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Valorization of orange peels as a source of natural antioxidants from pectin-extracted compounds

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Citrus peels, a major by-product of the fruit industry, are rich in pectic polysaccharides with potential bioactive properties. This study aimed to extract and characterize pectin from orange peels and to evaluate its physicochemical properties and antioxidant potential. The extracted pectin was analyzed for its physicochemical characteristics, and its antioxidant potential was assessed using three in vitro assays: DPPH, ABTS, and FRAP. The extraction yielded 19.0% dry material, with moisture (13.0%), ash (10.3%), total sugars (16.3%), and protein content (0.10%). Phytochemical analysis showed low levels of polyphenols (1.17 mg GAE/g DM), flavonoids (0.55 mg QE/g DM), and condensed tannins (0.10 mg CE/g DM). Despite these low phenolic contents, the pectic extract exhibited significant antioxidant activity in DPPH, ABTS, and FRAP assays, with IC_{50} values of 2.32, 2.00, and 1.85 mg/mL, respectively. These findings indicate that orange peel pectin is a promising natural antioxidant source with potential applications in the food, nutraceutical, and pharmaceutical industries, promoting the sustainable valorization of citrus by-products.

Keywords: Orange peel, Pectin, Extraction, Antioxidant activity, DPPH, ABTS, Citrus by-products

INTRODUCTION

Citrus fruits represent one of the most important fruit crops worldwide, with an estimated annual global production exceeding 115 million tons (Gonzatto and Santos, 2023). Among them, oranges are the most widely consumed due to their pleasant flavor, high nutritional value, and richness in bioactive compounds - over 170 phytochemicals have been identified (Ahmad and Langrish, 2012; Wang et al., 2007). Oranges are typically consumed fresh, or processed into marmalades and juices (Carmona et al., 2012).

In Tunisia, citrus cultivation is a major agro-economic activity, particularly concentrated in the Cap Bon region, which accounts for most of the country’s production (Ministère de l’Agriculture de Tunisie, 2014).

Orange peels are an abundant by-product of juice and marmalade industries and contain numerous nutritional and functional components, including water, proteins, sugars, minerals, essential oils, fibers, carotenoids, vitamin C, and phenolic compounds. The most common route of industrial valorization involves the extraction of essential oils, which serve as natural alternatives to synthetic fungicides (Singh et al., 2010; Fisher and Phillips, 2006). These oils are also employed in functional food formulations and in the confectionery industry (candies, jams, etc.) through direct incorporation of orange peel material (Bocco et al., 1998).

Moreover, citrus peels are increasingly used as feedstock and for biofuel and biogas production (Wilkins et al., 2007; Pourbafrani et al., 2010), as well as for the manufacture of biodegradable plastics (Byrne et al., 2004) and as corrosion inhibitors for metals and alloys (Da Rocha et al., 2010).

Citrus peels are particularly rich in dietary fibers (mainly pectin) and phenolic compounds, notably flavonoids and phenolic acids, which exhibit a broad spectrum of biological activities, including antioxidant, therapeutic, antiviral, antifungal, and antibacterial effects (Ma et al., 2009; Huang and Ho, 2010).

The extraction of pectin from orange peels has attracted growing scientific interest because pectin acts as a natural antioxidant and a valuable co-product derived from apple pomace and citrus peel residues. Pectin is widely used in the food industry as an emulsifier, stabilizer, and gelling agent. The global demand for pectin exceeds 30,000 tons per year and continues to grow at a rate of 4–5 % annually (Yeoh et al., 2008).

Recent studies have explored alternative fruit sources such as apricots, dates, and figs for pectin extraction, motivated by their abundance and local availability. From both economic and environmental perspectives, the valorization of such agro-industrial by-products into useful co-products like pectin represents a sustainable strategy for the food industry.

A major interest in pectic oligosaccharides (POS) lies in their structural diversity, which enables a wide range of functional and pharmacological applications. The development of pectin-derived oligosaccharides with prebiotic or pharmaceutical properties constitutes an emerging research area. These compounds have been associated with protective effects against colon cancer, antibacterial activity, suppression of hepatic lipid accumulation, inhibition of bacterial adhesion to epithelial cells, and stimulation of beneficial gut microbiota such as Bifidobacterium and Eubacterium rectale (Qiang et al., 2009; Manderson et al., 2005).

