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TUNISIAN *OPUNTIA FICUS-INDICA* FRUIT PEELS: BIOCHEMICAL AND MICROBIOLOGICAL CHARACTERIZATION AND POSSIBLE APPLICATIONS

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Abstract

In Tunisia, the number of prickly pear seed oil companies is currently increasing. However, a large amount of prickly pear fruit by-products is discarded. Proper utilization of these by-products, in particular fruit peels, could lead to the obtaining of a new important source of nutraceutical compounds. This investigation was aimed at conducting a phytochemical screening and assessing the antibacterial properties of prickly pear peels cultivar '*orange*'.

These samples were analyzed fresh and oven-dried at 45 °C to develop concepts for applications in the food industry as potential sources of fibers and antimicrobial "green" additives. The proximate compositions of both prickly pear peels were determined along with total phenolic compounds, carotenoids, and antioxidant activity. Free phenolic compounds were determined using liquid chromatography coupled with mass spectrometry. Furthermore, the antibacterial effectiveness of the prickly pear peel extracts was tested against selected foodborne pathogens.

The highest concentrations of dietary fiber (22.7 g/100 g d.m.) and carotenoids 10.90 mg/100 g d.m. were observed in oven-dried prickly pear peels, which also showed the highest inhibition of the DPPH radicals. The antibacterial activities showed a relevant growth inhibition against *Bacillus cereus* and a partial inhibition against *Staphylococcus aureus*. Prickly pear peel is a neglected nutritional and antibacterial source that should be widely valued as food additive.

Keywords: prickly pear, antioxidant, antimicrobial, phenolic acids, by-products, valorization

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Introduction

The growing concern on health promoting food alongside the consumers' awareness triggers food scientists to investigate new plant-based natural sources that allow obtaining bioactive compounds (Namir *et al.*, 2013; Monteiro and Claro, 2013). Furthermore, research studies have been focusing on tests to determine the suitability of these compounds as nutritional food additives and preservatives (Ayala-Zavala *et al.*, 2011). The extend of agro-industrial by-products with added nutritional value as new food supplements was investigated as a promising source of dietary fiber, oligosaccharides, and antioxidant compounds and their valorization could be economically attractive (Diaz-Vela *et al.*, 2013).

Prickly pear (PP) is the main edible fruit widely grown in Tunisia with 550,000 ha, as well as in many other Mediterranean countries such as Italy, Spain, and Morocco (Inglese *et al.*, 2017). Its global production is estimated at 1,862,413 tons (APIA, 2021). In Tunisia, this fruit is mainly consumed as fresh raw food. However, in the last few years, several prickly-pear derived products obtained from fruit industrial exploitation, such as jam, juice, and especially seed oil, came to the fore (Bouazizi *et al*., 2020).

Fruit peels are the main solid by-product originating from industrial processing and represent as high as 50% of the fresh weight. Another sobering fact is that around 500 kg of fruit peels are generated for each liter of prickly pear seed oil. Millions of kg of prickly pear fruit peels are discarded yearly in prickly pear seeds oil unities, causing huge disposal problems. The valorization of discarded peels can help reduce the impact on the environment and, at the same time, serve as a source of bioactive compounds, such as phenolics, carotenoids and other pigments, with high antioxidant activity, fibers, minerals (Nawirska and Kwaśniewska, 2005).

For all these reasons, this research study aimed at investigating the phytochemical characteristics as well as the antibacterial properties of fresh and oven-dried samples of prickly pear fruit peels cv. *orange*. Possible applications can be identified in the development of superfoods and nutraceuticals due to their potential source of antioxidants and "green" antimicrobial additives.

Materials and methods

Sampling and peel dehydration

PP fruit peels were collected from a local seed oil industry (Agroline, Bir Bou Rekba, Tunisia) during the oil extraction process, on the same day as the fruit peeling. Peels were then rinsed with tap water and sanitized using sodium hypochlorite solution (4%) for 30 min. The gross sample was divided in two portions: a sublot was frozen at -20 °C inside plastic bags, while the other one was oven-dried at 45 °C for 48 h (Electrothermal Blast Drying Oven WLG-45B, Tianjin, China).

