of the substances is shelf-life extension (USDA, 2008). In the last decade, there has been increased interest in using edible coating as aloe vera (Misir et al. 2014; Sicari et al., 2020) on fruits and vegetables compared to chitosan and alginate coatings to extend the postharvest quality and shelf life of kiwi slices.

Other studies highlighted that the recovered compounds from food wastes or by-products could be used as high added value (Chihoub et al., 2019) to fresh product with limited shelf life. Citrus processing industries produce huge amounts of waste and by-products every year, reach in commercially important high value-added compounds, which can be extracted and applied in food and processing industries, pharmaceutical companies, domestic use, etc. (Sharma et al., 2019). The main ones are essential oils, flavonoids, carotenoids, limonoids, phenolics, organic acids, vitamins, pectins and enzymes (Sharma et al. 2017), which can be used as ingredients with antioxidant and antimicrobial effects, and as active agent in dipping solutions with the aim to preserve and to extend the shelf life of food products.

In this work, the efficacy of an alginate coating formulation with lemon by-products phenolic extract applied on Raphanus sativus samples was tested by microbiological and physico-chemical analyses, to ensure quality preservation and shelf-life extension of the final product stored in polypropylene trays at 3°C.

4.1.2. MATERIALS AND METHODS

4.1.2.1. Raw materials

Lemon by-products samples (*Citrus limon* (L.) Osbeck), consisting of peels, pulp and seeds, were supplied by Agrumaria Reggina company, located in Gallico (Reggio Calabria, Italy), after the extraction of lemon juice and essential oils. Lemon by-products were transported to the Food Technology laboratory of the "Mediterranea" University of Reggio Calabria, immediately dried at a temperature of 50°C up to a final moisture content of 12% and stored in polyethylene bags under vacuum to avoid rehydration until subsequent extraction procedures of the bioactive compounds.

Radishes were supplied by a local distributor in the province of Reggio Calabria (Italy) and transported to the Food Technology laboratory of the "Mediterranea" University of Reggio Calabria, where leaves were completely removed before subjecting the radish samples to processing.

4.1.2.2. Preparation of lemon by-products extract (LP_E)

A total of 100 mL of ethanol:water (1:1, v:v) solution were mixed to 20 g of ground lemon by-products (LP) in a Sonoplus Ultrasonic homogeniser, Series 2000.2, HD 2200.2 (BANDELIN, Ultraschall seit 1955), for 60 min at 25°C. Subsequently, the sample was centrifuged (5000 rpm, 5 min, 4 °C, in a refrigerated centrifuge, (NF 1200R, Nüve, Ankara, Turkey), filtered (0.45 µm filter paper), and the resulting extract was made up to volume of 100 mL with the extraction solvent (food-grade ethanol:water, 1:1, v:v). The LP_E was subjected to qualitative and quantitative analysis before being used in the formulation of radish samples.

4.1.2.3. Characterization of LP_E

Physico-chemical properties, total polyphenol content (TPC_E), total flavonoid content (TF_E), antioxidant activity (DPPH and ABTS assays) and identification and quantification of the main antioxidant compounds of lemon by-products extract were carried out as reported by Imeneo et al., 2021.

4.1.2.4. Preparation of radish samples

Radish samples were subjected to a first washing in water and then in a chlorinated water for five minutes. After washing, radishes were cut into wedges giving firstly UCR sample (uncoated radishes). Successively an aliquot was submitted to dipping and coating treatment, according to Oms-Oliu et al. (2008). For the dipping treatments, 0.3% (w/v) citric acid solution and an aqueous solution containing the LP_E (1%, w/v) were prepared to obtain the samples DRa and DRb, respectively. Coating solutions were prepared by dissolving alginate (2%, w/v) in distilled water at 70°C under continuous stirring until the solution became clear. Glycerol was added as a plasticizer (1.5%, w/v) in alginate solution and, for the crosslinking of carbohydrate polymers, a calcium chloride solution (2%, w/v) was prepared to obtain the sample CRc. Another calcium chloride solution (2%, w/v) containing the LP_E (1%, w/v) was prepared for the formulation of CRd sample. All the samples (60g) were packaged in Polypropylene trays + Polypropylene/Polyethylene terephthalate film top by a thermo-sealed machine (VGP 25n, ORVED) and stored to 3°C for 14 days.

4.1.2.5. Headspace gas composition

The respiratory rate was determined according to the method reported by Del Aguila et al. (2008): 15g of radish sample were placed in sealed glass jars of 300 mL equipped with a rubber septum to insert the needle for the measurement of CO_2 and O_2 percentage using a CheckPoint handheld Gas Analyser (PBI Dansensor, Milan, Italy). The first measurement (time zero) was carried out 1 h after processing and after 1, 3 and 7 days. Results were expressed in CO_2 % and O_2 %.

4.1.2.6. Microbiological analysis

For the microbiological analyses total bacterial count (TBC) and total moulds (TM) were determined. An aliquot of sample (10 g) was diluted with a sterile Ringer's solution and was homogenized with a Stomacher (BagMixer ® interscience,Saint Nom, France) for 2 minutes. Decimal serially dilutions were prepared and plated on Petri plates with PCA-Plant Count Agar- growth land (Oxoid, Milan, Italy) for TBC at 26°C for 48 h and DRBC agar base (Dichloran Rose Bengal Chloranphenicol) for TM at 26°C for 3-4 days. The results were expressed as Log10 CFU g^{-1} (Fan and Song, 2008).

4.1.2.7. Physico-chemical analysis of radish samples

The samples were submitted to the following determinations: titratable acidity (% of citric acid g^{-1} f.w.) according to AOAC method (2000); pH by pH meter (Crison GLP, Barcellona, Spain); total soluble solids (°Brix) using a digital refractometer (PR-201a Atago); dry matter (%) by loss weight in an oven at 70 °C until constant weight according to the following equation:

$$\text{dry matter (\%)} = [(Fw - Mw)/Sw] * 100$$

Where: Fw = final weight (sample + melting pot); Mw = melting pot weight; Sw = fresh sample weight.

Colour analyses was performed with tristimulus colorimeter (model CM-700d, Konica Minolta, Osaka, Japan) on outer and inner portions of radish samples and was referred to the CIELab colour space for the parameters L*, a* and b*. Colour changes of radishes were measured through hue angle (h◦) parameter according to Oms-Oliu et al. (2006):

$$h° = \arctan b^*/a^*$$

Total colour difference (ΔE) in outer and inner sides of the samples at the 1st day and after 14 days of storage was obtained by the following formula, according to Thompson (2004):

$$\Delta E = \sqrt{((L^*-L^*_0)^2+(a^*-a^*_0)^2+(b^*-b^*_0)^2}$$

where L^*_0, a^*_0, and b^*_0 are the initial considered values (1st day).

4.1.2.8. Total polyphenol and anthocyanin determinations

The radish samples were submitted to methanolic extraction, according to Marotti and Piccaglia (2002). 10 g of sample were homogenised with 25 mL of methanol:water:acetic acid (50:42:8, v:v:v) for 2 minutes, centrifuged at 5,000 x g for 10 min at 4°C. The supernatant was collected and residues

were re-extracted. Both supernatant solutions were filtered through syringe filters (0.45 µm Chromafil RC-45/25), combined and diluted up to 25 mL volume with extraction solution.

Total phenolic content (TPC) was determined according to the method of Singleton and Rossi (1965) using the Folin–Ciocalteu reagent (Carlo Erba, Milan, Italy), by reacting 100 µL of methanolic extract. The solutions were spectrophotometrically analyzed at 760 nm in a UV–VIS spectrophotometer (Agilent, Santa Clara, California, USA) and the results were reported as mg gallic acid kg^{-1} of radish fresh weight (mg GAE kg^{-1} f.w.).

The total anthocyanin content (TAC) was determined spectrophotometrically according to the AOAC method (2005.02) on the methanol extract diluted (D=1:5, v:v) with pH 1.0 buffer (potassium chloride, 0.025 M) and pH 4.5 buffer (sodium acetate, 0.4M) and the absorbance was determined against the blank (distilled water) at both 520 and 700 nm. Anthocyanin pigment concentration was expressed as mg of cyaniding 3-glucoside kg^{-1} of radish fresh weight (mg C-3-GLUC kg^{-1} f.w.) and was calculated as follows:

$$\frac{A * MW * DF * 10^3}{\varepsilon * 1}$$

Where: A= (A_{520nm}-A_{700nm}) pH 1.0 - (A_{520nm}-A_{700nm}) pH 4.5; MW (molecular weight) = 449.2 g mol^{-1} for cyaniding 3-glucoside (cyd-3-glu); DF = dilution factor established in D; 1 = pathlength in cm; ε = 26900 molar extinction coefficients, in L*mol^{-1}*cm^{-1}, for cyd-3-glu; 10^3 = factor for conversion from g to mg.

4.1.2.9. Antioxidant activity

For the antioxidant activity determination of radish samples, two radical scavenging assays were performed: the Trolox equivalent antioxidant capacity (TEAC) and the DPPH radical scavenging activity. The first assay was evaluated according to the method of Re et al. (1999): 2975 µL of ABTS solution in ethanol and 25 µL of methanol extract were mixed and the absorbance was read spectrophotometrically at 734 nm after 6 min. The DPPH radical scavenging activity was determined by using DPPH methanolic solution, according to the method of Brand-Williams et al. (1995): 2950 µL of DPPH solution and 50 µL of methanol extract were mixed, kept in the dark for 15 min and the absorbance was recorded at 515 nm using a spectrophotometer. The results were expressed as µM Trolox equivalent kg^{-1} of radish fresh weight (µM TE kg^{-1} f.w.).

4.1.2.10. Statistical analysis

The results were reported as mean ± standard deviation (mean±SD) of three replicates. Significance of the results and statistical differences were analysed using SPSS software version 15. Analyses of variance (ANOVA and Multivariate analysis) were performed to compare mean values of different preservative treatment and Tukey's multiple range elaboration was used as post-hoc test ($p<0.05$).

4.1.3. RESULTS AND DISCUSSION

4.1.3.1. Characterisation of lemon by-products extract (LP_E)

Results of LP_E characterization are reported in Table 25. TPC_E and TF_E determinations denoted mean values of 4.73±0.15 mg GAE g^{-1} d.w. and 1.19±0.04 mg CE g^{-1} d.w. respectively, with the predominant compounds represented by eriocitrin and hesperidin, as confirmed by literature (Sharma et al., 2017).

Table 25: Physico-chemical characterization of LP_E.

pH	3.76 ± 0.03
Colour:	L*: 32.09 ± 0.40
	a*: 0.91 ± 0.17
	b*: 2.18 ± 0.19
TPC_E (mg GAE g^{-1} d.w.)	4.73 ± 0.15
TF_E (mg CE g^{-1} d.w.)	1.19 ± 0.04
DPPH (µmol TE g^{-1} d.w.)	6.85 ± 0.37
ABTS (µmol TE g^{-1} d.w.)	15.16 ± 0.51
Eriocitrin (mg 100 g^{-1} d.w)	116.20 ± 0.02
Hesperidin (mg 100 g^{-1} d.w.)	129.18 ± 0.07

GAE: Gallic acid equivalent; TPC: Total phenolic compounds; LP_E (lemon by-products extract); TF: Total flavonoids; DPPH and ABTS: Total antioxidant activity assays.

4.1.3.2. Headspace gas composition of radish samples

Gas composition (O_2% and CO_2%) evaluated in the headspace of the sealed glass jars is shown in Figure 14. The general trend is that the O_2 concentrations decreased from the 1st to the 7th day of monitoring, while an opposite trend was observed for CO_2 concentrations, confirming what was described by different authors (Rojas-Graü et al., 2008; Ayranci and Tunc, 2003). The formulation of coating with the addition of LP_E (CRd) significantly ($p<0.01$) reduced the respiration process compared to the other radish samples, showing the highest O_2% and the lowest CO_2%. These results highlighted the effectiveness of the synergistic effect of the extract and the coating formulation (CRd) to delay the deteriorative processes and to extend the radish shelf life, thanks to the preservative action

exerted by the antioxidant extract (LP_E) plus the presence of calcium ions contained in the coating formulation on radish, rather than the effect of oxygen barrier properties by coatings as suggest by Wong et al., (1994) and Lee et al., (2003) in cut apples.

Indeed, both samples DRb and CRc, respectively characterized only by the presence of LP_E or the alginate-based coating, showed the lowest O_2% values and the highest CO_2% values, demonstrating the inefficiency of these two treatments to reduce the respiration rate.

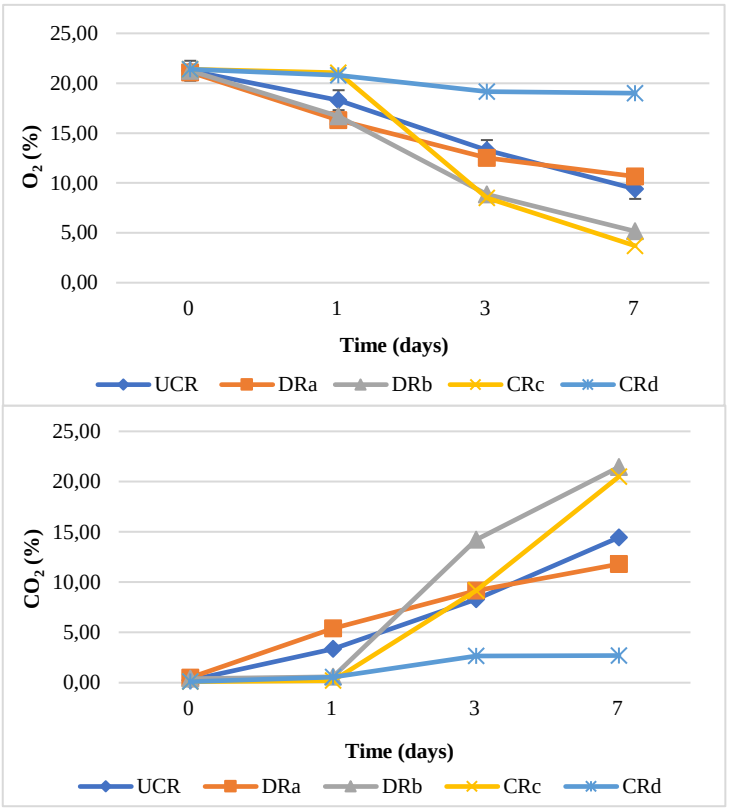


Figure 14: Headspace gas composition in sealed glass jars containing dipped and coated radish samples.

4.1.3.3. Microbiological activity and physico-chemical characterization of radish samples

For microbial count there were differences among treatments after 7 days of storage as shown in Table 26 ($p<0.01$). The lowest aerobic bacteria count was found in CRc sample (2.58±0.16 Log CFU g^{-1}): probably, the O_2 decrement during the storage period, as reported in Figure 14, caused an anaerobic condition inside the package, with a consequently minor aerobic bacteria growth. Browning on the surface of this sample and softening were the first obvious and visible consequences defining the end of shelf life of CRc on the 7th day. The other samples showed a microbial count increment over time: UCR did not significantly ($p>0.05$) differ from dipped and coated samples, confirming what found by Oms-Oliu et al. (2008), not exceeding 6 Log CFU g^{-1}. The presence of LP_E in alginate-based coating formulation (CRd) did not express a great variation for this qualitative parameter, as

instead demonstrated by Rojas-Graü et al. (2008) and Raybaudi-Massilia et al. (2007) on cut apples active coating.

Table 26: Microbiological results, acidity, pH, soluble solids and dry matter values of radish samples.