Accordingly, the present study was devoted to the extraction and physicochemical characterization of pectin from orange peels (PEOP), followed by the evaluation of its in vitro antioxidant potential using DPPH, ABTS, and FRAP assays.

MATERIALS AND METHODS

Reagents

Folin–Ciocalteu, Ethanol, sodium carbonate, DPPH (2,2-diphenyl-1-picrylhydrazyl) ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid, methanol, ethanol, epinephrine, NaCl, hydrochloric acid (HCl), and sodium hydroxide (NaOH) were purchased from sigma-Aldrich Co., Munich (Germany). All the other chemicals used were of analytical grade.

Preparation of orange peel powder

A single batch of “Maltaise demi-sanguine” oranges was collected in March 2019 through an agronomist from the Menzel Bouzalfa region. The harvested fruits were at commercial maturity, exhibiting an orange color and an average weight of 185 ± 7.07 g. Upon arrival at the laboratory, the oranges were washed with tap water, and the peels were carefully separated from the fresh fruits. The fresh peels were oven-dried at 50 °C for three days, finely ground using an electric blender, and sieved to obtain a uniform powder. The resulting powder was stored in tightly sealed, dark-labeled containers at room temperature until further use for pectin extraction.

Extraction of pectin

Most soluble pectin from fruits is released into the juice during processing. The extraction process aims to solubilize the remaining pectins in the peel using a diluted acid under heat. The main steps of the extraction procedure were as follows:

• Preparation of raw material and solubilization of protopectin.

• Alcoholic precipitation of pectin.

• Washing, drying, and conditioning of the extracted pectin.

Pectins were extracted from the orange peel cell wall material using diluted hydrochloric acid (HCl). The extraction conditions (pH, temperature, and duration) were optimized to achieve the highest possible yield.

Orange peels were separated and oven-dried at 50 °C for three days, then ground using a blender, and the resulting powder was stored at room temperature (25 °C). For extraction, 10 g of peel powder were mixed with 495.8 mL of distilled water and 4.2 mL of HCl, adjusting the pH to 3. The mixture was stirred and heated at 100 °C for 10 min, then cooled to room temperature. The suspension was centrifuged at 5000 × g for 20 min, and the supernatant was collected and adjusted to pH 7. Absolute ethanol (twice the supernatant volume) was then added under gentle stirring for 5 min at room temperature, followed by incubation for 12 h to allow pectin precipitation. The precipitate recovered by centrifugation, washed with 96% ethanol, and oven-dried at 40 °C until a constant weight was obtained. The dried pectin was finally dissolved in water to prepare the pectic extract of orange peels (PEOP) for further analyses.

Physicochemical characterization

Yield

After extraction, the wet pectin was dried at 40 °C to a constant weight, cooled in a desiccator, and weighed. The yield (%) was calculated relative to the dry weight of the raw material using the following formula:

The moisture content and dry matter content were determined according to standard gravimetric methods.

Ash and organic matter content

The ash content and organic matter content (MO) were determined following the AOAC (1990) method. One gram of the dry powder was placed in a pre-weighed porcelain crucible and incinerated in a muffle furnace at 550 °C for 3 h. The loss in weight corresponds to organic matter, while the residue represents the mineral (ash) content (Cunniff and Washington, 1997).

where:

PI = initial weight (crucible + sample before incineration),

PF = final weight (crucible + ash),

PE = weight of the sample.

Determination of Total Polyphenols

The total phenolic content (TPC) was determined using the Folin–Ciocalteu method as modified by Singleton et al. (1988). Briefly, 25 µL of extract were mixed with 2.125 mL of Folin–Ciocalteu reagent and 2 mL of distilled water. After vortexing, the mixture was left to stand for 3 min, then 375 µL of 10% sodium carbonate was added. The reaction mixture was incubated in a water bath at 37 °C for 2 h. The absorbance was measured at 760 nm using a spectrophotometer (Singleton, 1999).

The TPC was calculated from a calibration curve prepared with gallic acid and expressed as mg gallic acid equivalent per g of dry matter (mg GAE/g DM).