Chemical parameters and proximate composition

pH and titratable acidity were determined on 10 g of ground sample in 20 mL of deionized water (NF V 05-101, 1974). Moisture content, ash, protein, and fat contents of fresh and dried peels were determined according to AOAC official methods (2000). Fibers were measured using the method AACC-32-10 (2000), cellulose, hemicellulose, and hydrosoluble polysaccharides were determined using the method described by Sun *et al.* (2003), and finally, carotenoids content was analyzed through the specific AOAC method (2000).

Free soluble phenolic acids determination

Phenolic acid determination of oven-dried peels only was performed using the method described by Montevecchi *et al.* (2018). An Agilent Technologies liquid chromatography (Agilent 1200 series, Santa Clara, CA, U.S.A.) equipped with a degasser, a binary pump, an autosampler, and coupled with a 6410B triple-quadrupole mass spectrometer was used.

Preparation of prickly pear peel extracts

Aliquots of homogenized samples (5 g) of both fresh and dried peels were separately extracted by stirring for 4 h at 25 °C with either 150 mL of methanol or deionized water to obtain methanol prickly pear peel extracts (MOP) and water prickly pear peel extracts (WOP), respectively. The tubes containing the samples were centrifuged at 6000 × *g* for 20 min at 4 °C. The clear supernatants were filtered (membrane pore size: 0.45 µm) and then concentrated under reduced pressure at 40 °C and further oven-dried at 40 °C overnight. Dried extracts were finally weighed and dissolved in either 1.5 mL of distilled sterile water for an antibacterial activity or in methanol for total phenolic content (TPC) and radical scavenging activity (RSA) determinations.

Determination of total phenolic content

The TPCs of MOP and WOP were determined using the Folin-Ciocâlteu reagent (Chu and Chen, 2006) and the results were expressed as g gallic acid equivalents (GAE)/100 g dry matter (d.m.).

Determination of radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\cdot}$) RSA was measured using the method described by Licciardello *et al.* (2015). An aliquot of 100 µL of the sample was added to 900 µL of DPPH solution (100 µM in MeOH) and vortexed. Trolox was used as a standard substance and the results were expressed as mmol Trolox equivalents (TE)/g d.m. Spectrophotometric measurements were carried out at 517 nm.

Microbiological analysis

An aliquot of 10 g of sanitized sample was taken and weighed to the nearest 0.01 g with a Bunsen burner and then mixed in 90 mL of sterile buffered peptone water into a stomacher bag apparatus and homogenized. The stock solution thus constitutes the 10^{-1} dilution. This suspension is homogenized and left at room temperature for 20 min, for the revivification of the germs. Serial dilutions were prepared using peptone

and buffered water. Homogenate samples were plated on appropriate media in Petri dishes. Plate count agar (30 °C for 72 h) was used for total mesophilic count (TMC), oxytetracycline-glucose-yeast extract agar (25 °C for 5 days) for yeasts and molds, and violet red bile glucose agar for *Enterobacteriaceae* (30 °C – 24 h for total coliforms and 44 °C – 24 h for fecal coliforms) (CQIASA, 2009).

Antibacterial sensitivity testing

The antibacterial effectiveness of the extracts was assessed against five foodborne pathogen bacterial strains (*Listeria monocytogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Bacillus cereus*) using the diffusion disc method (Amírez-Moreno *et al.*, 2017). An aliquot of 100 µL of the standardized inoculum of the test microorganisms (10^8 CFU/mL) was placed into sterile Petri dishes before pouring 20 mL of sterilized soybean casein digest agar (~45 °C), followed by gentle and thorough mixing. Holes with a diameter of 9 mm were punched aseptically into the solidified media, and 100 µL of extracts were introduced into the so-formed wells. The Petri dishes were incubated at 37 °C until a visible growth of test microorganisms in the control plates. The antimicrobial activity was expressed as the diameter size (mm) of the inhibition zones (including the well diameter) produced by the extracts against test microorganisms.

Statistical analyses

All analyses were carried out in triplicate. Results were expressed as mean values ± standard deviations.

Results and discussion

Proximate composition of fresh and oven-dried prickly pear fruit peels

Table 1 shows the chemical composition of fresh PP peels. The pH average value was slightly lower than the pH values of the pulp juice. However, it was not low enough to be considered sufficient for long preservation of the material. The data on the fibrous fraction is noteworthy at 4.78 g/100 g f.m. and includes a remarkable amount of hemicelluloses (1.35 g/100 g f.m.).