	Time (days)	Samples					
		UCR	**DRa**	**DRb**	**CRc**	**CRd**	**Sign.**
Total aerobic count (Log CFU g^{-1})	1	2.55 ± 0.55^{C}	2.68 ± 0.11^{C}	2.43 ± 0.20^{B}	2.16 ± 0.35^{B}	2.74 ± 0.26^{B}	**n.s.**
	3	4.29 ± 0.56^{B}	4.46 ± 0.66^{AB}	4.58 ± 0.38^{A}	3.79 ± 0.27^{A}	4.48 ± 0.69^{AB}	**n.s.**
	7	4.60 ± 0.03^{Ba}	4.53 ± 0.70^{ABa}	4.38 ± 0.11^{Aa}	2.58 ± 0.16^{Bb}	4.22 ± 0.04^{ABa}	**
	10	$5.27\pm0.67A^{Ba}$	3.94 ± 0.65^{BCb}	4.75 ± 0.32^{Aab}	n.d.	5.09 ± 0.46^{Aab}	**
	14	6.05 ± 0.34^{A}	5.67 ± 0.51^{A}	5.01 ± 1.13^{A}	n.d.	4.49 ± 1.43^{AB}	**n.s.**
	SIGN.	**	**	**	**	*	
Acidity (% citric acid)	1	0.14 ± 0.00^{Aa}	0.10 ± 0.01^{Ac}	0.11 ± 0.01^{Abc}	0.13 ± 0.00^{ab}	0.12 ± 0.02^{Aabc}	**
	3	0.08 ± 0.02^{Bbc}	0.07 ± 0.00^{Bc}	0.07 ± 0.00^{Bc}	0.13 ± 0.00^{a}	0.10 ± 0.01^{ABb}	**
	7	0.07 ± 0.00^{Bc}	0.10 ± 0.00^{Aab}	0.08 ± 0.02^{Bbc}	0.12 ± 0.00^{a}	0.09 ± 0.00^{Bbc}	**
	10	0.06 ± 0.03^{B}	0.08 ± 0.00^{B}	0.08 ± 0.00^{B}	n.d.	0.09 ± 0.00^{B}	**n.s.**
	14	0.08 ± 0.02^{Bb}	0.08 ± 0.00^{Bb}	0.09 ± 0.00^{ABab}	n.d.	0.11 ± 0.00^{ABa}	**
	SIGN.	**	**	**	**N.S.**	*	
pH	1	6.36 ± 0.02^{Ba}	6.26 ± 0.01^{Ca}	6.26 ± 0.00^{Ca}	5.84 ± 0.01^{Ab}	5.97 ± 0.13^{Bb}	**
	3	6.48 ± 0.08^{Bb}	6.62 ± 0.05^{ABa}	6.51 ± 0.06^{Bab}	5.70 ± 0.01^{Bd}	6.17 ± 0.00^{Bc}	**
	7	6.67 ± 0.13^{ABa}	6.41 ± 0.25^{BCa}	6.59 ± 0.01^{Ba}	5.68 ± 0.04^{Bb}	6.50 ± 0.11^{Aa}	**
	10	6.91 ± 0.06^{Aa}	6.87 ± 0.04^{Aa}	6.84 ± 0.01^{Aa}	n.d.	6.46 ± 0.13^{Ab}	**
	14	6.66 ± 0.28^{ABa}	6.91 ± 0.11^{Aa}	6.75 ± 0.10^{Aa}	n.d.	6.18 ± 0.03^{Bb}	**
	SIGN.	**	**	**	**	**	
Soluble solids (°Brix)	1	2.95 ± 0.07^{b}	1.90 ± 0.14^{Bc}	1.75 ± 0.07^{Bc}	3.05 ± 0.07^{Bab}	3.25 ± 0.07^{a}	**
	3	3.05±0.35	2.75 ± 0.64^{AB}	3.00 ± 0.14^{A}	3.65 ± 0.64^{AB}	4.30±0.85	**n.s.**
	7	3.10 ± 0.42^{ab}	2.30 ± 0.42^{ABb}	2.50 ± 0.42^{ABb}	3.90 ± 0.14^{Aa}	3.70 ± 0.57^{a}	**
	10	2.80 ± 0.00^{ab}	2.05 ± 0.07^{ABb}	3.35 ± 0.78^{Aa}	n.d.	3.50 ± 0.57^{a}	**
	14	2.60 ± 0.28^{b}	2.90 ± 0.14^{Ab}	2.75 ± 0.21^{Ab}	n.d.	$3.65s\pm0.35^{a}$	**
	SIGN.	**N.S.**	*	**	**	**N.S.**	
Dry matter (%)	1	4.07 ± 0.44^{b}	4.26 ± 0.42^{ABb}	4.06 ± 0.69^{b}	5.54 ± 0.03^{a}	6.06 ± 0.37^{a}	**
	3	$3.77 \pm 1,12^{b}$	4.08 ± 0.42^{ABab}	4.18 ± 0.28^{ab}	6.02 ± 1.19^{a}	5.26 ± 0.19^{ab}	*
	7	4.72 ± 0.84^{ab}	4.84 ± 0.17^{Aab}	3.67 ± 1.58^{b}	6.41 ± 0.67^{a}	5.14 ± 0.32^{ab}	*
	10	4.16 ± 0.29^{b}	4.58 ± 0.28^{ABab}	5.04 ± 0.12^{ab}	n.d.	5.31 ± 0.79^{a}	**
	14	4.17 ± 0.59	3.96 ± 0.01^{B}	4.84 ± 0.36	n.d.	5.34 ± 1.09	**n.s.**
	SIGN.	**N.S.**	*	**N.S.**	**N.S.**	**N.S.**	

The data are presented as means ± SD (n=3). Means within a row or column with different letters are significantly different by Tukey's post hoc test. Abbreviation: Sign., significance; ns, not significant. n.d., not detected. **Significance at $p<0.01$; *Significance at $p<0.05$.

Regarding total acidity, a highly significant ($p<0.01$) reduction in this value was noted for all samples over time, except for CRc, which showed similar value until the 7th day of storage. An opposite trend

to that described above was found for total soluble solid values, due to the reduction in the metabolic activity of vegetables (Das et al., 2013), showing a significant increase over time, except for UCR and CRd. The different development of acidity and °Brix values could be explained by the progressive vegetable maturation process characterised by a change in organic acids content, which are used as respiration substrates or transformed into other compounds resulting in changes in taste. In addition to organic acids, an important respiration substrate are sugars, which are transformed into simpler molecules, such as CO_2 and water (Vanaclocha, 2014). Indeed, the higher °Brix values found for CRc on the 7th day could explain the sharp reduction in O_2% and increase in CO_2%, shown in Figure 14.
Colour results, expressed as hue angle (h°) and ΔE, respectively showed in Table 27 and Figure 15. After three storage days, a slight increase in h° values was observed in the inner surface of the samples, except for CRc which showed a decrease in h° values over time, confirming a noticeable colour change probably due to the development of browning in the radishes inner surface associated with the high degree of senescence that appeared on the seventh day. At the end of monitoring, CRd showed the highest value of h° angle. Regarding the outer side, all samples showed lower values than inner part, thanks to the natural darker colour of radish outer surface. In general, a gradual but non-linear reduction in values over time was observed, CRd showed higher values on the last day than on the first day of monitoring.

Table 27: Hue angle (h°) values of radish samples.

Outer						
Days / Sample	**1**	**3**	**7**	**10**	**14**	**SIGN.**
UCR	11.16 ± 0.22^{Ab}	10.61 ± 0.23^{Ac}	7.57 ± 0.10^{Bc}	-2.10 ± 0.14^{Dd}	3.61 ± 0.12^{Cc}	**
DRa	13.55 ± 0.15^{Aa}	12.63 ± 0.14^{Bb}	9.46 ± 0.10^{Cb}	2.58 ± 0.04^{Dc}	-0.28 ± 0.09^{Ed}	**
DRb	13.67 ± 0.12^{Ba}	14.62 ± 0.31^{Aa}	9.46 ± 0.21^{Cb}	10.23 ± 0.13^{Ca}	6.71 ± 0.24^{Db}	**
CRc	11.21 ± 0.26^{Cb}	15.28 ± 0.19^{Ba}	23.28 ± 0.16^{Aa}	n.d.	n.d.	**
CRd	10.18 ± 0.23^{Bc}	8.58 ± 0.22^{Cd}	6.22 ± 0.23^{Ed}	7.47 ± 0.28^{Db}	12.59 ± 0.23^{Aa}	**
Sign.	**	**	**	**	**	
Inner						
Days / Sample	**1**	**3**	**7**	**10**	**14**	**SIGN.**
UCR	72.69 ± 0.30^{Cb}	64.39 ± 0.22^{Eb}	71.58 ± 0.09^{Dc}	75.30 ± 0.27^{Bd}	78.52 ± 0.14^{Ac}	**
DRa	76.57 ± 0.28^{Ca}	59.36 ± 0.32^{Dc}	76.71 ± 0.18^{Cb}	84.42 ± 0.14^{Aa}	81.66 ± 0.41^{Bb}	**
DRb	77.26 ± 0.32^{Ca}	56.51 ± 0.29^{Ed}	80.31 ± 0.21^{Ba}	81.71 ± 0.40^{Ab}	74.47 ± 0.15^{Dd}	**
CRc	30.68 ± 0.23^{Ad}	26.60 ± 0.16^{Be}	19.36 ± 0.36^{Cd}	n.d.	n.d.	**
CRd	40.59 ± 0.34^{Ec}	68.56 ± 0.23^{Da}	80.45 ± 0.23^{Ba}	76.28 ± 0.15^{Cc}	85.60 ± 0.35^{Aa}	**
Sign.	**	**	**	**	**	

The data are presented as means ± SD (n=10). Means within a row or column with different letters are significantly different by Tukey's post hoc test. Abbreviation: Sign., significance; ns, not significant. n.d., not detected. **Significance at $p<0.01$; *Significance at $p<0.05$.

Figure 14 showed the colour variation (ΔE) regarding the differences between the last day (14th day) and the first day of monitoring. The use of alginate-based coating with the addition of the lemon by-products extract (CRd) promoted a protection against colour variation both on outer and inner portions of vegetable wedges. In fact, CRd was characterized by the lowest ΔE values of 3.13±0.01 (outer) and 1.09±0.00 (inner). DRb, dipped in LP_E solution, also exhibited a limited colour variation, especially in inner surface, suggesting that LP_E did not affect the natural colour of radishes. Differently, CRc and DRa presented the most evident colour variations over time on both vegetable surfaces. By colour results, alginate-based coating and dipping solution with LP_E were effective in avoiding browning, confirming what was noted by Rojas-Graü et al. (2007) on fresh-cut coated pears.

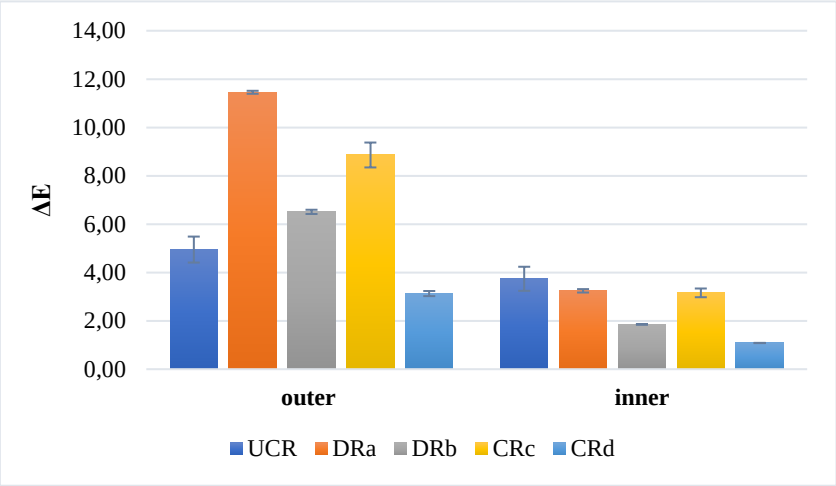


Figure 15: Colour variation values of dipped and coated radish samples.

4.1.3.4. Characterization of bioactive compounds and antioxidant activity of radish samples

Bioactive compounds (total polyphenols and anthocyanin content) and antioxidant activity are reported on Table 28. The total polyphenol content (TPC) did not vary either among samples or over time, contrary to what was found for the total anthocyanin content (TAC). The decrease in TAC was highly significant at the end of monitoring in CRd, with 189±9 mg C-3-Glu kg^{-1} f.w. on the 14th day. In general, looking at the data for TPC and TAC it can be stated that the presence of the extract in the final product, whether in the form of dipping or coating, did not significantly influence the total content of bioactive compounds. At the same time, from the comparison of the two coating formulations, the presence of LP_E contributed to prolonging the shelf life of the vegetable beyond seven days, slowing down the phenomenon of senescence favoured by the stress condition induced by the initial processing operations and by the presence of the coating itself.

A similar situation to that presented above was found in terms of antioxidant activity, for both the DPPH and ABTS assays. Significant statistical differences for DPPH scavenging (%) were found

among samples after three and seven storage days ($p<0.01$) and for ABTS scavenging (%) on the 10th day: higher values for both assays were observed in sample DRb, treated with LP_E dipping solution, with 38.62 ± 4.92 % and 35.85 ± 0.89 inactivation %, respectively. The only use of edible coating (CRc) did not seem to substantially contribute to enhancement the radish antioxidant capacity as just declared by Oms-Oliu et al. (2008), contrary to what showed by coating formulated with the addition of an ingredient with antioxidant properties such as lemon by-products extract (Galvis-Sánchez et al., 2003). A general but not significant decrease of antioxidant activity was found in all samples for the DPPH assay after seven days of storage, in contrast to the constant trend determined for the ABTS assay: Reyes et al. (2007) suggested that the antioxidant activity in fruits and vegetables is correlated with the observed value in total phenolic content. Indeed, in this work positive correlation values were found after 10 days between DPPH scavenging and anthocyanin content ($r = 0.915$) and between ABTS scavenging and both polyphenolic and anthocyanin content ($r = 0.748$ and 0.913, respectively).

Table 28: Total polyphenol content (TPC), Total anthocyanins content (TAC) and expression of antioxidant activity (DPPH and ABTS assays) values of radish samples.

	Days	**UCR**	**DRa**	**DRb**	**CRc**	**CRd**	**SIGN.**
TPC mg GAE kg^{-1} f.w.	1	399±79	382±25	433±46	443±12	467±65	**N.S.**
	3	397±59	402±28	442±29	380±47	440±10	**N.S.**
	7	428±32	460±53	429±24	493±15	493±16	**N.S.**
	10	374±21	373±7	400±25	n.d.	362±47	**N.S.**
	14	389±26	334±19	424±72	n.d.	393±7	**N.S.**
	Sig.	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	
TAC mg C-3-Glu kg^{-1} f.w.	1	195±37	205±16	236±49	200±9^{a}	246±23^{a}	**N.S.**
	3	202±45	211±41	235±18	137±8^{b}	225±17^{ab}	**N.S.**
	7	241±28	265±35	225±8	197±0^{a}	264±7^{a}	**N.S.**
	10	202±14^{A}	206±7^{A}	199±7^{A}	n.d.	160±4^{Bc}	*
	14	205±0^{A}	162±1^{B}	194±4^{A}	n.d.	189±9^{Abc}	**
	Sig.	**n.s.**	**n.s.**	**n.s.**	**	**	
DPPH Inactivation %	1	34.26±5.31	34.28±1.17^{ab}	37.18±5.63	22.87±4.08	33.34±5.17	**N.S.**
	3	33.50±1.79^{A}	34.37±0.43^{Aab}	34.92±0.86^{A}	21.87±0.16^{B}	34.52±1.94^{A}	**
	7	38.04±4.60^{AB}	38.08±2.59^{Aa}	38.62±4.92^{A}	18.79±5.20^{B}	30.31±6.02^{AB}	*
	10	36.05±7.37	34.49±0.88^{ab}	35.00±3.18	n.d.	23.62±2.35	**N.S.**
	14	29.53±4.69	27.28±2.98^{b}	33.09±0.11	n.d.	27.98±2.37	**N.S.**
	Sig.	**n.s.**	*	**n.s.**	**n.s.**	**n.s.**	
ABTS Inactivation %	1	32.47±5.64	32.52±0.86	38.45±2.32	29.84±4.33	36.71±2.04	**N.S.**
	3	29.57±0.70	34.59±1.44	34.07±6.43	21.66±2.09	33.26±6.35	**N.S.**
	7	46.55±5.84	39.64±2.01	35.65±6.21	30.31±6.98	40.23±6.70	**N.S.**
	10	31.88±4.07^{AB}	32.68±1.58^{AB}	35.85±0.89^{A}	n.d.	23.72±1.72^{B}	*
	14	36.48±8.84	32.96±4.34	41.29±2.52	n.d.	31.42±0.74	**N.S.**
	Sig.	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	

The data are presented as means ± SD (n=10). Means within a row or column with different letters are significantly different by Tukey's post hoc test. Abbreviation: Sign., significance; ns, not significant. n.d., not detected. **Significance at $p<0.01$; *Significance at $p<0.05$.