Determination of total flavonoids

Total flavonoid content (TFC) was determined according to the method of Zhishen et al.. Briefly, 0.5 mL of PEOP was mixed with 120 µL of 5% $NaNO_2$. After 5 min, 120 µL of 10% $AlCl_3$ and 800 µL of 1 M NaOH were added. The absorbance was recorded at 510 nm. The TFC was calculated from a calibration curve using quercetin as a standard and expressed as mg quercetin equivalent per g of dry matter (mg QE/g DM) (Zhishen et al., 1999).

Determination of condensed tannins

Condensed tannins were quantified using the vanillin–HCl method described by Price et al. (1978). In an acidic medium, vanillin reacts with condensed tannin units to form a red complex measured at 550 nm. Quantification was performed using a catechin calibration curve, and results were expressed as mg catechin equivalent per g of dry matter (mg CE/g DM) (Price et al., 1978).

Protein content

Protein concentration was determined by the Bradford method (Bradford, 1976), which is based on the binding of Coomassie Brilliant Blue G-250 dye to proteins, causing a shift in absorbance maximum from 465 to 595 nm. The absorbance was measured at 595 nm, and the protein content was calculated using a bovine serum albumin (BSA) standard curve.

Total sugar content

Total neutral monosaccharides were estimated calorimetrically according to Dubois et al. (1956). One milliliter of extract was mixed with 50 µL of 75% phenol, followed by rapid addition of 2.5 mL of concentrated sulfuric acid. The mixture was kept at room temperature in the dark for 30 min, and the absorbance was measured at 485 nm. Total sugars were quantified using a glucose calibration curve and expressed as mg glucose equivalent per g of dry matter (mg GE/g DM) (DuBois et al., 1956).

Antioxidant activity

The antioxidant potential of the extracted pectin was evaluated using three complementary in vitro assays: the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging test, the ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) assay, and the FRAP (Ferric Reducing Antioxidant Power) assay.

ABTS radical cation scavenging assay

The ABTS assay was performed according to Siddhuraju and Becker (2006). An ABTS solution (7 mM) was mixed with 2.45 mM potassium persulfate and incubated in the dark for 16 h at room temperature to generate the ABTS•+ radical cation. The solution was diluted to an absorbance of 0.70 ± 0.02 at 734 nm. Then, 1 mL of PEOP (at various concentrations) was mixed with 3 mL of ABTS•+ solution and incubated in the dark for 60 min (Siddhuraju 2006). Absorbance was measured at 734 nm, and the percentage inhibition was calculated as:

where A_0 is the absorbance of the control and As is the absorbance of the sample.

DPPH radical scavenging assay

The DPPH assay was performed following Gorinstein et al. (2004). A mixture of 200 µL of different concentration of PEOP and 2.8 mL of methanolic DPPH solution (0.1 mM) was shaken vigorously for 30 s and incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm, and the percentage inhibition of DPPH radicals was calculated using the same formula as above (Gorinstein et al., 2004).

FRAP assay

In this study, the FRAP assay was performed following the method of Chokri et al. (2024), with slight modifications. The reaction mixture consisted of 1.25 mL of 0.2 M phosphate buffer (pH 6.6), 1.25 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$], and 1 mL of pectin extract (PEOP) at various concentrations (ranging from 2 to 60 µM). The mixture was incubated at 50 °C for 20 min to allow the reduction of ferricyanide to ferrocyanide. After incubation, 1.25 mL of 10% trichloroacetic acid was added to stop the reaction, and the mixture was centrifuged (Eppendorf centrifuge, Hamburg, Germany) at 4000 rpm for 10 min to remove any precipitate. An aliquot of the supernatant (1.25 mL) was then combined with 1.25 mL of distilled water and 0.25 mL of freshly prepared 0.2% ferric chloride ($FeCl_3$). The resulting solution was incubated in the dark for 10 min, and absorbance was measured at 700 nm using a UV–Vis spectrophotometer (Techcomp S1020). This assay evaluates the formation of a ferric ion complex (Prussian blue), where higher absorbance values indicate greater reducing power of the pectin extract (Chokri et al., 2024).

RESULTS

Phytochemical study

Physicochemical characterization

The evaluation of several physicochemical parameters of the Extracted Pectic Extract from Orange Peels (PEOP) revealed the following results: yield 19%, moisture content 13%, ash content 10.32%, organic matter 89.86%, dry matter 87%, protein content 0.1%, and total sugars 16.26% (Table 1).

Phenolic composition analysis

The phytochemical screening (Table 2) showed that the pectic extract from orange peels contained low amounts of total polyphenols, flavonoids, and condensed tannins.