Protein and fat contents in the present study were higher compared to El-Beltagi *et al*. (2019) results. These substances can be conveniently extracted to exploit their emulsifying properties for various uses in food technology. Since the increase in protein and fiber content in oven-dried samples did not correspond to water removal from the fresh sample, a partial alteration of them might have occurred during the prolonged heating process. For this reason, protein and fiber should be extracted from fresh samples for higher yields.

Carotenoids represent the main water-insoluble pigments in the PP peels and are heat sensitive. Their concentration in fresh samples was as high as 6.47 mg/100 g f.m., similar to the results obtained by Hernàndez García *et al*. (2020), however carotenoids showed a large reduction in oven-dried samples, as did protein and fibers. Because of the hydrophobic nature of carotenoids, their extraction effectiveness is heavily affected by the water content of the substrate. Furthermore,

thermal dehydration methods could cause the degradation of those compounds (Saini and Keum, 2018).

Table 1 shows that oven-dried peels have a suitable moisture content of < 10% to ensure long storage when transformed into a fine powder. PP peels also showed a considerable amount of ash, 14.57 g/100 g. This result is in line with what has been described by Silva *et al*. (2021), who have suggested that PP peels are rich in several crucial dietary minerals, namely potassium, magnesium, and calcium. Magnesium and calcium are particularly beneficial in osteoporosis prevention (Sunyecz *et al*., 2008; Castiglioni *et al*., 2013).

The crude fiber content (20.7 g/100 g d.m.) of oven-dried peels is noteworthy. This figure is higher than the values found by El-Sharnouby *et al*. (2012) regarding wheat flour (1.60 g/100 g d.m.), date powder (9.4 g/100 g d.m.), and wheat bran (15.4 g/100 d.m.).

The protein content makes the PP peel powder a good emulsifier for the preparation of biscuits and cakes, whereas the fat should be suitably stabilized to avoid rancidity phenomena.

Table 1. Proximate composition of fresh and oven-dried prickly pear fruit peels cv *orange* (mean of three measures ± standard deviation).

Chemical analysis (Unit of measure)	Fresh peels	Oven-dried peels
Titratable acidity (g citric acid eq./100 g f.m.)	0.06 ± 0.01	0.06 ± 0.01
pH (- log [H^+])	4.67 ± 0.13	4.78 ± 0.12
Moisture (g/100 g f.m.)	90.38 ± 0.06	9.11 ± 0.07
Ash (g/100 g f.m.)	2.03 ± 0.02	14.57 ± 0.02
Protein (g/100 g f.m.)	0.91 ± 0.04	3.31 ± 0.23
Fat (g/100 g f.m.)	0.28 ± 0.01	2.72 ± 0.20
Crude fiber (g/100 g f.m.)	4.78 ± 0.13	20.70 ± 0.06
Cellulose (g/100 g f.m.)	1.84 ± 0.01	7.48 ± 0.15
Hemicelluloses (g/100 g f.m.)	1.35 ± 0.00	8.14 ± 0.64
Hydrosoluble polysaccharides (g/100 g f.m.)	0.46 ± 0.01	1.57 ± 0.05
Carotenoids (mg/100 g f.m.)	6.47 ± 0.05	10.90 ± 0.03

Free phenolic acids determination using RP-LC-MS-TQ

Table 2 shows the quantification of free phenolic acids in oven-dried prickly pear peels.

Table 2. Free phenolic acids of oven-dried fruit peels.

	Concentrations (mg/kg f.m.)
Gallic acid	Not detected
Vanillic acid	6,218 ± 295
Caffeic acid	251 ± 6
Syringic acid	928 ± 24

Vanillin	368 ± 18
p-Coumaric acid	501 ± 6
trans-Ferulic acid	11,448 ± 373
Sinapic acid	1,118 ± 46
Salicylic acid	158 ± 6
trans-Cinnamic acid	78 ± 1
Total	*20,990 ± 681*

Gallic acid was not detected, while *trans*-ferulic acid showed the highest content followed by vanillic acid, sinapic acid, and syringic acid. The rest of free phenolic acids showed concentrations lower than 900 mg/kg f.m. Such data are in agreement with some previous studies (Hernàndez García *et al.*, 2020; García-Cayuela *et al.*, 2019).