4.1.4. CONCLUSIONS

The coating with only sodium alginate (CRc) results in a short radish shelf life due to the absence of some antioxidant agents and higher stress conditions. A higher consumption of O_2 was observed with a reduced growth of aerobic bacteria and the appearance of an unacceptable colour. On the other hand, using of alginate-based coating with the lemon by-products extract (CRd) on the minimally processed Raphanus s. contributed to slow down the respiration process, to limit colour variation, to reduce the microbial growth and to improve the shelf life up to 14 days. In addition, the added value of the LP_E also used in the formulation of the dipping solution (DRb) was highlighted by the content of bioactive compounds and their correlations with the antioxidant activity assays and by the reduced microbial load observed. A further positive effect of the extract was particularly found in relation to the respiration typical of minimally processed vegetables, slowing down the process and thus the degree of senescence. This allowed a better preservation of the radish sample, characterised over time by a lesser colour variation, both on the outside and on the inside, thanks to a greater containment of oxidative processes affecting polyphenolic compounds. The latter, in fact, did not show significant variations over time as well as the expression of antioxidant activity.

The present study suggests that coating containing an antioxidant extract was effective in microbiological, physico-chemical and sensorial quality, improving the shelf life of a common commercial Raphanus sativus up to 14 days.

4.2. SHELF-LIFE EXTENSION OF FRESH-CUT CARROTS WITH PECTIN-BASED COATING ADDED WITH AN ANTIOXIDANT EXTRACT FROM LEMON BY-PRODUCTS

4.2.1. INTRODUCTION

Consumption of fresh fruit and vegetables is known to be beneficial to human health. Carrot consumption is becoming increasingly popular due to its nutritional value, playing an important role in the world economy. Indeed, carrots are a good source of bioactive compounds, such as carotenoids, phenolic compounds, vitamin C and fibre, and antioxidant properties. (Arscott & Tanumihardjo, 2010; López-Gámez et al., 2020a). However, fresh fruit and vegetables are highly perishable and during post-harvest handling and storage, significant losses of vitamins and other phytonutrients can occur, depending on the nutrient, genotype, physical damage, temperature and storage environment. (Olivas & Barbosa-Cánovas, 2005; Wang, 2007, Guerrieo et al., 2016).

Edible coatings have proven to be a valid strategy to boost food appearance and preservation thanks to their environmentally favourable nature, acting as moisture and oxygen barriers during fruit and vegetables processing, handling and storage, thus slowing down food spoilage. Moreover, they could increase safety, due to the possibility to the incorporation of antimicrobial and antioxidant compounds (Hassanpour, 2015; Guerriero et al., 2016). The use of edible coatings enriched with bioactive molecules resulted efficient in maintaining the quality of many fruits and vegetables during storage (Antunes et al., 2012; Campos et al., 2011; Guerreiro et al., 2015; Oms-Oliu et al., 2010; Zúñiga et al., 2012). In this context, polysaccharide-based edible coatings, such as pectin, are often used considering their ability to form rigid and stable gels (Campos et al., 2011; Salmieri and Lacroix, 2006; Guerriero et al., 2015). In particular, the hydrocolloidal and polyelectrolyte properties of pectin define its unique capacities, such as intense water retention in colloidal systems and their stabilization, facilitated plasticization with glycerol thanks to the ability of its hydrophobic groups to adsorb organic lipoid substances (Baeva & Panchev, 2005). Pectin represents a relevant polysaccharide with food applications due to its ability to form a gel in the presence of Ca^{2+} ions or a solute at low pH (Thakur, Singh, & Handa, 1997).

The aim of this study was to determine the effect of an antioxidant extract obtained from lemon by-products when incorporated in polysaccharide edible pectin-based coating, to extent the shelf-life of ready-to-eat minimally processed carrots.

4.2.2. MATERIALS AND METHODS

4.2.2.1. Chemical and reagents

Food grade low-methoxyl pectin (~30% esterified) (Sigma–Aldrich Chemic, Steinhein, Germany), glycerol (Merck, Whitehouse Station, NJ, USA) and calcium chloride (Sigma–Aldrich Chemic, Steinhein, Germany) were used. Folin-Ciocalteu reagent and ethanol were acquired from Scharlau S.L. (Barcelona, Spain), sodium carbonate was purchased from Fisher Scientific Scharlau Chemie (Loughborough, UK). DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis acid (3-ethylbenzothiazolin-6-sulfonic acid), gallic acid and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium hydroxide was purchased from Acros Organics (New Jersey, USA). Methanol was acquired from J.T. Baker S.A. (Sowińskiego, Poland). Acetone was purchased from Fisher Chemical (Loughborough, UK). Phenolphthalein was purchased from POCH S.A. (Sowińskiego, Poland). PCA, GCA and buffer peptone water were purchased from Biokar Diagnostics (Beauvais, France). Sodium hypochlorite was purchased from Productes Sant Manteu (Barcelona, Spain).

4.2.2.2. Sampling

Lemon by-products samples (*Citrus limon (L.) Osbeck)*, consisting of peels, pulp and seeds, were supplied by Agrumaria Reggina company located in Reggio Calabria (Italy) after the extraction of lemon juice and essential oils. Lemon by-products were initially dried at a temperature of 50°C up to a final moisture content of 12% and stored in polyethylene bags under vacuum to avoid rehydration until subsequent extraction procedures of the bioactive compounds.

Carrots (*Daucus carota cv. Nantes*) were obtained in a local supermarket in Lleida (Spain). The carrots were transported to the laboratory of the University of Lleida and immediately processed for this study. Food grade low-methoxyl pectin (~30% esterified) (Sigma–Aldrich Chemic, Steinhein, Germany) was the carbohydrate biopolymer used to prepare the coating formulations. Glycerol (Merck, Whitehouse Station, NJ, USA) was added as plasticizer. Calcium chloride (Sigma–Aldrich Chemic, Steinhein, Germany) was used to induce a crosslinking reaction.

4.2.2.3. Lemon by-products extract (AE)

The extraction was performed according to Papoutsis et al. (2018) with some modifications.

Briefly, 50 mL of ethanol:water (1:1, v/v) were added to 10 g of dried and ground lemon by-products and placed on a heating magnetic stirrer for 30 minutes at 70°C, monitored by a digital thermometer. Subsequently, sample was centrifuged (NF 1200R, Nüve, Ankara, Turkey) at 5000 rpm for 5 min at 4°C, filtered through a Büchner apparatus with 0.45 μm filter paper and the resulting extract was

made up to volume of 50 mL with the extraction solvent. The antioxidant extract (AE) was then filtered with 0.45 µm nylon filters and stored at -20°C until subsequent analyses.

- **Determination of extract total phenolic content (TPC_{AE})**

TPC_{AE} was determined according to the method reported by González-Molina et al. (2009), with appropriate modifications. 0.2 mL of antioxidant extract were placed inside a 25 mL flask and mixed with 5 mL of deionized water and 1 mL of Folin-Ciocalteu reagent. After 8 minutes, 10 mL of saturated sodium carbonate solution (Na_2CO_3) at 20% (w/v) was added and made up to volume with deionized water. At the same time, the solution used as a blank was prepared. The mixtures were incubated for two hours at room temperature and in the dark.

The absorbance of the samples was measured at 765 nm against a blank using a double-beam ultraviolet-visible spectrophotometer (Perkin-Elmer UV- Vis λ2, Waltham, Massachusetts, U.S.) and comparing with a gallic acid calibration curve (concentration between 1 and 10 mg L^{-1}). The results were expressed as mg of gallic acid g^{-1} of lemon by-products dry weight (mg GAE g^{-1} d.w.).

- **Antioxidant activity determination**

It was tested by two assays, based on $DPPH^{\cdot}$ and $ABTS^{+}$ extinction, which often do not give the same results because of the two different action mechanisms and the two distinct radicals involved.

DPPH assay

The DPPH assay was carried out as reported in Section 1.2.4.3., adding 50 µL of AE to 2950 µL of a 6 x 10^{-5} M of methanol solution of $DPPH^{\cdot}$.The results were expressed as µM Trolox equivalents g^{-1} of lemon by-products dry weight (µM TE g^{-1} d.w.), comparing with a Trolox calibration curve (from 3 to 18 µM).

ABTS assay

The ABTS assay was carried out as described in Section 1.2.4.3. by mixing 10 µL of AE and 2990 µL of $ABTS^{+}$ ethanol solution. The results were expressed as µM Trolox equivalents g^{-1} of lemon by-products dry weight (µM TE g^{-1} d.w.), comparing with a Trolox calibration curve (from 3 to 18 µM).

4.2.2.4. Preparation of the dipping and coating solutions

The dipping solutions consisted of water and an aqueous solution containing the antioxidant extract previously obtained from lemon by-products (1%, v/v).

The concentrations of coating ingredients used in the formulations were set up according to (Oms-Oliu et al., 2008). Coatings were prepared by dissolving pectin (2%, w/v) powder in distilled water

and heating at 70 °C while stirring until the solution became clear. Glycerol was added as a plasticizer at 1.5% (w/v) in pectin solutions. For the crosslinking of carbohydrates polymer, calcium chloride aqueous solution (2%, w/v) was prepared. The antioxidant extract was added (1%, v/v) to the calcium chloride solution.

4.2.2.5. Vegetable coating and packaging

Whole carrots (length of 17±3 cm) were sanitized in a 200 µL L^{-1} NaClO solution for 2 min, rinsed with tap water, and dried prior to peeling and cutting operations. Carrots were peeled and the remaining vegetable was cut lengthwise into two parts and then into two half spheres (diameter 28±2 mm, height 20±2 mm). The carrot pieces were dipped in an aqueous solutions of lemon by-products extract at 1% (v/v) or dipped into distilled water for 2 minutes (W+AE and W, respectively).

The other carrot pieces were dipped into the polysaccharide solutions for 2 min. The excess of coating material was allowed to drip off for 1 min before submerging the samples again for 2 min in the calcium chloride solutions, containing the lemon by-products extract (PBC+AE). As controls, carrot pieces were coated without incorporation of extract (PBC).

Then, 100 g of each carrot samples pieces were placed in polyethylene terephthalate trays (150 mL) and stored at 4±1°C in darkness up to withdrawal for analyses after 1, 3, 7, 10 and 14 days.

Two trays of each sample were taken at each sampling time to perform replicate analyses throughout 14 days of storage.

Table 29: Carrot samples denomination.

Dipping	**W**	Water
	W+AE	Water solution containing the antioxidant extract obtained from lemon by-products (1%, v/v).
Coating	**PBC**	Pectin-based coating
	PBC+AE	Pectin-based coating containing the antioxidant extract obtained from lemon by-products (1%, v/v).

4.2.2.6. Physico-chemical properties of carrot samples

Titratable acidity (% of citric acid) was determined according to AOAC method (2000) and pH by using a Crison micropH 2000 (Crison Instruments S.A., Alella, Barcelona, Spain) at 25 °C.

Total soluble solids (°Brix) were determined by measuring the refraction index with a digital refractometer (PR-32, 3412-J01, Atago Company Ltd., Tokyo, Japan) at 25 °C.

The dry matter (%) of carrot samples was determined by loss weight in an oven (P-Selecta) at 70 °C to constant weight.

- **Colour evaluation**

Colour values of carrots cortical tissue (external side) and vascular cylinder (internal side) were directly measured with a colorimeter (Minolta Chroma Meter Model CR-400, Minolta Sensing Inc., Osaka, Japan) each day of monitoring, performing ten readings in each replica of all treatments by changing the position of the carrot in each measure. Colour changes of fresh-cut carrots were measured through total colour difference (ΔE) at first storage day and after 14 days obtained by the following equation, according to Thompson (2004):

$$\Delta E = \sqrt{[(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2]}$$

where L_0, a_0, and b_0 are the initial considered values (1st day) and L, a and b refer to data collected at the last day of monitoring (14th day), and the whiteness index (WI) determined at each day of monitoring, according to the formula reported by Piscopo et al. (2019):

$$WI = 100 - \sqrt{[(100 - L^2) + a^2 + b^2]}$$

- **Hardness evaluation**

Two trays were taken at each sampling time to perform the analysis, and five carrot pieces (diameter 28±2 mm, height 20±2 mm) from each replicate were randomly withdrawn to carry out repetitions. Hardness of cortical tissue and vascular cylinder of carrots were determined with a TA-XT2 texture analyser (Stable Micro Systems Ltd., Surrey, England), equipped with a 4-mm-diameter cylinder steel probe, which penetrated 10 mm the carrot tissue at a constant rate of 5 mm s^{-1} and automatically returns. The motion of the probe was perpendicular to the surface of carrot pieces. Hardness (N s^{-1}) was determined as area under the curve between the graph of y (force) and x (time) (Ribas-Agustí et al., 2019).

4.2.2.7. Microbiological analysis

Total aerobic bacterial count (TBC) and total yeast and mould populations TM were evaluated during storage of fresh-cut carrots. Two counts were obtained each time from each of two replicate packages. In sterile conditions, 10 g of sample were homogenized for 3 min with 90 mL of 0.1% sterile peptone water with a Stomacher Lab Blender 400 (Seward Medical, London, England). Serial dilutions of sample homogenates were poured in plate count agar (PCA; Biokar Diagnostics. Beauvais, France) at 25± 1°C for 48 h for TBC and chloramphenicol glucose agar (GCA) at 27 ± 1°C for 5 d for TM. The results were expressed as Log10 CFU g^{-1} carrot (Fan and Song, 2008).

4.2.2.8. Respiratory activity

A gas analyser (Micro-GC CP 2002, Chrompack International, Middelburg, The Netherlands), equipped with a thermal conductivity detector, was used to analyse the samples respiratory activity.

A static system was used to determine the carrots respiratory activity, as reported by Lopez-Gamez et al. (2020b), with some modifications. At each monitoring time considered, 50 g of carrots were individually placed in hermetic containers of 250 mL and gas sample (1.7 mL) was withdrawn from the headspace through a rubber septum with a syringe. Respiration as carbon dioxide production ($RRCO_2$) and as oxygen consumption (RRO_2) was expressed as mg Kg^{-1} h^{-1}, according to Tappi et al. (2014).