In vitro antioxidant activity evaluation

The antioxidant activity of the pectic extract of orange peel (PEOP) was evaluated using three complementary assays: DPPH radical scavenging, ABTS radical scavenging, and reducing power tests.

DPPH radical scavenging activity

As shown in the first graph (Figure 1A), the DPPH inhibition percentage of PEOP increased in a concentration-dependent manner, indicating that its free radical scavenging capacity rises with higher concentrations. However, ascorbic acid (vitamin C) exhibited stronger activity across all tested concentrations. At the highest concentration (5 mg/mL), PEOP achieved approximately 80–85% inhibition, compared to nearly 95–100% for ascorbic acid.

PEOP possesses a notable ability to neutralize DPPH radicals, though it remains slightly less potent than the standard antioxidant, ascorbic acid.

ABTS radical scavenging activity

The ABTS inhibition graph (Figure 1B) also shows a dose-dependent increase for both PEOP and ascorbic acid. The scavenging activity of PEOP is comparable to that of ascorbic acid, especially at higher concentrations (3–4 mg/mL), where both exceed 80% inhibition.

The pectic extract displays a strong ABTS radical scavenging capacity, suggesting the presence of bioactive compounds capable of donating electrons or hydrogen atoms to stabilize free radicals. The calculated half maximal inhibitory concentrations (IC_{50}) were 2.32 mg/mL for DPPH and 2.00 mg/mL for ABTS. A lower IC_{50} value indicates a higher antioxidant capacity. The pectic extract exhibited strong antioxidant potential, with IC_{50} values comparable to those of ascorbic acid, used as the reference compound (Table 3).

Reducing power (OD 700 nm)

In the reducing power assay (Figure 1C), PEOP demonstrated an increasing absorbance with concentration, confirming its ability to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions. However, its reducing potential was lower than that of BHA (Butylated Hydroxyanisole), a synthetic antioxidant. Although PEOP shows moderate reducing power compared to BHA, it still indicates effective electron-donating capacity, reinforcing its antioxidant potential.

DISCUSSION

The fruit peel, or pericarp, consists of two layers: the outer epicarp (flavedo or zest), brightly colored due to flavonoids, and the inner mesocarp (albedo), which is white and spongy (Rana et al., 2023). Citrus peel composition varies with variety, climate, and environment. Pectins, plant-derived polysaccharides abundant in fruit cell walls, are industrially extracted from citrus peels and apple pomace and represent valuable bioactive compounds (Voragen et al., 1995).

In the present study, we first demonstrated that the pectic extraction yield was approximately 19%. However, this yield varies among different fruits and depends on the extraction conditions such as pH, temperature, duration, and the type of acid used for solubilization (Yapo and Koffi, 2006; Faravash and Ashtiani, 2008).

Regarding the physicochemical characterization, our results showed that the moisture and total sugar contents were consistent with the values established by FAO/WHO standards, indicating acceptable levels for good stability during storage. Conversely, the ash content of PEOP was about 10.3%, a value close to that reported by Delluca and Joslyn (1957), who found that the ash content of pectin precipitated by mineral salts ranges between 8 and 25%. These levels increase with the pH of precipitation and the concentration of hydrochloric acid, whereas the use of organic solvents such as ethanol tends to reduce ash content by enhancing the removal of impurities during pectin precipitation. According to Cheftel, mineral salts are co-precipitated with pectin when precipitation occurs in alcohol concentrations of 50–60% (Cheftel, 1980). However, Nithish et al. obtained lower ash contents in pectin precipitated from lemon peels using aluminum chloride (Nithish et al., 2025).

Li et al. (2007) reported that orange peels exhibit a notable affinity for metal ions such as Ni, Cu, Pb, Zn, and Co, suggesting potential non-food applications, particularly the use of pectins as heavy metal chelating agents in wastewater treatment.

The dry matter, organic matter, and mineral matter contents were 87.0%, 89.9 %, and 10.3%, respectively. These values may reflect the climatic conditions of the sampling area (North-East Tunisia), a mild and humid region characterized by high rainfall (Rejeb et al., 1993).

