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The residues of fruit and vegetable processing: from "waste" to "resource" of natural phytochemical compounds

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Abstract: The project of Sant'Anna School, in line with the Italian legislation on limiting waste and promoting the redistribution of surpluses and unused goods, aimed to study the potential healthy value of residues obtained from the transformation of fruit and vegetable products that represent a cost, as they must be handled, stored and disposed according to stringent actual regulations. Two "model" species (potato and apple) were considered to test the possibility of using industrial processing waste for food applications. The extracts, obtained with "green" methods from potato and apple peels, were evaluated as natural antioxidants in the preparation of minimally processed fresh-cut apple. Results suggest the possibility to use these novel byproduct extracts as valuable alternative treatments to traditional chemical additives employed for minimally processed apples.

1. Introduction

The Italian law n. 166 (2016) defined as "antisprechi" has been formed with the aim of limiting waste, while promoting the redistribution of surpluses and unused goods. This law relies on two fundamental principles guaranteed by Italian Constitution: subsidiarity and solidarity. For the first time, it formally defines the terms "waste" and "surplus", constituting a reference point for dialogues between different institutions opening up new perspective for investigation on new products recovered from byproducts. In agreement with these regulations, the Italian Ministery of Agriculture (Mipaaft) established financial contributions to support innovative projects, related to research and technological development, in the field of food shelf life and packaging, which ensure a concrete application of the results achieved. The project of Sant'Anna School was one out of 10 selected and financed by Mipaaft in 2017.

The Italian food industry is constantly growing, with an estimated increase of + 1.5% in 2018 equal to 134 billion euro (there were 132 in 2016). The industrial production of the agri-food sector has grown by more than 5% in the last fifteen years (Caroli *et al*., 2019).

The transformation activities produce large quantities of organic waste whose disposal represents an additional cost for the food industries as they must be moved, stored and disposed. In Italy, the annual residues deriving from the canning industry are estimated at around 1400 kt of d.m. (Balsari *et al*., 2011). Consequently, the reuse of waste is a strategic theme in the search for 'renewability' for the industrial application of raw materials of vegetable origin, also to meet the interest of users towards eco-sustainable products.

Processing waste from the agri-food industry, in particular fruit and vegetables, are generally considered an excellent source of bioactive compounds with antioxidant activity such as vitamins, phenols and carotenoids. Plants produce a wide range of secondary metabolites, mainly phenolics, with different functions: pigmentation, growth, reproduction, resistance to pathogens (Castoria *et al*., 2009). The positive effects on human health of these phytochemicals have been widely documented and concern the antioxidant, anti-inflammatory and anticarcinogenic action (Auger *et al*., 2004; Manach *et al*., 2005; Bitler *et al*., 2007). Phytochemicals extracted from various species such as olive, citrus, tomato, oregano, green tea, grapes and garlic have been shown to have the ability to inhibit lipid oxidation in various model systems. Therefore, the transformation of waste byproducts into synthetic food additives appears particularly promising (Goni and Hervert-Hernández, 2011).

Two widely worldwide consumed fresh or processed foods, such as potatoes and apples, generate a large waste amount. The by-product management of potatoes (*Solanum tuberosum*) is considered an important problem faced by food processing companies, as they cannot be discharged into the environment due to their high polluting potential (Rodriguez Amado *et al*., 2014). Potato is a source of different bioactive compounds such as starch, dietary fiber, amino acids, minerals, vitamins, and phenolics (Akyol *et al*., 2016). The apple is the most widely consumed fruit, with a wide range of varieties with different organoleptic characteristics. Numerous scientific studies demonstrated these fruits possess a wide range of chemical compounds with beneficial effects on human health: dietary fibers and proteins, secondary metabolites such as vitamins, phenols and carotenoids, which have proven anti-inflammatory and anticarcinogenic antioxidant effects (Boyer and Liu, 2004). The main structural classes of apple constituents include hydroxycinnamic acids, di-hydrochalcones, flavonols, catechins and oligomeric procyanidins, as well as anthocyanin in red apples (Jakopic *et al*., 2007; Veberic *et al*., 2007). In particular, considering the antioxidant capacity of compounds present in apple tissues, this fruit may be considered a valid challenge in the chain of ready to eat products. One of most critical points in fresh-cut fruit slices is the appearance of cut surface browning as a consequence of physical stresses imposed on cells during preparation. To avoid the loose of quality, chemical treatments have been used as food additives (Paiva-Martins *et al*., 2007). However, some of the most common synthetic compounds such as BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) are suspected of harmful effects; thus, alternative antioxidant additives or disinfectants would be needed (Chen *et al*., 2016).