4.2.2.9. Extraction and determination of total phenolic compounds

Phenolic compounds were extracted following the methodology proposed by Formica-Oliveira et al. (2017), with slight modifications. 5 g of carrot were homogenised with 20 mL of methanol with an Ultra-Turrax T25 (IKA® WERKE, Germany) for 2 minutes. Homogenates were centrifugated at 13,500g at 4°C for 20 minutes (Centrifuge AVANTITM J-25, Beckman Instruments Inc., Fullerton, CA, USA). The supernatants were collected and then filtered through a Whatman no. 1 filter.

The total phenolic content (TPC) was determined according to the Folin–Ciocalteu procedure adapted to 96-wells microplates (Lopez-Gamez et al., 2020a). An aliquot of 30 µL of methanolic extract was placed into a microplate; then, 150 µL of 10% (v/v) Folin-Ciocalteu's reagent and 120 µL of Na_2CO_3 7.5% (w/v) were added. After an incubation period of 90 min at room temperature in darkness, the absorbance was measured at 765 nm using a microplate reader (Thermo Scientific Multiskan GO, Vantaa, Finland). The results were expressed as mg of gallic acid equivalents per 100 g of fresh weight (mg GAE 100 g^{-1} f.w.) relative to those of untreated carrots. Phenolic content was extracted twice per replica and spectrophotometrically determined four times.

4.2.2.10. Total carotenoids content

Total carotenoids content (TCC) was determined spectrophotometrically (CECIL CE 2021; Cecil Instruments Ltd., Cambridge, UK) following the methodology proposed by González-Casado et al. (2018), with slight modifications. A carrot sample (2 g) was homogenised with 25 mL of acetone:ethanol (1:1, v/v) with an Ultra-Turrax T25 (IKA® WERKE, Germany). Sample was extracted in the dark, filtered through Whatman No. 4 filter paper, and washed with the acetone:ethanol solution until the residue was colourless. Samples were adjusted to 100 mL, and the absorbance was read at 470 nm versus a blank of acetone:ethanol. TCC was calculated using the following equation:

$$Total\ Carotenoids\ Content = \frac{A_{470} * \mathrm{V} * 10^4}{A_{1cm}^{1\%} * G}$$

where A_{470} is the absorbance at 470 nm, V is the total volume of extract (mL), $A_{1cm}^{1\%}$ is the extinction coefficient of a mixture of carotenoids established in 2500 by Gross (1991) and G is the sample weight (g). Total carotenoids were expressed as mg per 100 g of fresh weight (mg TCC 100 g^{-1} f.w.) relative to those of untreated carrots.

4.2.2.11. Antioxidant activity

The antioxidant capacity of fresh-cut carrots was studied through the determination of free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, according to the procedure described by Ribas-Agustí et al. (2019), with some modifications, and by ABTS (2,2'-azino-bis acid 3- ethylbenzothiazolin-6-sulfonic acid) assay (Re at al., 1999), using a 96-wells microplates reader (Thermo Scientific Multiskan GO, Vantaa, Finland).

To perform the DPPH assay, an aliquot of 20 µL of methanolic extract (Section 4.2.2.9) was placed into a microplate and 280 µL of 6 x 10^{-5} M of methanol solution of DPPH were added. The homogenate was kept in darkness for 30 minutes under continuous stirring and the absorption of the samples was measured at 515 nm against a blank of methanol without DPPH. The results were expressed as µM Trolox equivalents per 100 g of fresh weight (µM TE 100 g^{-1} f.w.) relative to those of untreated carrots, comparing with a Trolox calibration curve (from 6 to 30 µM).

For the ABTS assay, an aliquot of 40 µL of methanolic extract (Section 4.2.2.9) was placed into a microplate and 260 µL of ABTS ethanol solution were added. The homogenate was kept in darkness for 6 minutes under continuous stirring and the absorption of the samples was measured at 734 nm against a blank of ethanol without ABTS. The results were expressed as µM Trolox equivalents per 100 g of fresh weight (µM TE 100 g^{-1} f.w.) relative to those of untreated carrots, comparing with a Trolox calibration curve (from 30 to 120 µM).

4.2.2.12. Statistical analysis

All the experimental results were expressed as mean value (n=4) ± standard deviation (mean ± SD). Significance of the results and statistical differences were analysed using SPSS Software (Version 15.0, SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) and a series of multiple comparisons, with Tukey's post hoc test, were performed to determine individual significant differences ($p<0.05$). The Pearson's correlation test was employed for the determination of correlation coefficients (r) among the extracted polyphenolic compounds and antioxidant assays.

4.2.3. RESULTS AND DISCUSSION

4.2.3.1. Characterisation of lemon by-products extract (AE)

Results of AE characterization are reported in Table 30. TPC_{AE} determination denoted mean values of 8.98±0.02 mg GAE g^{-1} d.w. and the antioxidant activity was of 17.65±2.28 µmol TE g^{-1} d.w. and of 9.30±0.04 µmol TE g^{-1} d.w., by ABTS and DPPH respectively.

Table 30: Physico-chemical characterisation of AE.

pH	3.64 ± 0.01
Colour:	L*: 31.98 ± 0.19
	a*: 1.16 ± 0.04
	b*: 1.72 ± 0.12
TPC_{AE} (mg GAE g^{-1} d.w.)	8.98 ± 0.02
DPPH (µmol TE g^{-1} d.w.)	9.30 ± 0.04
ABTS (µmol TE g^{-1} d.w.)	17.65 ± 2.28

Data are presented as means ± SD (n=3).
GAE: Gallic acid equivalent; TPC_{AE}: Total phenolic compounds; DPPH and ABTS: Total antioxidant activity assays.

4.2.3.2. Physico-chemical characterisation of carrot samples

From the results shown in the Table 31, the main differences among the tested carrot samples were found in acidity and °Brix determination. In terms of % of citric acid, all samples showed significant differences (p<0.01) among each other and over time, up to the 14th day of monitoring. Only PBC+AE showed a greater stability of acidity values over time, showing no significant differences. A similar trend was found in relation to total soluble solids (°Brix), where all samples showed significant differences among them, with the PBC+AE characterized by the highest values and the samples without the extract (W and PBC) with by the lowest ones. Furthermore, the latter did not show significant changes (p>0.05) in values over time, unlike the samples with the extract.

Table 31: Acidity, pH, soluble solids and dry matter values of carrot samples.

	Storage time (days)					
	1	**3**	**7**	**10**	**14**	
Acidity (% citric acid)						**SIGN.**
W	0.10 ± 0.00^{abA}	0.07 ± 0.01^{C}	0.07 ± 0.01^{bBC}	0.08 ± 0.01^{bB}	0.10 ± 0.00^{aA}	******
W+AE	0.10 ± 0.01^{abA}	0.07 ± 0.00^{BC}	0.11 ± 0.00^{aA}	0.08 ± 0.00^{bAB}	0.05 ± 0.02^{bC}	******
PBC	0.11 ± 0.00^{aA}	0.08 ± 0.01^{B}	0.08 ± 0.00^{bB}	0.12 ± 0.03^{aA}	0.07 ± 0.00^{abB}	******
PBC+AE	0.09 ± 0.01^{b}	0.07 ± 0.01	0.09 ± 0.01^{ab}	0.07 ± 0.01^{b}	0.09 ± 0.02^{a}	**NS**
Sign.	*****	**ns**	******	******	******	
pH						**SIGN.**
W	6.34 ± 0.04^{BC}	6.32 ± 0.03^{C}	6.43 ± 0.01^{AB}	6.6 ± 0.03^{aA}	6.49 ± 0.10^{aB}	******
W+AE	6.27 ± 0.09	6.46 ± 0.10	6.54 ± 0.08	6.51 ± 0.25^{a}	6.43 ± 0.00^{a}	**NS**
PBC	6.31 ± 0.05^{A}	6.38 ± 0.01^{A}	6.40 ± 0.06^{A}	6.04 ± 0.26^{bB}	6.21 ± 0.00^{bAB}	******
PBC+AE	6.31 ± 0.01^{C}	6.29 ± 0.16^{C}	6.52 ± 0.13^{B}	6.73 ± 0.06^{aA}	6.24 ± 0.06^{bC}	******
Sign.	**ns**	**ns**	**ns**	******	******	
Soluble solids (°Brix)						**SIGN.**
W	3.6 ± 0.14^{d}	3.25 ± 0.07^{c}	3.60 ± 0.57^{c}	3.70 ± 0.28^{c}	3.90 ± 0.99^{b}	**NS**
W+AE	5.65 ± 0.21^{bA}	5.20 ± 0.42^{bAB}	5.00 ± 0.42^{bB}	5.65 ± 0.07^{bA}	5.60 ± 0.28^{aA}	*****
PBC	5.35 ± 0.07^{c}	5.40 ± 0.14^{b}	4.90 ± 0.42^{b}	5.30 ± 0.28^{b}	5.35 ± 1.34^{ab}	**NS**
PBC+AE	6.4 ± 0.14^{aA}	6.05 ± 0.07^{aC}	6.25 ± 0.07^{aABC}	6.35 ± 0.21^{aAB}	6.10 ± 0.14^{aBC}	******
Sign.	******	******	******	******	******	
Dry matter (%)						**SIGN.**
W	9.24 ± 0.18^{b}	9.94 ± 0.94^{a}	9.99 ± 1.15	9.60 ± 0.45^{ab}	9.50 ± 0.07	**NS**
W+AE	10.47 ± 0.78^{a}	9.71 ± 0.21^{a}	10.48 ± 1.76	10.11 ± 0.32^{a}	9.19 ± 0.41	**NS**
PBC	10.96 ± 0.50^{aA}	9.73 ± 0.10^{aB}	9.18 ± 0.18^{B}	9.41 ± 0.03^{bB}	9.31 ± 0.84^{B}	******
PBC+AE	9.20 ± 0.36^{b}	8.68 ± 0.28^{b}	8.73 ± 0.68	9.55 ± 0.50^{ab}	9.22 ± 1.53	**NS**
Sign.	******	******	**ns**	*****	**ns**	

The data are presented as means ± SD (n=4). Means within a row or column with different letters are significantly different by Tukey's post hoc test. Abbreviation: Sign., significance; ns, not significant. **Significance at $p<0.01$; *Significance at $p<0.05$.

The colour analysis was carried out on both outside (cortical tissue) and inside (vascular cylinder) of the carrots, determining the colour variation (ΔE, Table 32) from the first to the last day of monitoring and the whiteness index (WI, Figure 13), which is considered one of the main defects of carrots.

Table 32: Colour variation of carrot samples.

	ΔE	
	outside	**inside**
W	2.08 ± 0.72	4.79 ± 1.70
W+AE	1.26 ± 0.63	5.38 ± 1.96
PBC	1.47 ± 0.81	3.67 ± 1.98
PBC+AE	1.57 ± 0.86	4.40 ± 0.66
Sign.	**ns**	**ns**

Abbreviation: Sign., significance; ns, not significant.

Looking at the ΔE values, all samples showed little colour variation in the outside (ΔE< 3), but a clear variation in the inside of the carrot with ΔE >3 was found. As reported in literature, appreciable differences start to be visible from ΔE >3 values (Fai et al., 2016), indicating a very distinct colour change. According to Shigematsu et al. (2019), comparing the carrot treatments with and without coating, the addition of the antioxidant extract to the coating formulation (PBC+AE) or in the dipping solution (W+AE) did not modify the product's colour characteristics. The colour and appearance of minimally processed carrots are critical attributes because they quickly lose their characteristic bright orange colour due to dehydration, leading to a white blush on the surface, reducing their acceptability (Mastromatteo et al., 2012). As reported by Bourtoom (2008), the polysaccharide-based edible coatings can slow down the loss of water from fresh fruits and vegetables thereby prolonging their shelf life. Indeed, looking at the WI data (Figure 16), the coated samples (PBC and PBC+AE) showed significant lower values both outside and inside than the other samples.

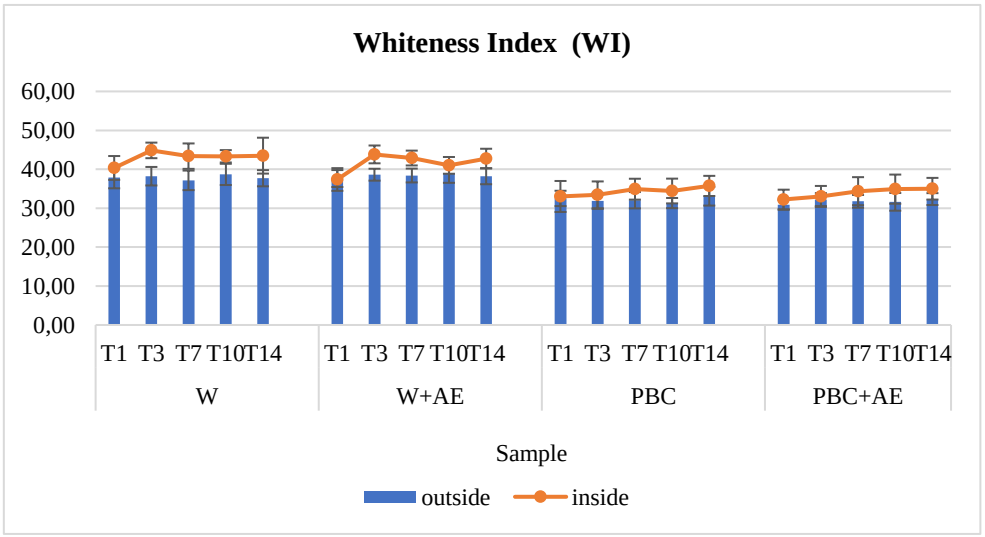


Figure 16: Changes in Whiteness Index values of carrot samples. The data are presented as means±SD (n=3).

Surface white discoloration on peeled carrots during storage could be due to both physical and physiological feedbacks to wounding, which affects their quality and shelf life. The physical reaction consists in a reversible surface dehydration of the outer surface (Cisneros-Zevallos et al., 1995); the physiological one represents the developing of lignin (Bolin and Huxsoll, 1991; Howard and Griffin, 1993), an irreversible constituent of colour change. Carrots' sensitivity to surface white discoloration development is affected by temperature (Buick and Damoglou, 1987), relative humidity (Avena et al., 1994), surface moisture, degree of abrasion and cutting tool type (Tatsumi et al., 1991). White discoloration because of surface dehydration could be prevented through relative humidity increase and by ensuring excess of moisture on carrot surface before storage (Cisneros-Zevallos et al., 1995). A hydrophilic substance, such as pectin, could promote hydration of the carrot surface and reduce the formation of white discolouration. The efficacy of these materials might be associated to the absorption capacities of the hydrophilic materials rather than their water barrier properties (Cisneros-Zevallos et al., 1997). Confirming what was previously discussed for ΔE, none of the sample showed a significant change ($p>0.05$) in WI values over time.

Regarding texture analysis (Table 33), carrot samples with and without coating showed no relevant differences in texture until the 14th day of storage, when PBC+AE sample showed the highest values, both on outside and inside. Firmness loss may be related with the deterioration of compounds responsible for vegetable structural rigidity, primarily insoluble pectin and protopectin. During maturation, pectinesterase and polygalacturonase activities improve, producing the solubilisation of pectin substances (Ferrari et al., 2013). The enriched edible coating applied to the carrots probably acted on these enzymes, decreasing their performances, and preserved the firmness of the sample, which suggested that the coating was very effective in maintaining the texture of the product (Shigematsu et al., 2019).

Table 33: Firmness changes of carrot samples.