TCP and RSA of prickly pear fruit peel extracts

Figure 1 shows the TPC and the RSA of the PP peel extracts. Results displayed that methanolic extracts (MOP) obtained from both fresh and oven-dried PP fruit peels yielded similar values in TPC (31.3-34.1 g GAE/100 g d.m.) and RSA (329.6-334.2 mmol TE/g d.m). This could be due to the drying temperature (45 °C) used in the process, which did not affect the phenolic content and the radical scavenging activity. Previous research studies have reported similar results (Al-Rawahi *et al.*, 2013). Hernández Garcia et al. (2020) indicated that PP peels possess a higher DPPH scavenging activity than other plant substrates such as pomegranate pulp.

The TPC and RSA of the aqueous extracts (WOP) showed significantly lower values than those referred to the MOP extracts, due to the lower extraction power of the solvent.

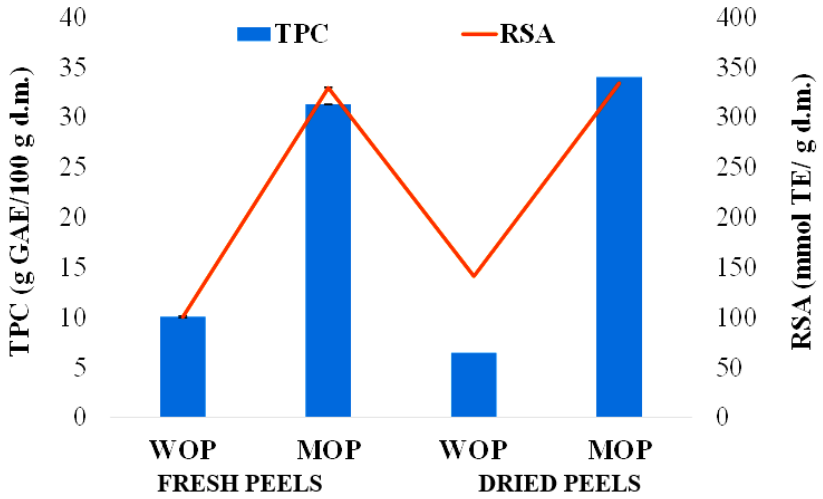

Figure 1. Total phenolic content (TPC) and radical scavenging activity (RSA) of the samples (n = 3). WOP: water extract. MOP: methanol extract. TE: Trolox equivalents. GAE: gallic acid equivalents.

Microbial analysis

The microbiological quality assessment of fresh peels was necessary to control the hygiene and safety of the raw material after sanitization with NaClO 4%. Oven-dried peels were also subjected to microbiological counts to assess possible contamination during the process. Table 3 shows the counts of total mesophilic bacteria, yeasts and molds, total coliforms, and fecal coliforms present in the PP fresh and oven-dried peels.

Microbial counts showed that the amounts of TMC (7.4×10^2 CFU/g) and YM (1.3×10^2 CFU/g) were both lower than the critical threshold of 1.0×10^3 CFU/g, whereas total and fecal coliforms were absent. This confirms the high hygienic quality of the raw material.

The microbiological evaluation of oven-dried PP peels showed that the endogenous flora included total mesophilic microorganisms (3.3×10^2 CFU/g) while yeasts and molds, total coliforms, and fecal coliforms were absent. These results are essentially lower than the legal limits which require that the TMC count does not exceed 20×10^4 CFU/g according to ISO 4833, and that yeasts and molds do not exceed 1.0×10^4 CFU/g according to ISO 7954 (CQIASA, 2009). These outcomes exhibit the great safety properties of the process conditions for obtaining dried samples.

Table 3. Contamination of fresh and oven-dried prickly pear peel. Antibacterial activity (diameter of the inhibition zone measured in mm) of dried prickly pear peels' extracts.