The aim of the project was to evaluate, in two fruit and vegetable "model" species (potato and apple), the possibility of using industrial processing waste for food applications. In this work the attention was focused on the effect of extracts obtained from organic potato and apple peels on the preservation of the physicochemical quality parameters of fresh-cut apples.

2. Materials and Methods

Preparation of fresh-cut apples

Fresh-cut apples were prepared from organic undamaged 'Golden Delicious' (*Malus domestica* Borkh) purchased from a local large retailer. The experimental procedure is showed in figure 1, according to safety statements and recommendations for minimally processed apples (Dávila-Aviña *et al*., 2015). Apples were washed in running water, hand-peeled and cored with a ceramic knife, longitudinally cut into cubes (12 per apple) and completely dipped in different preserving solutions for 2 min, manually stirring. Dipping treatments were performed comparing usually used preservative media (1% butylated hydroxytoluene - BHT - and 1% citric acid - CA) and novel extracts from potato (P) and apple (A) peels obtained by water (W) and 10%

ethanol (Et). After dipping, the apple cubes were drained on absorbent paper and packaged in plastic lidded containers (150 cc). Packaging were put in controlled chambers at 20±1°C to stimulate a short browning reaction or stored at 4±1°C (dark conditions up to 5 days) to simulate the average standard cool retail storage condition. Each dipping treatment consisted of 3 replicates represented by 3 different containers.

Procedure for potatoes and apple peel extracts

The extracts were prepared starting from i) organic yellow-paste potatoes cv. Bologna and ii) organic apples cv. Fuji by cryomaceration of peels which were maintained to direct contact with solid CO_2 (ratio peels/CO_2 1/1 w/w) over 24 hours, according to Venturi *et al*. (2019). The extraction was carried out using as solvents water and Et at 10% following the steps reported in figure 2 and the extracts were stored at -20°C in test tubes saturated with nitrogen, until dipping treatments.

Characterization of peel extracts

The total phenols content was determined calorimetrically at 700 nm, using the Folin-Ciocalteau reagent (Waterhouse, 2001). Phenols content was expressed as gallic acid equivalents. Antioxidant capacity was determined according to the TEAC antioxidant assay which was performed spectrophotometrically at 734 nm following Venturi *et al*. (2017). The radical cation ABTS (2,2′-azino- di-[3-ethylbenzthiazoline sulphonate]) was generated as described by Pellegrini *et al*. (1999). The activities of the extracts were expressed in terms of Trolox equivalent antioxidant capacity (TEAC).

Quality evaluation of fresh-cut apples

Fresh-cut apple characteristics were evaluated by a short test, 3h after cutting at 20°C, and by a cold storage test maintaining the cubes at 4°C until 5 days. The main quality parameters such as browning for the short test, and total solid sugars and firmness for the cold storage test were analyzed.

The level of browning was determined by color of apple cubes surface using a colorimeter (Eoptis, Mod. CLM-196 Benchtop, Tn., Italy) according to Buera *et al*. (1985). Results were expressed as Δ Browning Index (ΔBI) values by the follow equation:

$$\Delta BI = BIf - BIs$$

where
BIf = BI at the end of each observation time,
BIs = BI at the start of each experiment.

$$BI = 100^* (x-0.31)/0.172$$

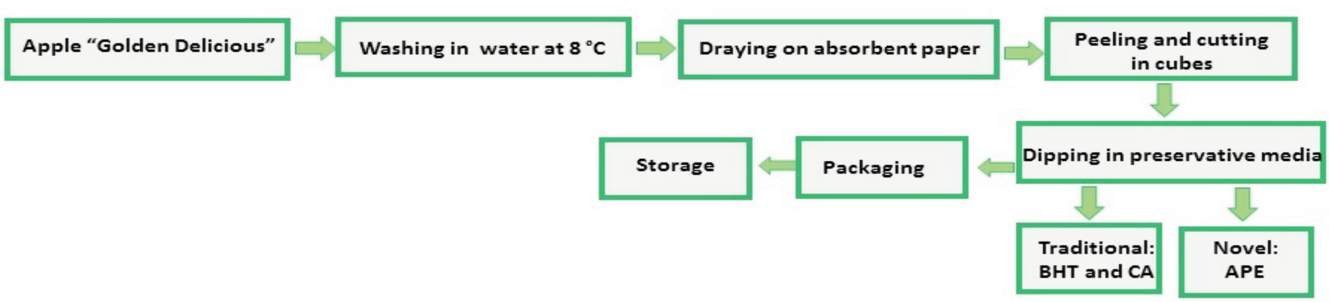

Fig. 1 - Experimental procedure to compare different preservative treatments on fresh-cut apple cubes with traditional (1% butylated hydroxytoluene - BHT - and citric acid - CA) and novel extracts from potato and apple (APE) peels.