	Outside (N s^{-1})					
	Storage time (days)					
	1	**3**	**7**	**10**	**14**	**SIGN.**
W	57.70 ± 3.73^{aA}	51.05 ± 4.86^{B}	49.39 ± 4.15^{B}	50.46 ± 5.47^{B}	48.87 ± 4.03^{abB}	**
W+AE	54.34 ± 6.49^{abA}	51.07 ± 5.93^{AB}	51.24 ± 8.09^{AB}	48.06 ± 5.62^{AB}	44.52 ± 6.79^{bcB}	*
PBC	51.27 ± 5.27^{bAB}	53.37 ± 2.52^{A}	47.99 ± 4.16^{B}	52.89 ± 4.18^{AB}	41.56 ± 4.18^{cC}	**
PBC+AE	50.54 ± 4.73^{b}	48.47 ± 4.10	48.69 ± 3.96	47.73 ± 4.11	52.70 ± 4.25^{a}	ns
Sign.	*	ns	ns	ns	**	
	Inside (N s^{-1})					
	Storage time (days)					
	1	**3**	**7**	**10**	**14**	**SIGN.**
W	45.34 ± 4.19	43.85 ± 3.69	43.18 ± 1.91^{a}	$43,32 \pm 3.80$	42.25 ± 3.66^{b}	ns
W+AE	43.55 ± 3.27	42.28 ± 3.47	39.25 ± 4.31^{b}	40.15 ± 4.65	43.10 ± 5.07^{ab}	ns
PBC	$44,53 \pm 3.74$	46.00 ± 2.99	43.07 ± 3.67^{ab}	45.70 ± 4.78	41.22 ± 4.80^{b}	ns
PBC+AE	44.67 ± 2.47^{AB}	45.84 ± 4.56^{AB}	45.61 ± 2.41^{aAB}	42.41 ± 4.95^{B}	48.22 ± 4.01^{aA}	*
Sign.	ns	ns	**	ns	**	

The data are presented as means ± SD (n=10). Means within a row or column with different letters are significantly different by Tukey's post hoc test. Abbreviation: Sign., significance; ns, not significant. **Significance at $p<0.01$; *Significance at $p<0.05$.

In addition, the observed decrease in hardness of the fresh-cut carrots may be linked not only to pectinolytic enzymes action, but it may also be provoked by the increased activity of glycolytic enzymes, which contribute to the hydrolysis of hemicellulose and other cell wall components and that might be activated as a defence mechanism in case of microbiological attack and/or injury, such as throughout processing (Shigematsu et al., 2019).

4.2.3.3. Microbiological analysis

As reported in Figure 17, PBC+AE showed the lowest values of TBC until the 10th day of storage, after which a similar content in aerobic bacteria was detected for all the samples. In addition, the highest values were found for W and PBC, the sample without the antioxidant extract in their formulation. Similar results were reported by Amanatidou et al. (2000). The inclusion of natural antimicrobials in pectin-based coating allowed to improve the shelf-life of fresh-cut carrots. This could be associated to the preservation of the polyphenols compounds and by slowing down the release of bioactive ingredients. Based on these microbiological results, it is obvious that the addition of the antioxidant extract to the coating formulation permitted to contain the microbial load. The reduction in microbial population in carrots might be correlated to a more regulated release, a higher protection and a high stability maintenance of bioactive compounds over time, mainly against

oxidation (Ben-Fadhel et al., 2020). Yeast and moulds were not detected by count in carrot samples during the entire storage period.

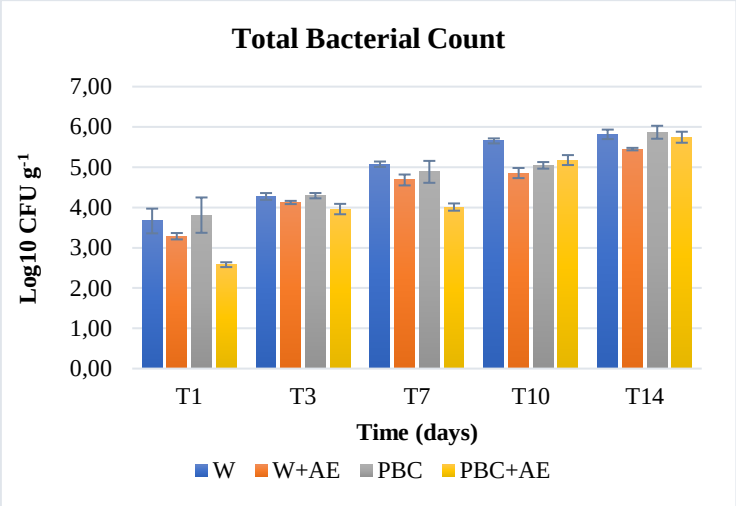


Figure 17: Total bacterial count (TBC) on carrot samples.
The data are presented as means ± SD (n=4).

4.2.3.4. Respiratory activity

It was detected in all carrots and expressed as oxygen consumption (RRO_2) and carbon dioxide production ($RRCO_2$) by the samples, as reported in Figure 18 and Figure 19, respectively.

At the beginning of the storage significance differences were found both in terms of oxygen consumption and carbon dioxide production: in particular, PBC and PBC+AE showed the lowest values of RRO_2, thanks to the presence of the polysaccharide-based coating which showed adequate gas barrier properties, although they showed the highest production of carbon dioxide. In all carrot samples tested, an increasing trend in RRO_2 was found until the 14th day of storage, when the highest values was registered for W sample. On the other hand, regarding $RRCO_2$, the lowest value was found for PBC+AE on the 14th day of storage, which confirms the role of pectin coating in regulating the gas exchanges.

The edible coating could behave as a barrier to gas transport and prolong commercial shelf-life of fresh products by controlling their internal atmosphere (Vargas et al., 2009). At the same time, the increase in the respiration process observed in coated samples could be ascribed to modifications in the barrier properties of coating matrix, which may be stimulated by the high values of carrots water activity stored in chilling conditions (Leceta et al., 2015). It could also be linked to vegetable tissue stress induced by minimal processing procedures, like trimming, peeling and cutting, or the application of coating matrix which generate stressed conditions around the product (Mastromatteo et al., 2012). Contrary to what reported by Ferrari et al. (2013), the control sample W did not show the highest CO_2 production at the beginning of storage time, unlike PBC. On the other hand, the use

of pectin coating with the antioxidant extract promoted a significant decrease of respiration rate in fresh-cut carrots compared to the control W, indicating that the interaction of calcium ions with pectin contributed to hinder gas exchange.

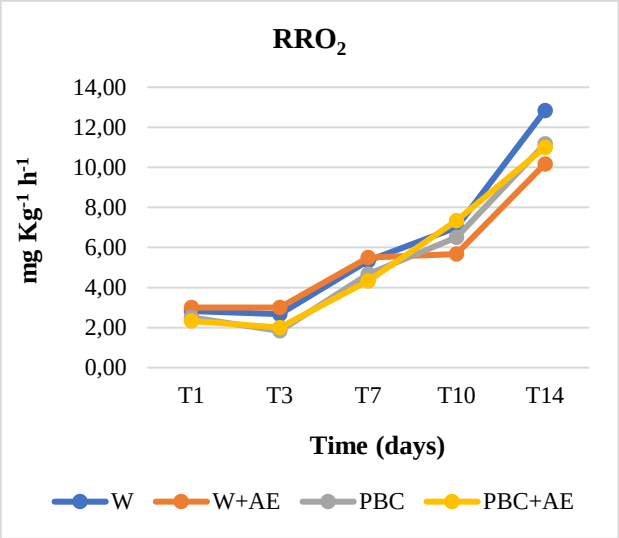


Figure 18: Oxygen consumption values of uncoated and coated carrots.

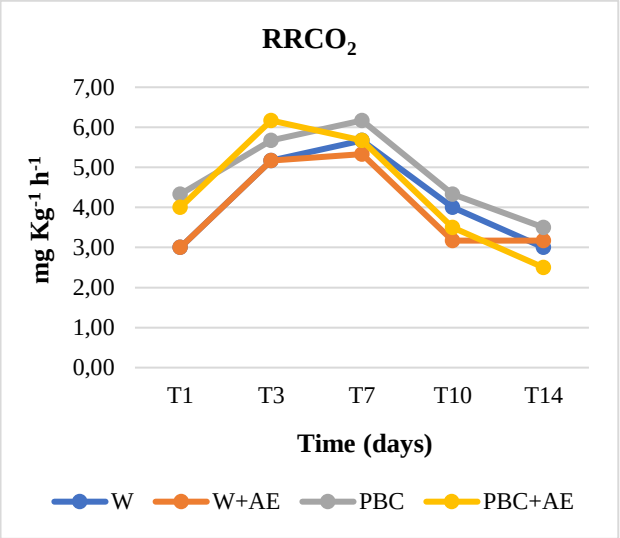


Figure 19: Carbon dioxide production values of uncoated and coated carrots.

4.2.3.5. Characterisation of bioactive compounds and antioxidant activity

Total carotenoid content (Table 34) reduced in all the treated samples during storage, with W and PBC characterized by an increase in values after the 10th day of storage probably due to a stress condition occurred at the end of monitoring, which was not detected in the samples characterised by the presence of the antioxidant extract, W+AE and PBC+AE. Indeed, carotenoid production could be encouraged by enzymatically-mediated softening mechanisms initiated by reactive oxygen species (ROS) caused by exposure to oxidative stress (González-Casado et al., 2018). A similar trend was found in TPC and antioxidant activity values (Figure 20).

Table 34: Total carotenoids content (TCC) of carrot samples.

TCC (mg TCC 100 g^{-1})	Storage time (days)					
	1	**3**	**7**	**10**	**14**	**SIGN.**
W	11.43 ± 0.58^{cB}	11.12 ± 0.04^{bB}	9.49 ± 0.08^{cC}	8.53 ± 0.64^{cD}	13.26 ± 0.26^{aA}	**
W+AE	16.83 ± 0.67^{aA}	14.03 ± 0.16^{aB}	13.78 ± 0.07^{aB}	13.29 ± 0.75^{aB}	10.33 ± 0.07^{bC}	**
PBC	11.47 ± 0.67^{cB}	11.44 ± 0.56^{bB}	11.11 ± 0.83^{bB}	8.83 ± 0.22^{cC}	12.68 ± 0.03^{aA}	**
PBC+AE	13.91 ± 0.44^{bA}	13.97 ± 0.81^{aA}	13.55 ± 0.51^{aA}	12.07 ± 0.04^{bB}	11.01 ± 0.90^{bB}	**
Sign.	**	**	**	**	**	

The data are presented as means ± SD (n=4). Means within a row or column with different letters 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.

The inclusion of the antioxidant extract into pectin coating formulation assured a protection of the bioactive compounds and their higher stability during storage. TPC results clearly proved that pectin matrix led to TPC stabilization over time, indicating that bioactive compounds were protected against oxidation phenomena (Ben-Fadhel et al., 2020). Among coated and uncoated samples, PBC+AE and W+AE showed a constant total polyphenols content over time until the 10th day of storage, resulting more stable than W and PBC, which showed a decrease in values until the 7th day of storage, a trend followed soon after by a significant increase in TPC as well as antioxidant activity values, as observed also by Ranjitha et al., 2017. Generally, phenolic compounds are intensively produced if there are stressful environmental conditions to defend vegetable, by producing both antioxidant and antimicrobial effects (Ben-Fadhel et al., 2020). Increase in TPC is one of the most widely studied event in response to wounding in several fresh products, which has been further confirmed in the case of carrots. In addition, increase in TPC might also be related to the wound induced stimulation of the plant-enzyme phenylalanine ammonia lyase (PAL), which transforms L-phenilalanine into trans-cinnamic acid, that acts as a precursor for different phenylpropanoids, such as lignin (Ranjitha et al., 2017). In this regard, several authors assign whitening (Figure 16) to lignin synthesis in reply to cellular injuries, where the lignin performs as a new barrier (Vargas et al., 2009; Fonseca et al, 2002; Izumi et al., 1994).

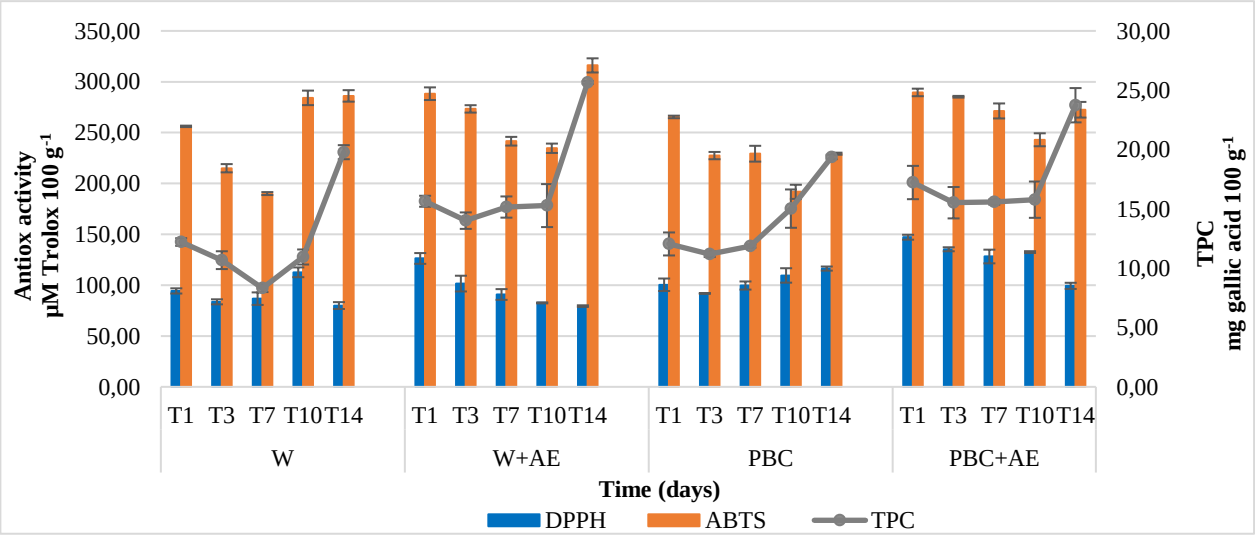


Figure 20: Total polyphenol content (TPC) and expression of antioxidant activity values (ABTS and DPPH) of carrot samples. The data are presented as means±SD (n=4).

4.2.4. CONCLUSIONS

The results of this study suggested that shelf-life of fresh-cut carrots can be extended by treating minimally processed carrots with pectin-based coating with the addition of an antioxidant extract obtained from lemon by-products. PBC+AE showed higher levels of total carotenoids and polyphenols contents and antioxidant activity, colour maintenance due to the inhibition of white blush formation on surfaces, preservation of texture over time and reduced microbial load during storage at 4°C, compared to the control sample. Encouraging results were also obtained with the carrot sample dipped in the solution enriched with the antioxidant extract (W+E).

Therefore, this study revealed the importance of food industry by-products, as a natural source of compounds with antioxidant and antimicrobial activities, in the formulation of new strategies to increase the shelf life of minimally processed vegetables and, therefore, at the industrial and marketing level of such products.