Our results also revealed that the protein content of the extracted orange peel pectin was within the acceptable limits established by Herbsthier and Fox, who reported a maximum protein level of 2.5% for high-quality pectin. The low protein values observed in our extract are probably due to partial hydrolysis by HCl (pH 3) during solubilization (Herbstreith and Fox, 2006). This hydrolysis depends directly on the type of acid used. Legentil et al. demonstrated that HCl is more effective in protein hydrolysis compared to cyclohexane-trans-1,2-diaminetetra-acetate (CDTA) (Legentil et al. 1995).

According to Yapo and Koffi, solubilization of pectin using acids such as citric acid may lead to co-solubilization of proteins, with protein levels ranging between 1.4 and 5.1%, compared to 0.9% for pectin solubilized in oxalate or water (Yapo and Koffi, 2006). These proteins are associated with pectins through arabinogalactan linkages (Seymour and Knox, 2002).

The total sugar content of our orange peel pectin (16.3%) was also close to the standard value (16.0%) reported by LEU and FAO/WHO (cited by Herbstreith and Fox, 2006).

Numerous studies have confirmed that cell wall polysaccharides, particularly pectins, exhibit qualitative and quantitative variations depending on factors such as fruit variety, maturity stage, geographic origin, and storage conditions (Kurz et al., 2008). Kurz et al. further noted that pectins are more affected by fruit ripening than other polysaccharides like cellulose and hemicellulose, which remain relatively stable. According to Rous and Crandallp, purer pectins have high galacturonic acid content and low ash content (Rouse and Crandall, 1978), while Levinge et al. (2002) reported values ranging from 29.0 to 52.8%.

The analysis of total polyphenols, flavonoids, and tannins in our extract revealed low concentrations of 1.17 mg GAE/g DM, 0.55 mg QE/g DM, and 0.1 mg CE/g DM, respectively. Similar findings have been reported by Ralet et al., and Yapo et al., who observed phenolic acid contents between 0.1 and 0.7%, gradually reduced through successive alcohol washings at different concentrations (Ralet et al., 2005; Yapo et al., 2007).

Moreover, Kurz et al. demonstrated that the polyphenolic composition of fruits varies both qualitatively and quantitatively depending on the fruit species, variety, maturity stage, geographical origin, and storage conditions of the raw material (Kurz et al. 2008).

Finally, the antioxidant activity of the pectic extract of orange peel (PEOP) was evaluated using DPPH, ABTS, and reducing power assays, and the results showed a clear concentration-dependent increase in activity across all tests (Figure 1). In the DPPH assay, PEOP exhibited a strong radical scavenging effect, reaching nearly 80% inhibition at 5 mg/mL, although slightly lower than ascorbic acid. Similarly, in the ABTS assay, PEOP demonstrated comparable scavenging activity to ascorbic acid at higher concentrations, confirming its efficiency in neutralizing free radicals. The reducing power of PEOP also increased with concentration but remained lower than that of BHA, indicating a moderate electron-donating ability. Overall, these results highlight that PEOP possesses significant antioxidant potential due to its richness in bioactive compounds such as polyphenols, flavonoids, and pectic polysaccharides, making it a promising natural source of antioxidants for food and pharmaceutical applications.

The evaluation of the antioxidant capacity of the orange peel pectic extract against DPPH and ABTS radicals revealed a significant activity, with IC50 values of 2.32 and 2 mg/mL, respectively. This richness in polysaccharides and antioxidant power suggests that pectin contributes to nutritional balance and promotes optimal physiological function. The presence of high sugar levels, mainly sucrose, is known to facilitate intestinal transit and improve bowel function (Rtibi et al., 2016; 2017), emphasizing the therapeutic relevance of these pectic compounds in the digestive system. Additionally, the hydroxyl groups in pectin molecules contribute to their antioxidant capacity. In fact, pectic polysaccharides from apple, commercial pectin, and citrus peel pectin have been shown to exhibit strong antioxidant activity (Wang and Lü, 2014; Wang et al., 2014).

CONCLUSION

Orange peel pectin was successfully extracted with a yield of 19% and displayed favorable physicochemical properties. Although it contained low amounts of polyphenols, flavonoids, and tannins, the extract exhibited significant antioxidant activity in DPPH, ABTS, and FRAP assays. Its polysaccharide-rich nature, combined with antioxidant potential, suggests applications in food, nutraceutical, and therapeutic fields. These findings highlight the value of orange peel as a sustainable source of bioactive pectin.

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