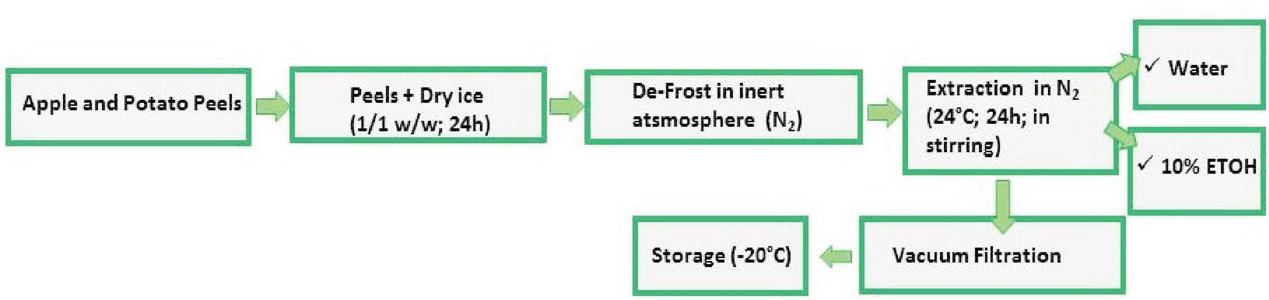

Fig. 2 - Procedure applied to obtain apple and potato extracts using as solvent water and 10% ethanol. Both extracts were stored at -20°C before use.

where
x = (a*+1.75L*)/(5.645L*+a*-3.012b*); L* defines the lightness, a* and b* define the red-greenness and blue-yellowness, respectively.

Total soluble solids (TSS), obtained from the fresh tissue sap of apple flesh cubes were measured using a hand refractometer (Mod. 2369-Bertuzzi, Milan, Italy) and expressed as °Brix.

Flesh firmness of apple flesh cubes was evaluated by a manual penetrometer (Mod. 53205, TR Turoni & C. Snc, Forlì, Italy), with a metal probe (8-mm diameter). The force needed to break parenchyma cells in the cortex was expressed in kilogram-force (kg/0.5 cm^2).

Statistical analysis

Statistical analysis of obtained data was conducted by PRISM 7 (GRAPHPAD, USA). Student *t* test and analysis of variance (ANOVA) with the test of mean comparisons according to Bonferroni were applied, with a level of significance at $p \leq 0.05$. All data are reported as mean values ± SE (standard error).

3. Results and Discussion

Peel antioxidant content

From the results reported in Table 1, a very remarkable total antioxidant activity was detected in both apple and potato peels extracts. However, on the basis of the high phenolic content, apple peels were characterized by the highest antioxidant capacity. It has been established that, both in red- and yellow-skinned apples, the highest nutraceutical power resides in the peel, representing the main source of antioxidants where values may reach 80% of total antioxidant capacity (Leccese *et al.*, 2009), mainly determined by polyphenols (Wojdylo *et al.*, 2008).

Table 1 - Phenolic content and antioxidant capacity of potato and apple peel water and ethanol extracts

	Phenolic content (gallic acid mg/g dry weight)	Antioxidant capacity (µmol TEAC/mL)
Apple Peel		
Water extract	9.25 ± 0.26	0.33 ± 0.03
10% ethanol extract	13.14 ± 0.20 *	0.73± 0.02 *
Potato Peel		
Water extract	2.92 ± 0.41	0.17 ± 0.02
10% ethanol extract	3.95 ± 0.02 *	0.21 ± 0.01 *

Data are reported as mean ± SE (n= 3). In the columns, and within species, asterisks indicate significant differences between solvents according to Student t test ($p \leq 0.05$).