	TMC	**YM**	**TC**	**FC**
Fresh PP peel (CFU/g)	$7.4 \times 10^2 \pm 0.963$	$1.3 \times 10^2 \pm 0.203$	Absent	Absent
Oven-dried PP peel (CFU/g)	$3.3 \times 10^2 \pm 0.126$	Absent	Absent	Absent
Bacterial strains	**Inhibition zone (mm)**			
	Peel extracts		**Control antibiotic**	
	WOP	**MOP**	**Ampicillin**	
Listeria monocytogenes FMCC 13-128	9 a	Nd	16 b	
Staphylococcus aureus ATCC 6538	Nd	7.5 a	16 b	
Bacillus cereus ATCC 168	27 a	31 b	22 c	
Escherichia coli ATCC 35150	6.5 a	7.5 a	20 b	
Salmonella ATCC 13076	8 a	9 a	14 b	

TMC: Total mesophilic bacteria, YM: yeasts and molds, TC: total coliform; FC: fecal coliform; Data indicate means ± SD. WOP: water extract; MOP: methanol extract; Nd: no inhibition shown

Antibacterial activities of dried prickly pear peels extracts

Table 3 shows the antibacterial activities of PP peels extracts, expressed as diameters of inhibition evaluated against three Gram positive (*S. aureus*, *B. cereus*, and *L. monocytogenes*) and two Gram negative (*E. coli* and *Salmonella sp.*) bacterial strains.

MOP did not exert visible effects on *Listeria monocytogenes*, while the WOP showed no inhibition zone against *Staphylococcus aureus*. On the basis of the inhibition zone around the hole diameter, *Bacillus cereus* was the most sensitive microorganism to both extracts. In addition, a bacteriostatic effect of WOP and MOP extracts toward this latter strain was observed (Figure 2).

These results proved that MOP was more effective than WOP against *Staphylococcus aureus* and *Bacillus cereus* in terms of bacteriostatic activity, while WOP was active against *L. monocytogenes*. The differences between extracts could be attributed to phenolic acids implicated and their amounts. For example, caffeic acid and *p*-coumaric acid displayed a further antimicrobial effect in methanolic extracts according to Mansour *et al.* (2013).

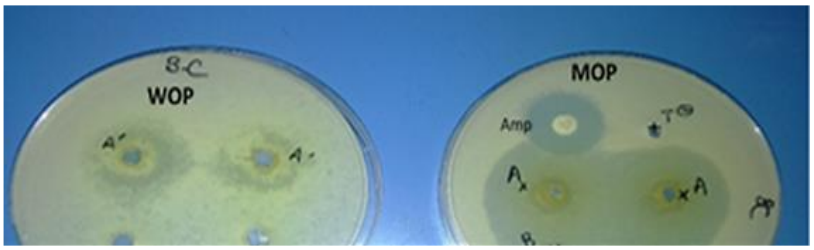

Figure 2. Growth inhibition of *Bacillus cereus* using water extract (WOP) and methanol extract (MOP) from prickly pear fruit peels. Amp: ampicillin (control antibiotic drug).

These results revealed the presence, in its composition, of a broad spectrum of antimicrobial compounds. Several major characterizations of compounds and assessment of the relationship between structure-potential activity should be also evaluated to better understand the physiological behavior of these compounds.

Applications

Figure 3 outlines the possible uses of PP peels based on the results obtained in this and previous studies. Fresh peels can be exploited at a technological level for the presence of mucilage and a high quantity of pigments, as well as at a microbiological technological level for the presence of antimicrobial substances in potential food applications, including the field of nanoparticles. The choice of the specific microorganisms in the antibacterial sensitivity testing was based on the association of those strains with spoilage of refrigerated food, thus envisaging a possible use of the PP peels as a natural food preservative.

The oven-drying of PP peels paired with their guaranteed prolonged conservation opens up the possibility of obtaining a "flour" to be used as an ingredient in the preparation of baked goods (Bouazizi *et al.*, 2020). Furthermore, extraction processes lead to the obtaining of potentially interesting additives for their antioxidant and antimicrobial properties.