4.3. ANTI-BROWNING EFFECT OF EDIBLE COATING ADDED WITH ANTIOXIDANT EXTRACT FROM ONION SOLID WASTE IN RAW MINIMALLY PROCESSED POTATOES

4.3.1. INTRODUCTION

Potato (*Solanum tuberosum*) is the fourth most common crop grown in the world after rice, wheat, and maize (Singh & Saldana, 2011). Consumers can use minimally processed potatoes in the preparation of meals getting advantages in terms of time-saving. The fresh-cut potatoes are washed, peeled and cut into smaller pieces. However, it is well known that these products are characterized by a limited shelf-life of 5 to 7 days at 4 to 5 °C, packed in vacuum or modified atmosphere, due to microbiological, sensory and nutritional deteriorations (Cantos et al., 2002; Ma et al., 2010). Indeed, peeling and cutting operations provoke potato tissue injury, which stimulate many physiological responses, such as browning development, texture degradation, nutritional loss, and microbial growth (Bilbao-Sainz et al., 2020). When fresh cut potatoes are not treated with inhibitors, changes in colour can occur in a few minutes, due to potato susceptibility to enzymatic browning caused by the enzymatic oxidation of phenolic compounds (Cacace et al., 2002; Sapers & Choi, 1995; Wang et al., 2015b; Cerit at el., 2020; Martinez & Whitaker, 1995). Peroxidase (POD) and polyphenol oxidase (PPO) are the main oxidoreductases enzymes responsible for browning in fresh-cut fruits and vegetables. POD can oxidize phenols to quinones in the presence of hydrogen peroxide; PPO can also catalyse two reactions, which consist in the hydroxylation of monophenols to diphenols and the oxidation of diphenols to quinones, involving the synthesis of melanoidins, high molecular weight pigments responsible for dark colours in matrices (Martinez & Whitaker, 1995; Queiroz et al., 2008; Troiani et al., 2003). Generally, PPO is in the plastids and phenolic substances are in vacuoles. When tissue is damaged, the enzyme and phenolic substrates come into contact, starting the browning development and affecting the acceptability of the final products by consumers (Yingsanga et al., 2008). Enzymatic browning in food represents an important issue for the food industry, which could be prevented by the addition of sulphites, ascorbic acid and its analogues, or cysteine (Ding et al., 2002; Jang et al., 2002; Negishi & Ozawa, 2000; Kim et al., 2005). In this regard, there is an increasing demand by consumers for substituting synthetic compounds with natural substances as food ingredients (Jang et al., 2002).

In this study, onion solid waste extract was considered as a natural inhibitor of potato browning and as a functional ingredient to improve total polyphenols content in fresh cut potatoes, evaluating its influence on the expression of peroxidase (POD), polyphenol oxidase (PPO) and pectin

methylesterase (PME) activities in fresh cut potatoes. The choice of onion (*Allium cepa L.*) was driven by the fact that its processing and consumption generate about 450.000 tonnes of onion waste per year (Schieber et al., 2001). Since this kind of waste is not appropriate as animal feed, its utilization for alternative purposes would be desirable to reduce its polluting load and recover added-value compounds (Osman et al., 2008).

4.3.2. MATERIALS AND METHODS

4.3.2.1. Chemical and reagents

Sodium chloride was purchased from POCH S.A. (Sowińskiego, Poland), sodium hydroxide was purchased from Acros Organics (New Jersey, USA), citrus pectin, bromothymol blue, catechol and D-galacturonic acid were acquired from Sigma Aldrich (St. Louis, MO, USA), p-phenylendiamine was purchased from Merck (Hohenbrunn, Germany), polyvinylpyrrolidone was acquired from Fischer Scientific (Geel, Belgium), hydrochloric acid and Sodium dihydrogen phosphate were purchased from Panreac Química S.A. (Barcelona, Spain), hydrogen peroxide was acquired from Chemlab (Zedelgem, Belgium). Folin-Ciocalteu reagent was acquired from Scharlau S.L. (Barcelona, Spain), sodium carbonate was acquired from Fisher Scientific Scharlau Chemie (Loughborough, UK), gallic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). Food grade deacylated gellan gum (Kelcogel®, CPKelco, Chicago, IL, USA), glycerol (Merck, Whitehouse Station, NJ, USA), calcium chloride (Sigma–Aldrich Chemic, Steinhein, Germany) were used. Ascorbic acid, dipotassium hydrogen phosphate and disodium hydrogen phosphate were purchased from Scharlab S.L. (Sentmenat, Spain). Potassium dihydrogen phosphate was purchased from Prolabo (Fontenay S/Bois). Sodium hypochlorite was acquired from Productes Sant Manteu (Barcelona, Spain).

4.3.2.2. Sampling

Red onions (*Allium cepa L.*) were supplied by a local producer in the province of Reggio Calabria (Italy). The bulbs were transported to the laboratory of "Mediterranea" University of Reggio Calabria and the outer dry and semi-dry layers were separated, as well as the apical and basal trimmings, which were considered as onion solid waste (OSW) and used in the extraction process. The OSW were initially dried at 50°C up to a moisture content of 17%, then pulverised in a domestic blender and stored in polyethylene bags under vacuum to avoid rehydration until subsequent extraction procedure of the bioactive compounds.

Potato (*Solanum tuberosum cv. Monalisa*) were obtained in a local supermarket in Lleida (Spain). The potatoes were transported to the laboratory of the University of Lleida and immediately processed for this study. Food grade deacylated gellan gum (Kelcogel®, CPKelco, Chicago, IL, USA) was the

carbohydrate biopolymer used to prepare the coating formulations. Glycerol (Merck, Whitehouse Station, NJ, USA) was added as plasticizer. Calcium chloride (Sigma–Aldrich Chemic, Steinhein, Germany) was used to induce a crosslinking reaction.

4.3.2.3. Onion solid waste extract (OSW_E)

The extraction was performed according to Mi Jang et al. (2013), with some modifications.
Briefly, 50 mL of ethanol:water (1:1, v/v) were added to 2.5 g of dried and ground onion solid waste and placed on a heating magnetic stirrer for 60 minutes at 40°C, monitored by a digital thermometer. Subsequently, sample was centrifuged (NF 1200R, Nüve, Ankara, Turkey) at 5000 rpm for 5 min at 4°C, filtered through a Büchner apparatus with 0.45 µm filter paper and the resulting extract was made up to 50 mL with the extraction solvent. The extract was then filtered with 0.45 µm nylon filters and stored at -20°C until subsequent analyses.

4.3.2.4. Characterization of onion solid waste extract (OSW_E)

- **Total polyphenols content (TPC_E)**

TPC_E was determined according to the method reported by González-Molina et al. (2009), with appropriate modifications. 0.2 mL of extract were placed inside a 25 mL flask and mixed with 5 mL of deionized water and 1 mL of Folin-Ciocalteu reagent. After 8 minutes, 10 mL of saturated sodium carbonate solution (Na_2CO_3) at 20% (w/v) was added and made up to volume with deionized water. At the same time, the solution used as a blank was prepared. The mixtures were incubated for two hours at room temperature and in the dark.
The absorbance of the samples was measured at 765 nm against a blank using a double-beam ultraviolet-visible spectrophotometer (Perkin-Elmer UV- Vis λ2, Waltham, Massachusetts, U.S.) and comparing with a gallic acid calibration curve (concentration between 1 and 10 mg L^{-1}). The results were expressed as mg of gallic acid g^{-1} (mg GAE g^{-1} d.w.) of onion solid waste dry weight.

- **Antioxidant activity determination**

DPPH assay

The DPPH assay was performed as reported in Section 2.2.3.3. The results were expressed as µM Trolox equivalents g^{-1} of onion solid waste dry weight (µM TE g^{-1} d.w.), comparing with a Trolox calibration curve (from 6 to 21 µM).

ABTS assay

The antioxidant activity of the extracts was determined as described in Section 2.2.3.3. The quenching of initial absorbance was plotted against the Trolox concentration (from 3 to 18 μM) and the results were expressed as μM Trolox equivalents g^{-1} of onion solid waste dry weight (μM TE g^{-1} d.w.).

4.3.2.5. Preparation of the dipping and coating solutions

The dipping solutions consisted of four different formulations: water (W), aqueous solution with ascorbic acid (1%, w/v) used as antibrowning agent (W+A), aqueous solution with the hydroalcoholic antioxidant extract (1%, v/v) previously obtained (W+E) and aqueous solution with ascorbic acid (1%, w/v) and the hydroalcoholic extract (1%, v/v) (W+A+E).

The concentrations of coating ingredients used in the formulations were set up according to (Oms-Oliu et al., 2008). Coatings were prepared by dissolving gellan gum powder (0.5%, w/v) in distilled water and heating at 70 °C while stirring until the solution became clear. Glycerol was added as a plasticizer at 0.6% (w/v) in gellan gum solutions. For the crosslinking of carbohydrate polymer, calcium chloride solution (2%, w/v) was prepared (GBC). The ascorbic acid was added (1%, w/v) to the calcium chloride solution for the sample coated with the gellan based coating enriched with the antibrowning agent (GBC+A); the onion solid waste extract (1%, v/v) were added to the calcium chloride solution for the sample coated with the gellan based coating enriched with the antioxidant extract (GBC+E); both ascorbic acid (1%, w/v) and onion solid waste extract (1%, v/v) were added to the calcium chloride solution for the sample coated with the gellan based coating enriched with both substances (GBC+A+E). All samples' formulations are reported in Table 35.

Table 35: Potato samples denomination.

Dipping	**W**	Water
	W+A	Water and ascorbic acid (1%, w/v).
	W+E	Water and antioxidant extract from onion solid waste (1%, v/v).
	W+A+E	Water, ascorbic acid (1%, w/v) and antioxidant extract from onion solid waste (1%, v/v).
Coating	**GBC**	Gellan gum-based coating
	GBC+A	Gellan gum-based coating and ascorbic acid (1%, w/v).
	GBC+E	Gellan gum-based coating and antioxidant extract from onion solid waste (1%, v/v).
	GBC+A+E	Gellan gum-based coating, ascorbic acid (1%, w/v) and antioxidant extract from onion solid waste (1%, v/v).

4.3.2.6. Potatoes coating and packaging

Whole potatoes were sanitized in a 200 µL L^{-1} NaClO solution for 2 min, rinsed with tap water, and dried prior to peeling and cutting operations. Potatoes were peeled and the remaining vegetable was cut into sticks (height 30±2 mm, base 13±2 mm). The uncoated potatoes pieces were dipped in W, W+A, W+E and W+A+E solutions for 2 minutes.

The other potato pieces were dipped into the polysaccharide solutions for 2 min. The excess of coating material was allowed to drip off for 1 min before submerging the samples again for 2 min in the different calcium chloride solutions (GBC, GBC+A, GBC+E and GBC+A+E). Then, 80 g of each potatoes samples pieces were placed in polypropylene trays and thermosealed using a packaging machine ILPRA Food Pack Basic V/6 (ILPRA Systems, CP, Vigevono, Italy). The O_2 and CO_2 permeance of the sealing film were 5.2419×10^{-13} mol O_2 m^{-2} s^{-1} Pa^{-1} and 2.3825×10^{-12} mol CO_2 m^{-2} s^{-1} Pa^{-1} at 23 ◦C and 0% RH, respectively (ILPRA Systems Espana, S.L. Matarò, Spain). The packages were stored at 4±1°C in darkness up to random withdrawal for analyses at the day of production (time 0) and after 2, 4, 8 and 14 days. Two trays of each sample were taken at each sampling time to perform replicate analyses throughout 14 days of storage.

4.3.2.7. Colour evaluation

Colour determination was carried out directly on potatoes surface with a colorimeter (Minolta Chroma Meter Model CR-400, Minolta Sensing Inc., Osaka, Japan), performing ten different readings in each replica. The equipment was set up for a D65 illuminant and 10° observer angle. Ten potato sticks from each of two replicates trays were evaluated for each treatment at each sampling time. Colour changes of fresh-cut potatoes were measured through total colour difference (ΔE) at first storage day and after 14 days obtained by the following equation, according to Thompson (2004):

$$\Delta E = \sqrt{[(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2]}$$

where L_0, a_0, and b_0 are the initial considered values (1st day) and L, a and b refer to data collected at the last day of monitoring (14^{th} day);

the whiteness index (WI) determined according to the formula reported by Piscopo et al. (2019):

$$WI = 100 - \sqrt{[(100 - L^2) + a^2 + b^2]}$$

where L, a and b refer to data collected at each day of monitoring;

and the browning index (BI), according to Palamutoglu (2020):

$$BI = \frac{[100\ (x - 0.31)]}{0.172}$$

where x = $\frac{(a^*+1.75L^*)}{(5.645L+a^*-3.012b^*)}$ and L*, a* and b* refer to data collected at each day of monitoring.

4.3.2.8. Texture analysis

To measure potato cutting force at each day of monitoring, two trays were taken at each sampling time to perform the analyses, and five potatoes sticks (height 30±2 mm, base 13±2 mm^2) from each replicate were randomly withdrawn to carry out repetitions. Cutting of potatoes tissue was determined with a TA-XT2 texture analyser (Stable Micro Systems Ltd., Surrey, England), equipped with a knife blade, which penetrated 10 mm the potatoes tissue at a constant rate of 5 mm s^{-1} and automatically returns. The motion of the probe was perpendicular to the surface of potatoes pieces. Cutting (N∗s) was determined as area under the curve between the graph of y (force) and x (time) (Ribas-Agustí et al., 2019).

4.3.2.9. Determination of enzyme activities

- **Pectin methylesterase (PME) activity**

The PME extraction and activity determination were performed as described by López-Gámez et al. (2020b), adapted to 96-well microplates. All the solutions must be previously adjusted to pH 7.5 (Crison micropH 2000, Crison Instruments S.A., Alella, Barcelona, Spain). The reaction mixture consisted of 30 µL of PME extract, 70 µL of distilled water, 180 µL of citrus pectin 0.5 % (w/v) solution and 30 µL of bromothymol blue 0.01 % (w/v). PME activity was determined by monitoring the colour change during 3 min at 620 nm in a microplate spectrophotometer (Thermo Scientific Multiskan GO, Vantaa, Finland). Galacturonic acid was used to make a standard curve and calculate PME activity, which was expressed as micromoles of galacturonic acid produced per second and per gram of sample (µmol galact. ac. s^{-1} g^{-1} sample).

- **Polyphenol oxidase (PPO) activity**

PPO extraction was based on the procedure described by López-Gámez et al. (2020b). In this case, PPO was extracted from potatoes tissues (4 g) adding 15 mL of cold phosphate buffer (0.1 M; pH 6.5) and 0.4 g of polyvinylpyrrolidone. Then, samples were vortexed for 1 min and centrifuged at 20,000 × g for 15 min at 4°C (Centrifuge AVANTITM J-25, Beckman Instruments Inc., Fullerton, CA, USA). The resulting supernatant was filtered across Whatman No. 1 filter. During the whole procedure, samples were maintained in an ice-bath to prevent protein denaturation.

PPO activity was assayed spectrophotometrically measuring the catechol oxidation rate at 420 nm for 2 min (Thermo Scientific Multiskan GO, Vantaa, Finland). The reaction mixture was adapted to

96-well microplate, which contained 10 μL of enzymatic extract and 290 μL of catechol (0.05 M) prepared in extraction buffer just before the analysis. Results were expressed as Units of Activity of the enzyme that causes a change in absorbance at 420 nm per minute per gram of sample (UA min^{-1} g^{-1} sample).

- **Peroxidase (POD) activity**

POD extraction was carried out following the same procedure as previously described for PPO extraction (López-Gámez et al., 2020b). Determination was performed placing 10 μL of enzyme extract into 96-well microplate. Then, 260 μL of extraction buffer, 20 μL of p-phenylenediamine 1% (w/v) and 10 μL of H_2O_2 1.5% (v/v) were added. Spectrophotometric readings at 485 nm were registered every 10 seconds during 10 minutes of incubation in a spectrophotometer (Thermo Scientific Multiskan GO, Vantaa, Finland). POD activity was expressed as Units of Activity of the enzyme that causes a change in absorbance at 485 nm per minute per gram of sample (UA min^{-1} g^{-1} sample).