A great influence of solvent composition on antioxidant capacity of the extracts was stood out. The presence of a low ethanol percentage increased the extraction of antioxidant compounds like polyphenols in comparison with solely water in both species. In any case, cryomaceration of vegetal byproducts by means of solid carbon dioxide can be profitably applied to improve the green extraction of bioactive compounds, favoring the processes in solid/liquid extraction as demonstrated on other plant materials (Andrich *et al.*, 2003; Zinnai *et al.*, 2015; Nari *et al.*, 2018; Ascrizzi *et al.*, 2019). According to the literature, potato and apple peels resulted a good source of total phenolics which can have health beneficial properties, mainly due to the presence of chlorogenic acid and caffeic acid (Wolfe and Wu, 2003; Friedman *et al.*, 2017).

Short-time test: effect of dipping on browning of fresh-cut apples

In this study different preservative solutions have been used, in particular those commonly used by the IV gamma industry such as BHT and CA. The activity of these traditional solutions has been compared with that carried out by alternative solutions, deriving from agro-industry waste such as potato and apple peels. Nowadays, consumer awareness about green products is increasingly and in this contest the recovery of waste from the agro-industry can be considered as an evaluable source of chemical compounds of natural origin.

The main parameter negatively affecting the visual quality of fresh cut fruits, like apple, is the presence of oxidation on the surface and in the under layer flash tissue which occurs immediately after cutting. As reported in literature (Queiroz *et al.*, 2008) and in previous studies (Venturi *et al.*, 2019), it has been observed that the first hours after cutting are very critical due to the appearance of the initial oxidative process symptoms. Thus, the evaluation of browning in a short-time interval can be considered a useable and effective test to screen the efficiency of conservative treatments.

In figure 3 the Δ Browning Index (ΔBI) values determined at 20°C after 3 hours from dipping treatments with commercial and novel compounds are reported. The browning appearance was much more evident in water control dipping, while the tissue browning was significantly reduced when the preservative agents were added. Both apple and potato extracts obtained by 10% ethanol (EtA and EtP), showed the strongest anti-browning effects whilst water extracts (WA and WP) had values comparable to those obtained with the use of commercial compounds BHT and CA. The beneficial effect exerted by apple and potato extracts could be linked to their phenolic content (Table 1). In the case of potato and its byproducts, the inhibition of the polyphenoloxidase (PPO) activity has been well recognized (Akyol *et al*., 2016). For this reason, potato extracts have been studied for their capacity in reducing lipid oxidation and improving nutritional properties of processed foods (Franco *et al*., 2016). As regard apple, phenolic rich peel extracts were found to be effective inhibitors of polyunsaturated fatty acid oxidation as demonstrated by a stronger inhibitory effect on fish oil oxidation (Sekhon-Loodu *et al*., 2013).

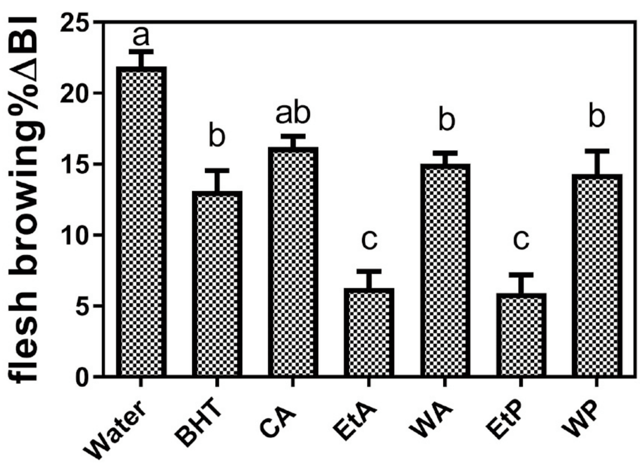

Fig. 3 - Short-time test: effect of dipping on flesh browning of fresh-cut apples. Δ Browning Index (ΔBI) percentage determined at 20°C, after 3 hours from dipping treatments: water; BHT (1% butylated hydroxytoluene); CA (1% citric acid); EtA (10% ethanol apple extract); WA (water apple extract); EtP (10% ethanol potato extract); WP (water potato extract). Data are means ± SE.

Cold storage test: effect of dipping on quality of fresh-cut apples

Temperature is considered the most important factor in the conservation of perishable products. The temperature strongly influences the respiration of the tissues, so much so that as the temperature increases, the respiration also increases (Fagundes *et al*., 2013). An increase of 10°C has been observed to induce an increase in respiratory activity and an acceleration of the aging process of approximately 2-3 times. Therefore, the temperature must be as low as possible according to the tolerance thresholds of the different species. In general, most products can be stored at temperatures close to zero (0-1°C) with the exception of those are considered as a chilling sensitives (tomato, melon etc.) which must be stored at temperatures of 7-13°C. Storage at non optimal temperatures generates an alteration of cell membranes (Saltveit, 2002) which accelerate respiration and activate the enzymes involved in the detoxification of free radicals, such as copper/zinc superoxide dismutase, catalase and the enzymes of the ascorbate-glutathione cycle. In particular, minimally processed apples must be stored at a temperature not exceeding 4-6°C and need stabilizing treatments to maintain the quality level of fruits during cold storage (Senesi, 2008). Thus, the use of conservative compounds have to be appropriately tested also under cold conditions to address the requirement of keeping fruit quality during all the distribution chain.