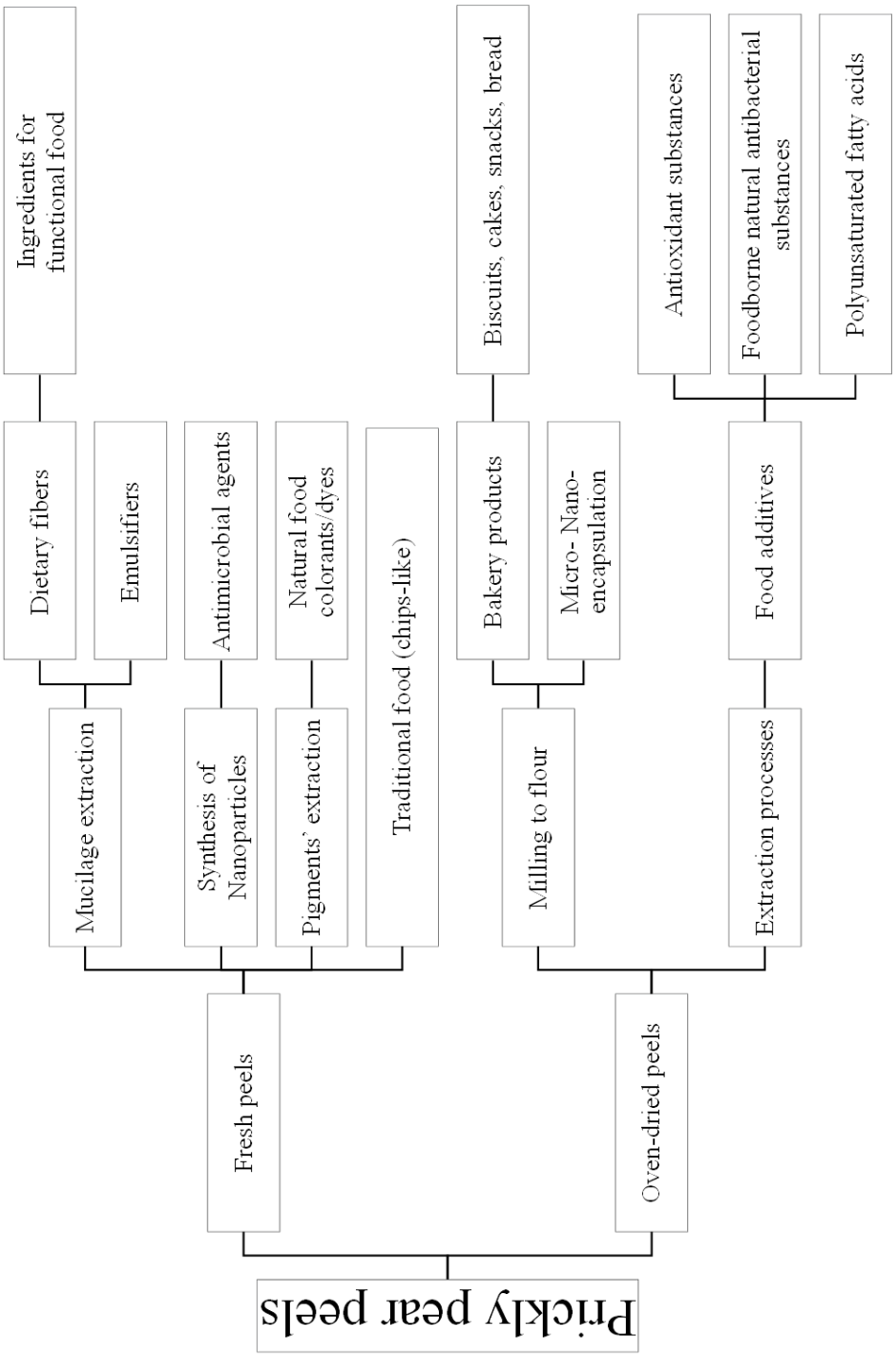

Figure 3. Possible uses of prickly pear peels in food industry.

Conclusions

Food process by-products, such as prickly pear fruit peels are neglected nutritional and antibacterial sources which deserve to be widely valorized as food additives. Results obtained so far show that peels are promising inedible waste products for phytochemical valuable compounds, in particular phenolic compounds which are characterized by powerful antioxidant and antimicrobial activities, as well as fibers suitable for use as additive food.

Overall, the powerful antioxidant and antimicrobial activities are mainly influenced by the extraction conditions, especially by the solvent polarity (water/methanol). Therefore, the optimization of the extraction methodology should be carried out to obtain a safeguard food supplement with high recovery in bioactive components.

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