4.3.2.10. Extraction and determination of total polyphenols content in potato samples

Phenolic compounds were extracted following the methodology proposed by Abalos et al. (2020), with slight modifications. 8 g of potatoes were homogenised with 25 mL of methanol with an Ultra-Turrax T25 (IKA® WERKE, Germany) for 2 minutes. Homogenates were immersed in an ultrasonic bath for 15 minutes at 40°C (Ultrasons-H, J.P. Selecta s.a., Barcelona, Spain) and then were centrifuged at 11,200g for 5 minutes. The supernatants were collected and filtered through a Whatman no. 1 filter. The total polyphenols content (TPC) was determined according to the Folin–Ciocalteu procedure adapted to 96-wells microplates (Moura et al., 2021), with some modifications. An aliquot of 12.5 μL of methanolic extract was placed into a microplate; then, 200 μL of distilled water and 12.5 μL of Folin-Ciocalteu reagent were added. After 3 minutes, 50 μL of Na_2CO_3 20% (w/v) were added. After an incubation period of 60 minutes at room temperature in darkness, the absorbance was measured at 720 nm using a microplate reader (Thermo Scientific Multiskan GO, Vantaa, Finland). The results were expressed as mg of gallic acid equivalents per 100 g of fresh weight (mg GAE 100 g^{-1} f.w.) relative to those of untreated potatoes. Phenolic content was extracted twice per replica and spectrophotometrically determined four times.

4.3.2.11. Antioxidant activity

The antioxidant capacity of fresh-cut potatoes was studied through the determination of free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, according to the procedure

described by Ribas-Agustí et al. (2019), with some modifications, and by ABTS (2,2'-azino-bis acid 3- ethylbenzothiazolin-6-sulfonic acid) assay (Re at al., 1999), using a 96-wells microplates reader (Thermo Scientific Multiskan GO, Vantaa, Finland).

To perform the DPPH assay, an aliquot of 25 µL of methanolic extract (Section 4.3.2.10.) was placed into a microplate and 275 µL of 6 x 10^{-5} M of methanol solution of DPPH were added. The homogenate was kept in darkness for 30 min. under continuous stirring and the absorption of the samples was measured at 515 nm against a blank of methanol without DPPH. The results were expressed as µM Trolox equivalents 100 g^{-1} of fresh weight relative to those of untreated potatoes (µM TE 100 g^{-1}), comparing with a Trolox calibration curve (from 6 to 30 µM).

For the ABTS assay, an aliquot of 25 µL of methanolic extract (Section 4.3.2.10.) was placed into a microplate and 275 µL of ABTS ethanol solution were added. The homogenate was kept in darkness for 6 min. under continuous stirring and the absorption of the samples was measured at 734 nm against a blank of ethanol without ABTS. The results were expressed as µM Trolox equivalents 100 g^{-1} of fresh weight relative to those of untreated potatoes (µM TE 100 g^{-1}), comparing with a Trolox calibration curve (from 30 to 120 µM).

4.3.2.12. Microscope observation

To better observe changes in texture, potato samples were subjected to microscope observation (Olympus BX41TF). Changes in the cell walls and in distribution of starch granules within them were observed over time. Specifically, each potato sample was sliced (<1 mm thickness), three slices for each sample were placed in a slide and covered with a coverslip for microscopic observation.

4.3.2.13. Statistical analysis

All the experimental results were expressed as mean value (n=4) ± standard deviation (mean ± SD). Significance of the results and statistical differences were analysed using SPSS Software (Version 15.0, SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) and a series of multiple comparisons, with Tukey's post hoc test, were performed to determine individual significant differences ($p<0.05$). The Pearson's correlation test was employed for the determination of correlation coefficients (r) among the extracted polyphenolic compounds and antioxidant assays.

4.3.3. RESULTS AND DISCUSSION

4.3.3.1. Characterisation of onion solid waste extract

Results of OSW_E characterization are reported in Table 36. TPC_E determination denoted mean values of 21.55 ± 0.07 mg GAE g^{-1} d.w. and the antioxidant activity was of 33.57±1.62 µmol TE g^{-1} d.w. and of 78.28±1.00 µmol TE g^{-1} d.w., by DPPH and ABTS respectively.

Table 36: Physico-chemical characterization of OSW_E.

Colour:	L*: 31.81 ± 0.27
	a*: 1.58 ± 0.10
	b*: 0.42 ± 0.16
TPC_E (mg GAE g^{-1} d.w.)	21.55 ± 0.07
DPPH (µmol TE g^{-1} d.w.)	33.57 ± 1.62
ABTS (µmol TE g^{-1} d.w.)	78.28 ± 1.00

Data are presented as means ± SD (n=3).
GAE: Gallic acid equivalent; TPC_E: Total phenolic compounds; DPPH and ABTS: Total antioxidant activity assays.

4.3.3.2. Potato colour variations

Colour and appearance of fresh-cut potatoes are critical attributes because they rapidly develop superficial whitening, due to dehydration, and browning, provoked by enzyme activities, reducing their acceptability. In this study, all the tested potato samples showed significant overall colour variations over time with consequent development of whitening and browning (Figure 21). A significant difference (p<0.01) in terms of ΔE was found among the samples tested. W showed the significantly highest colour variation value of 8.19±1.22. The other samples, containing either ascorbic acid (A) or the antioxidant extract (E) or both (A+E), showed almost similar values of ΔE, which could suggest a colour-preserving activity over time exerted by the antioxidant extract, comparable to that of ascorbic acid. The sample with the significantly lowest value of 1.48±0.04 was the one dipped in both ascorbic acid and antioxidant extract (WAE), probably due to the synergistic action of these two compounds.

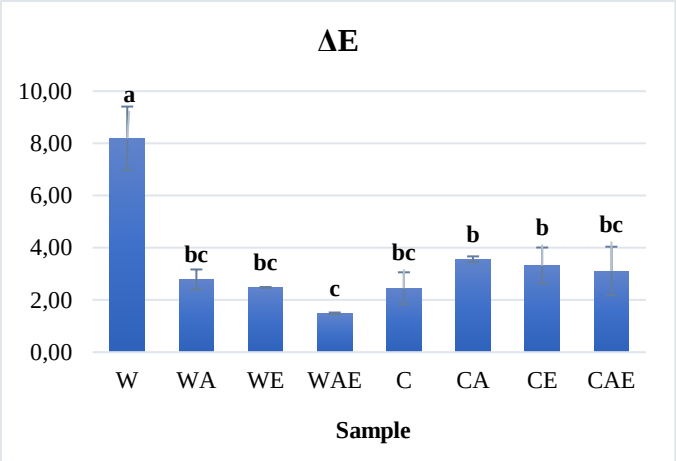


Figure 21: Colour variation values of potato samples.

Regarding the whiteness index (Table 37), all the dipped samples showed higher values than the coated ones until the last day of monitoring. This aspect confirms the relevant role of polysaccharide-based coating in delaying the loss of water from vegetables, reducing surface dehydration and decolouration (Bourtoom, 2008, Tatsumi et al., 1991).

Table 37: Changes in Whiteness Index values of potato samples.

Whiteness Index (WI)						
	Storage time (days)					
	0	**2**	**4**	**8**	**14**	**SIGN.**
W	60.81 ± 1.77^{aAB}	61.70 ± 0.18^{abA}	60.95 ± 0.29^{aA}	61.41 ± 0.06^{aA}	58.28 ± 1.25^{bcB}	*
WA	59.31 ± 0.30^{abc}	61.43 ± 2.30^{ab}	61.76 ± 0.86^{a}	60.95 ± 1.10^{a}	61.66 ± 0.40^{a}	NS
WE	60.42 ± 0.30^{ab}	60.54 ± 2.08^{ab}	60.44 ± 0.65^{a}	58.77 ± 0.73^{bc}	58.55 ± 1.12^{bc}	NS
WAE	61.02 ± 0.04^{aBC}	62.75 ± 1.01^{aA}	61.63 ± 0.57^{aAB}	59.75 ± 0.16^{abC}	59.73 ± 0.12^{abC}	**
C	54.70 ± 0.31^{eA}	55.37 ± 0.11^{cA}	53.01 ± 0.69^{cB}	55.23 ± 0.25^{deA}	52.64 ± 0.05^{eB}	**
CA	57.51 ± 0.90^{cdBC}	58.15 ± 0.23^{bcAB}	60.07 ± 0.23^{aA}	57.14 ± 1.37^{cdBC}	55.93 ± 0.43^{dC}	**
CE	56.07 ± 0.84^{deAB}	56.19 ± 0.04^{cAB}	57.87 ± 0.20^{bA}	54.90 ± 0.38^{eAB}	56.73 ± 1.23^{cdB}	**
CAE	58.16 ± 0.83^{bcd}	56.18 ± 1.45^{c}	56.18 ± 1.22^{b}	56.77 ± 0.09^{de}	57.45 ± 0.80^{bcd}	NS
Sign.	**	**	**	**	**	

Data are presented as means ± SD (n=10). Means within a row or column with different letters 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.

About the development of browning (Table 38), ascorbic acid and OSW_E in dipping solution and their combination in coating formulation were able to play the role of anti-browning agents (Ben-Fadhel et al., 2020). These observations suggested that the colour of W+A, W+E and C+A+E was well maintained during storage, confirming also by ΔE values (Figure 20). The colour of fresh cut potato could be changed because of enzymatic oxidation, such as PPO activity in presence of oxygen.

In this study, calcium salt incorporated in coating formulation probably also worked as an anti-browning agent in synergy with the presence of ascorbic acid and the OSW_E, inhibiting the PPO activity due to the calcium ion interaction at the PPO activity site (Ferrari et al., 2013). Indeed, C+A+E showed the lowest value of BI among the coated samples. In addition, this phenomenon could be ascribed to the stabilization of the extract phenolic compounds by the coating matrix against oxidation, since browning could be related to oxidation of phenols (Amanatidou et al., 2000).

Table 38: Changes in Browning Index values of potato samples.

Browning Index (BI)						
	Storage time (days)					
	0	**2**	**4**	**8**	**14**	**SIGN.**
W	24.77 ± 1.22^{bcdB}	25.42 ± 2.24^{abB}	24.78 ± 1.86^{cdB}	27.43 ± 0.85^{abcAB}	31.44 ± 0.80^{bA}	**
WA	22.04 ± 0.70^{de}	24.21 ± 1.22^{b}	23.02 ± 2.30^{d}	24.23 ± 0.03^{c}	24.32 ± 0.83^{d}	NS
WE	26.50 ± 1.82^{ab}	25.59 ± 1.84^{ab}	26.97 ± 0.11^{bcd}	26.02 ± 1.74^{bc}	28.03 ± 0.47^{c}	NS
WAE	20.84 ± 0.53^{eC}	24.03 ± 0.02^{bB}	24.79 ± 1.31^{cdAB}	24.75 ± 0.86^{bcAB}	26.48 ± 1.03^{cdA}	**
C	29.94 ± 0.45^{aC}	29.40 ± 3.15^{aBC}	31.78 ± 2.06^{aAB}	31.32 ± 1.56^{aAB}	35.11 ± 1.16^{aA}	**
CA	26.34 ± 1.91^{bB}	27.82 ± 0.66^{abB}	29.45 ± 0.35^{abB}	28.77 ± 2.37^{abB}	32.05 ± 0.34^{bA}	**
CE	26.11 ± 0.98^{bcB}	26.10 ± 0.52^{abB}	27.40 ± 0.29^{bcB}	27.39 ± 2.15^{abcB}	33.29 ± 0.49^{abA}	**
CAE	22.74 ± 1.43^{cde}	25.46 ± 0.93^{ab}	24.11 ± 0.93^{cd}	25.01 ± 0.83^{bc}	24.58 ± 1.56^{d}	NS
Sign.	**	*	**	**	**	

Data are presented as means ± SD (n=10). Means within a row or column with different letters are significantly different by Tukey's post hoc test. Abbreviation: Sign., significance; ns, not significant. **Significance at $p<0.01$; *Significance at $p<0.05$.

4.3.3.3. PPO and POD activity and changes in polyphenols content and antioxidant activity

The addition of natural antioxidant in dipping and coating formulations supported a shelf-life improvement of minimally processed potatoes, which could be related to the preservation of TPC and by slowing down the release of bioactive ingredients, due to the structure of the coating matrix (Ben-Fadhel et al., 2020). Indeed, as reported by Fai et al., 2013, when a coating is applied to the peeled vegetables surface, part of it migrates and is absorbed into the vegetable tissue, part by binding to the polymers of the cell wall and part persisting free on the surface. For this reason, the coating treatment could be implemented to include bioactive compounds that may limit oxidative damage, and thus contribute to maintain vegetables colour by inhibiting whitening or browning phenomena, the latter related to phenols oxidation (Amanatidou et al., 2000).

Looking at the data reported in Figure 22, PPO and POD activities showed a clear significant increasing trend ($p<0.01$) over time in all potato samples, according to Alegria et al. (2016), with the highest values detected in coated samples except for C+A+E. Indeed, the latter was characterized by the lowest values in terms of enzymatic activities but, at the same time, with the highest content of

total polyphenols. Higher PPO and POD activities was attributed to stressed conditions due to the processing operations of the matrix and the continuous vegetable ripening over time, confirmed also by BI values (Table 38). In general, the PPO trend over time reflected that described also by Palamutoglu (2020).

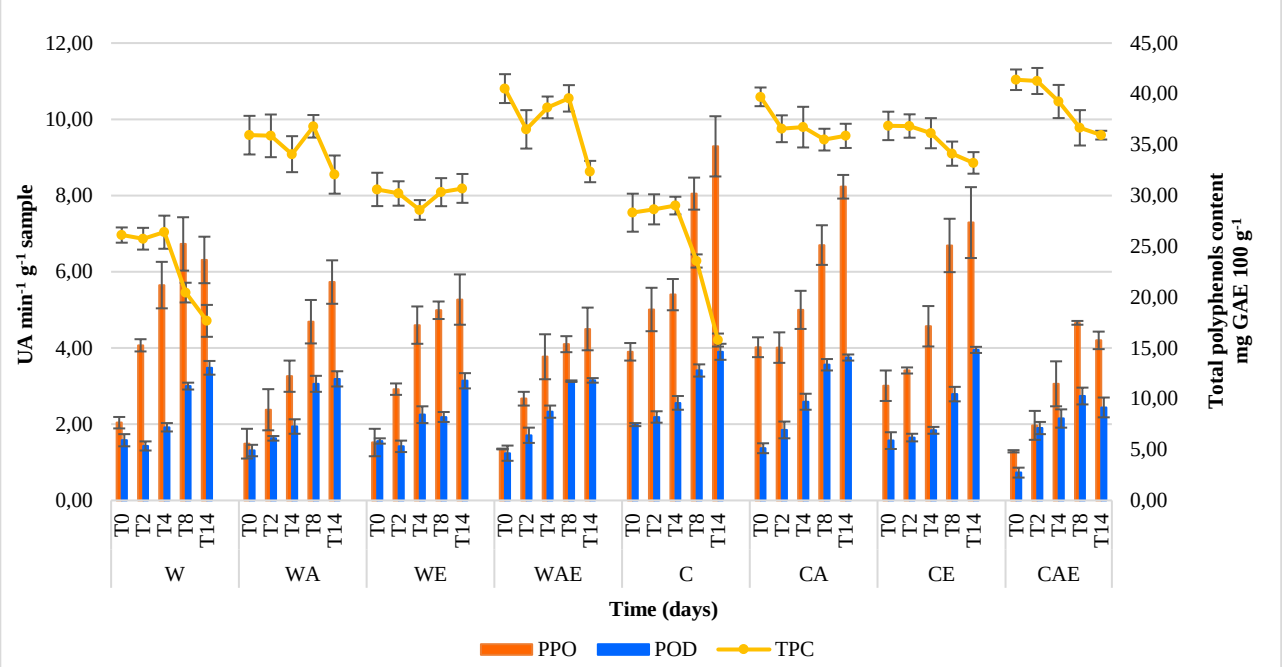


Figure 22: Changes in Polyphenol oxidase (PPO) and Peroxidase (POD) activities and total polyphenol content (TPC) values of potato samples.