In this work, during the storage at 4°C no significant differences were observed in the browning process in comparison with the phenomenon occurred after 3h at room temperature (data not shown). As a consequence, particular attention was focused on the maintenance of other quality parameters as firmness and TSS degree.

As concern apple tissue firmness, it is determined by cell size, biochemical and biophysical cell wall properties, cell-to-cell adhesion and tissue turgor (Toivonen and Brummell, 2008; Rux *et al*., 2017). The loss of texture and the degradation of tissues determine the softening of fruits not only during the ripening but also under particular storage conditions.

In the present study, when the processing procedures started, an average firmness of 2.70 kg/0.5 cm^2 was recorded. Under cold temperature at 4°C (Fig. 4), in water dipping treatment a texture decrease was observed with a tissue strength decline by about 25% and significant differences in comparison with all other treatments were observed. An interesting inhibition of tissue softening was recorded in the apple cubes treated with the peel extracts similarly to that observed using traditional media (BHT and CA). The positive effects of the natural novel compounds, confirmed also after 5 d of storage (Fig. 4), could be related to an attenuation of the physical and chemical changes affecting textural integrity as a consequence of enzymatic hydrolysis of cell wall pectic substances (Van Buggenhout *et al*., 2009). In particular, the softening increase observed in apple cubes after water treatment could be due to texture biochemical changes as reported in several minimally processed fruits and vegetables (Toivonen and Brummell, 2008).

As regard TSS content of Golden Delicious apple,

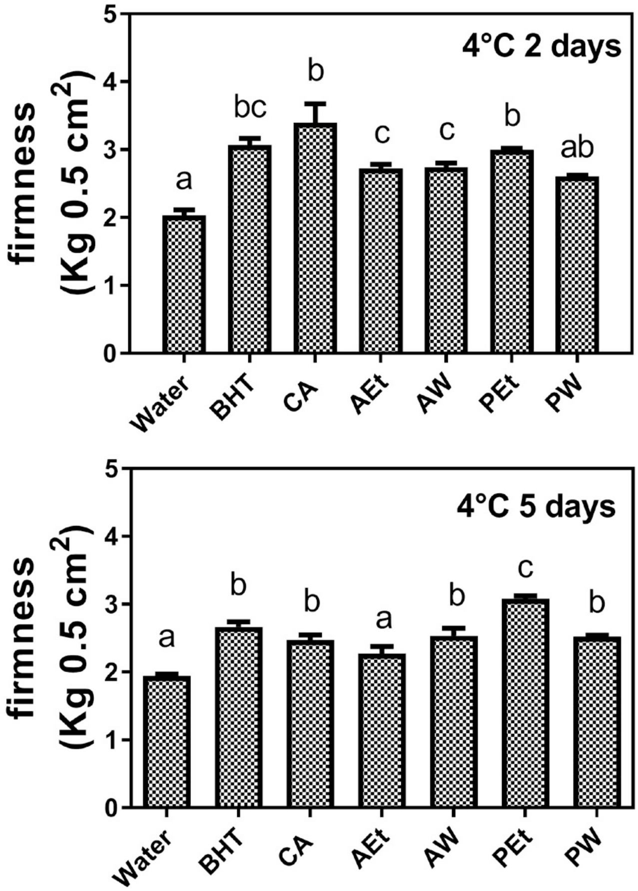

Fig. 4 - Cold storage test: effect of dipping on flesh firmness (kg/0.5 cm^2) of fresh-cut apples determined at 4°C after 2 and 5 days from dipping treatments: water; BHT (1% butylated hydroxytoluene); CA (1% citric acid); EtA (10% ethanol apple extract); WA (water apple extract); EtP (10% ethanol potato extract); WP (water potato extract). Data are means ± SE. Different letters indicate significant statistical difference at p≤0.05.