In addition, the trend of antioxidant activity (Table 39) confirmed the increased enzymatic activity and the decrease of TPC over time, with an average Pearson correlation factor of 0.81 between TPC and both DPPH and ABTS assays. The physiological role of PPO is to catalyse the oxidation of phenolic compounds into quinones, which are biologically active substances that cause browning of the foodstuff on which they are formed. Indeed, cutting operations on potatoes cause the cell structure to break down and thus the contact between PPO and the and the phenolic substrates. In this regard, comparing PPO (Figure 22) and browning values (Table 38), low enzymatic browning detected for WA, WE, WAE and CAE samples was associated to the lowest PPO activity, confirming what described by Holderbaum et al. (2010).

Table 39: Expression of antioxidant activity values (DPPH and ABTS assays) of potato samples.

DPPH (µM Trolox 100 g^{-1})						
	Storage time (days)					
	0	**2**	**4**	**8**	**14**	**SIGN.**
W	86.61 ± 5.67^{bcA}	83.36 ± 5.90^{cdA}	75.17 ± 5.85^{bAB}	77.06 ± 6.33^{cAB}	73.87 ± 6.36^{bcB}	*
WA	104.13 ± 4.53^{a}	97.88 ± 7.82^{abc}	102.35 ± 7.17^{a}	98.49 ± 5.98^{ab}	94.25 ± 6.78^{a}	NS
WE	98.90 ± 9.78^{abA}	86.34 ± 10.13^{bcdAB}	82.49 ± 8.49^{bAB}	76.26 ± 7.18^{cAB}	75.95 ± 6.35^{bcB}	*
WAE	106.41 ± 4.72^{aA}	102.69 ± 5.99^{abcAB}	102.98 ± 4.30^{aAB}	105.01 ± 3.29^{abA}	89.76 ± 6.09^{abB}	*
C	77.40 ± 3.96^{cA}	74.61 ± 5.85^{dA}	69.36 ± 3.54^{bAB}	75.26 ± 5.45^{cA}	65.39 ± 6.40^{cB}	*
CA	110.05 ± 1.65^{a}	106.96 ± 9.21^{ab}	106.17 ± 7.65^{a}	105.27 ± 8.94^{ab}	105.82 ± 7.01^{a}	NS
CE	84.39 ± 7.44^{bcAB}	84.58 ± 9.37^{cdAB}	83.33 ± 6.67^{bAB}	87.66 ± 6.65^{bcA}	75.27 ± 5.64^{bcB}	*
CAE	111.72 ± 2.07^{aA}	111.42 ± 2.90^{aA}	113.47 ± 1.26^{aA}	109.73 ± 6.03^{aAB}	104.51 ± 3.84^{aB}	*
Sign.	**	**	**	**	**	
ABTS (µM Trolox 100 g^{-1})						
	Storage time (days)					
	0	**2**	**4**	**8**	**14**	**SIGN.**
W	228.85 ± 22.08^{bA}	213.80 ± 14.18^{bcAB}	215.70 ± 15.08^{abAB}	171.67 ± 11.82^{dC}	181.39 ± 7.91^{cBC}	**
WA	274.56 ± 7.01^{abA}	261.11 ± 14.93^{aAB}	238.58 ± 10.20^{abAB}	248.05 ± 20.04^{abAB}	236.65 ± 13.85^{abB}	*
WE	281.97 ± 21.61^{abA}	235.97 ± 15.95^{abcB}	227.96 ± 19.08^{abB}	219.75 ± 4.85^{abcB}	227.83 ± 14.75^{abB}	**
WAE	277.34 ± 21.72^{abA}	245.54 ± 14.40^{abcAB}	252.92 ± 11.91^{aAB}	254.93 ± 16.21^{aAB}	243.95 ± 10.12^{aB}	**
C	254.41 ± 19.53^{bA}	201.00 ± 10.16^{cB}	203.32 ± 15.40^{bB}	202.20 ± 14.58^{cdB}	203.76 ± 9.50^{bcB}	**
CA	282.70 ± 23.51^{abA}	250.99 ± 20.27^{abAB}	230.95 ± 10.39^{abB}	209.86 ± 20.31^{bcdB}	214.95 ± 7.41^{abcB}	*
CE	271.69 ± 22.21^{abA}	230.96 ± 20.52^{abcB}	236.76 ± 17.41^{abB}	234.01 ± 13.82^{abcB}	228.77 ± 19.13^{abB}	**
CAE	324.21 ± 22.33^{aA}	260.31 ± 17.26^{aB}	237.93 ± 10.00^{abBC}	225.71 ± 14.57^{abcC}	233.32 ± 16.38^{abC}	**
Sign.	**	**	*	**	**	

Data are presented as means ± SD (n=10). Means within a row or column with different letters 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.

The reported data suggested that onion solid waste extract exerted an anti-browning effect on potato samples and controlled the enzymatic activities. Indeed, as found by Kim et al. (2005), onion is characterized by the presence of some components which can inhibit PPO activity. It was reported that Allium species, such as onion, contain different volatile sulphur compounds, like thiols, which could be considered as the active components responsible for the inhibitory effect of onion extracts on PPO (Negishi et al., 2002; Ding et al., 2002, Negishi & Ozawa, 2000). As confirmed also by Cerit et al. (2020), potatoes treated with thiol compounds were characterized by a significantly lower enzyme activity than untreated samples during storage. In addition, the lowest values in BI (Table 38) of CAE could be explained by the reaction of thiols with ascorbic acid and the binding of PPO to potato starch granules, which might also contribute to the differences in inhibition of enzymatic browning (Friedman & Bautista, 1995).

4.3.3.4. Characterisation of potato texture and PME activity

It is well known that pectin methylesterase (PME) is one of the main enzymes responsible for the degradation of cell wall pectin, producing a general relaxation of vegetable structure with consequential impacts in terms of texture. In this study the changing in PME activity were related to texture values, expressed as the cutting resistance exerted by the piece of potato (Figure 23). This parameter is linked to the product's resistance to fracture, which could be ascribed to tissue senescence and cell wall breakdown, as well as to sample water loss (Vargas et al., 2002). The reported data suggested that most of the potato samples showed significant variations in texture as well as in PME activity, contrary to what found for coated potato samples C+E and C+A+E. Indeed, these latter showed constant texture values, reflecting the same trend of PME activity values. On the other hand, the uncoated samples showed a significant reduction in texture values probably due to the progressive increase in PME activity in reply to injuries due to peeling and cutting operations, causing the hydrolysis of hemicellulose and other cell wall components that are presented in potato, confirming what reported by Shigematsu et al. (2019). The edible coating applied on potato samples in combination with the OSW_E might have reduced the performance of these enzyme and preserved the firmness of C+E and C+A+E, suggesting that the coating was very efficient in keeping products texture. Moreover, the calcium salt in the polysaccharide edible coating was used as a tissue structural preservative, since its ion performances as a cross-linking agent, creating complexes with the pectin of vegetable cell wall and middle lamella, boosting integrity of the structure and encouraging better tissue firmness (Ferrari et al., 2013).

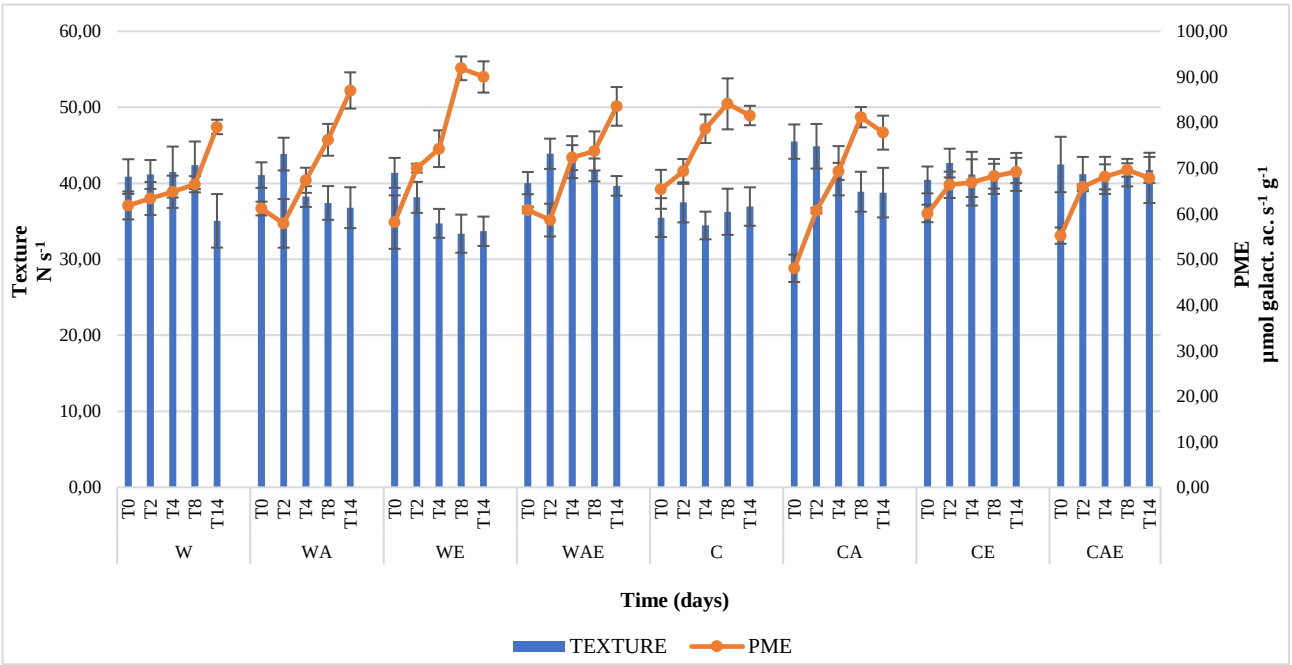


Figure 23: Changes in Texture and Pectin methylesterase (PME) activity values of potato samples.

4.3.3.5. Microscope observation

What has just been described previously about changes in texture and PME activity of potato samples (Section 4.3.3.4.) were observed also by the microscope observation. Main differences were detected between the first (T0) and the last day of monitoring (T14), focusing the attention on the contact area among the cell walls (Figures 24 and 25).

Specifically, after 14 days of monitoring, darker areas were noted between the cell walls identified by microscopic observation. Based on these considerations, comparing Figure 24 and 25, darker areas between the various cells are evident especially in samples W, WA, WE and CA, a phenomenon that could be associated with a progressive relaxation of the cell structure due to PME activity, at cell wall and median lamella level. This partly reflects what was previously discussed regarding the relationship between PME and the change in potato texture over time (Figure 23).

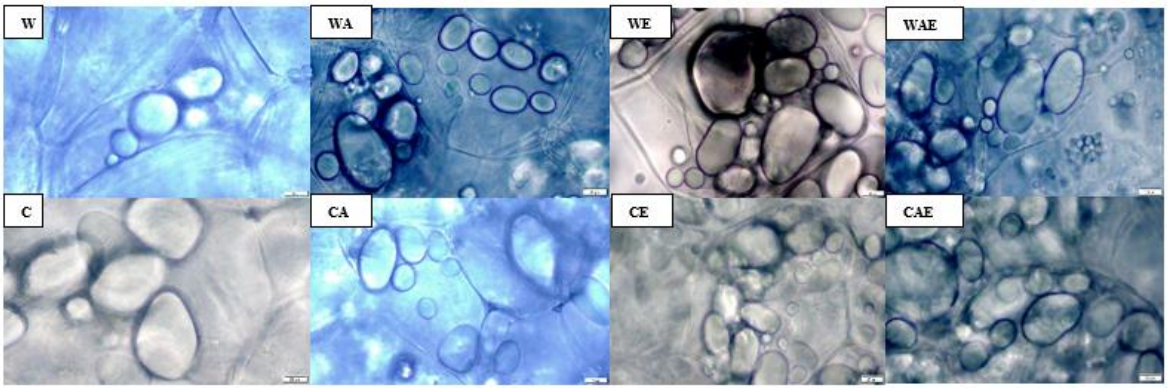


Figure 24: Microscopic detail of the cell wall and starch granules of potato samples at T0.

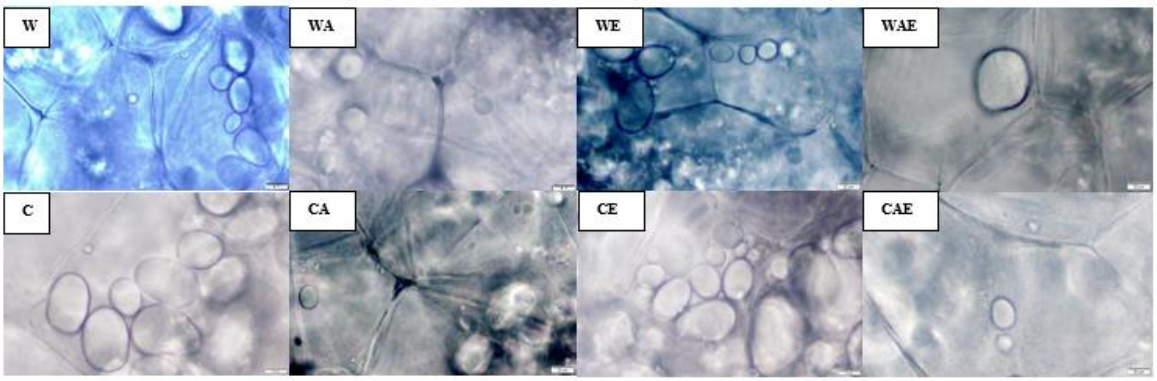


Figure 25: Microscopic detail of the cell wall and starch granules of potato samples at T14.

4.3.4. CONCLUSIONS

This study indicated that onion solid waste could be considered an important source of bioactive compounds, with antioxidant activity and might control the browning development of fresh-cut potatoes, achieving the best conservation results.

The coating and dipping treatments with OSW_E and its combination with ascorbic acid not only allowed a better preservation of the samples colour over time, contrasted the progress of whitening and browning on potatoes surface, but also increased the content in polyphenols compounds and antioxidant activity of the final products.

In addition, CAE resulted in a clear reduction in the activities of key enzymes such as PPO, POD and PME, helping to maintain the texture of the samples over time, compared with the other treatments.

Overall, edible coating with antioxidant extract obtained from onion solid waste could be considered an appropriate approach for controlling browning and preserving the quality of minimally processed potatoes.

CONCLUSIONS AND FUTURE PERSPECTIVE

In conclusion, the research work carried out during this PhD project demonstrated that by-products from the food industry are an important natural source of bioactive compounds with high antioxidant and antimicrobial properties and how they can be recovered and used in the production of new foods, through food grade and green methods.

On one hand, the inclusion of these compounds in two different types of bakery products (biscuit and toast bread) demonstrate that it is possible to produce functional foods rich in antioxidant and antimicrobial molecules, with positive effects on human health. In this regard, further research is needed to improve the techniques for including bioactive compounds in complex food matrices, studying their interactions and preservation over time, in order to ensure an ever-increasing supply of useful compounds through food consumption and prolonged their shelf life over time.

On the other hand, the development of new methods to improve the functional properties of edible coatings is one of the most critical issues for future research. Now, several studies on food applications have been conducted at a laboratory scale, but further research should be focused on a commercial level to provide more accurate and practical information that can be used to produce and commercialize fresh-cut vegetable products coated with functional edible coatings characterized by the presence of bioactive compounds, adding value to products while increasing their shelf-life. In addition, more studies are necessary to understand the interactions and influence of active ingredients (antimicrobials, antioxidants, and nutrients) on coating formulations when applied in new food products, focusing the attention especially on mechanical, sensory and functional properties that can be significantly affected, to develop new coating applications with improved performances.

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