at commercial stage the changes associated with ripening were already accomplished, so that the maximum soluble level was settled on about 13°Brix. During storage this value should be maintained to preserve the sensory quality of fresh cut apple (Augusto *et al*., 2016; Musacchi and Serra, 2018). The low temperature was effective to maintain the TSS degree, indeed a weak decrement was measured in samples without any conservation treatments stored at 4°C for 2 days (Fig. 5). This was in accordance with several researches reporting no substantial changes in apple slices of cv. Gala coated with alginate and stored at 5°C with values ranging from 14. 6 to 12.8 °Brix (Olivas *et al*., 2007). Analogous results were obtained using apple extracts in contrast to the traditional additives BHT and CA that caused a more drastic TSS reduction. After 5 d storage a notable TSS decrease was recorded in apple cubes dipped in water whilst no significant variations in all other treatments were detected, suggesting a protective effect in minimizing carbohydrate breakdown. The possibility to use natural extracts from other different sources as postharvest treatment, has been also evaluated in several minimally processed apples with profitable results (Augusto *et al*., 2016).

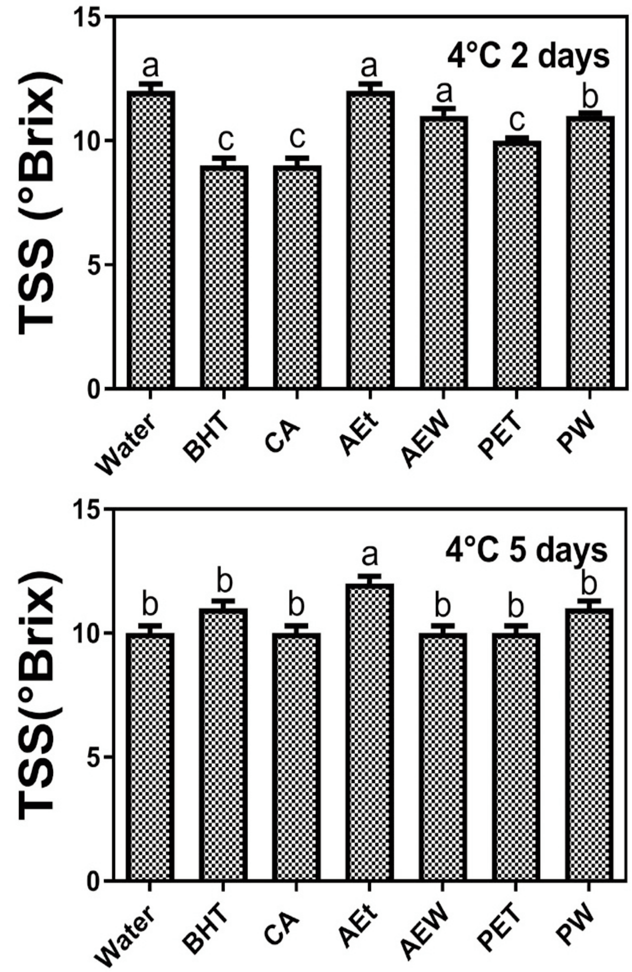

Fig. 5 - Cold storage test: effect of dipping on total solid content (TSS) expressed as °Brix of fresh-cut apples determined at 4°C after 2 and 5 days from dipping treatments: water; BHT (1% butylated hydroxytoluene); CA (1% citric acid); EtA (10% ethanol apple extract); WA (water apple extract); EtP (10% ethanol potato extract); WP (water potato extract). Data are means ± SE. Different letters indicate significant statistical difference at p≤0.05.

4. Conclusions

This study suggests that the residual waste of potato and apple can be considered as a exploitable source of valuable compounds for preservation of minimally processed apples. Potato and apple peel extracts based treatments positively affected the visual quality of fresh-cut apples as anti-browning agent starting from the beginning of the preparation process. During cold storage of apple cubes, the application of these novel extracts delay the degradation mechanism of the flesh constituents maintaining a good level of firmness as well as improving the nutritional quality.

On the basis of the obtained results, the proposed cryomaceration procedure to recover compounds from potato and apple peels proved to be effective to formulate potential natural additives for postharvest processing. Thus, synthetic preservatives in freshly stored vegetables could be replaced or at least reduced.

Furthermore, the employment of potato and apple peels proposed in this study may contribute to manage the environmental and economic problems caused by the increasing amount of wastes and residues from food processing industries.

Metabolomic characterization of the plant materials tested in this work is under investigation and the next step of our research will be to establish the relationship between the observed effects on fresh-cut apples and the chemical composition of these novel extracts.

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