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Immature *Opuntia ficus-indica* peel by-product as mayonnaise additive and natural anticoccidial drug

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Abstract: In recent years, the valorization of food by-products has attracted increased interest in the scientific community for ecological and economic reasons. *Opuntia ficus-indica* (OFI) peels are the primary by-product of prickly pears. Several studies claim that the beneficial bioactive compounds are concentrated in the fruit's peel. Therefore, the present investigation aimed to study OFI peel immature as a natural antioxidant in mayonnaise and its use as a natural treatment against coccidiosis, which is the main disease of broiler chickens. The finding confirmed that OFI peels are a source of phenolic compounds, including total phenolic compounds, flavonoids, tannins, and carotenoids (21.01±0.33, 1.12±0.28, 10.25±0.10 mg equivalent standard/g, and 5.62±0.53 mg β-carotene equivalent /100 g, respectively), having strong antioxidant activities over 87 percent (DPPH radical, iron chelation power, β-carotene bleaching). Using rancimat test OFI extract improves the mayonnaise's stability for 2.5 hours. Immature OFI peel extracts recorded a notable destruction rate of *Eimeria* oocysts. The current study promoted the use of immature OFI peel extracts as a natural food additive and as an antiparasitic treatment substitute.

Keywords: Opuntia ficus-indica; antioxidant; anticoccidial activity; chickens; mayonnaise

1. Introduction

Opuntia ficus-indica (OFI) fruit is much appreciated by consumers, yet its use in industry generates large quantities of by-products, with the peelers alone exceeding 40 percent [1]. Several studies have reported that fruit peels, especially those from OFI, are rich in various bioactive functional components [2,3]. With the aim of preserving the environment and minimizing industrial waste, the study of the composition of *Opuntia ficus-indica* peels has been the subject of several scientific studies. El-Said, *et al.* [4] finding shows the high nutritional value of OFI peels : proteins, vitamins, fiber, fat, and mineral. In addition, various phytochemical compounds belonging to different chemical classes have been found in OFI peels, with bioactive compounds such as flavonoids, tannins, betalains, and carotenoids being the main compounds [5,6]. The richness of OFI's composition leads to a constant increase in peel by-product investigations.

The use of food by-products as food additives is one of the first steps in the process of enhancing their value, such as their use as natural food colorants and as natural antioxidant additives [7,8]. Soltan, *et al.* [9] found that enrichment of mayonnaise with phenolic, and flavonoid-rich extracts from plants such as *Rosa canina* could be an effective substitute for synthetic preservatives and antioxidants.

The second approach is to use by-products as natural alternatives to synthetic drugs, such as antimicrobials, anti-inflammatories, antioxidants, and antidiabetics [10-12]. *In vitro* anticoccidial studies constitute a new line of research. Recently, Amrane-Abider, *et al.* [13] found that OFI flowers have a great anticoccidial property. Therefore, the present study aims to investigate the bioactive compounds of immature *Opuntia ficus-indica*, their antioxidant activities, and their use as natural antioxidants in mayonnaise. In addition, their use as a natural anticoccidial drug was also investigated.

2. Materials and methods

2.1. Plant material

In Talandjast village, Bejaia city, Algeria, immature *Opuntia ficus-indica* (OFI) fruits were gathered. The samples were washed and peeled. After being lyophilized, the peels were crushed and sieved. The peel powder was kept fresh in the fridge.

2.2. Chemical reagents

All chemicals products purchased by Sigma Chemical company (Sigma-Aldrich GmbH, Germany). Diclosol®, Avico, Arab Industry Veterinary Co., Amman, Jordan.

2.3. Microwave- assisted extraction

The extraction of bioactive compounds from OFI peel was carried out according to Amrane-Abider, Nerin, Cannelas, Zeroual, Hadjal and Louaileche [8].

2.4. Determination of bioactive compounds

Total phenolic and flavonoid content**s** in immature *Opuntia ficus-indica* peels were determined according to Velioglu, *et al.* [14] and Bahorun, *et al.* [15] respectively.

The condensed tannin contents was determined according to Porter, *et al.* [16].

Determination of carotenoid contents was carried out by the method reported by Amrane-Abider, *et al.* [17].

Gallic acid, quercetin, cyaniding, and β-carotene standards were respectively used for total phenolic, flavonoid, condensed tannin, and carotenoid contents determination (Table 1). With the exception of carotenoids, all other compounds are expressed in mg of standard equivalent (GAE) per g of dry weight (DW).

2.5. Determination of antioxidant activities

Radical scavenger 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay and ferric reducing power were determined according to Molyneux [18] *and Amarowicz, et al. [19], respectively*. The two activities were *expressed as a percentage of inhibition* (Table 1).While, the capacity of immature OFI peel extract to inhibit β-carotene bleaching was measured using the Morales, *et al.* [20] method.

2.6. Mayonnaise manufacture and oxidative stability

Mayonnaises were manually prepared at laboratory scale according to Soltan, Gazwi, Ragab, Aljohani, El-Ashmawy, Batiha, Hafiz and Abdel-Hameed [9], with slight modifications. Whole chicken eggs, sunflower oil, vinegar, sugar, and salt, in the following proportions: 20, 70, 6, 3, and 1 g/100 g, were the main ingredients in the mayonnaise recipe. Three types of mayonnaise were prepared in triplicate. The difference between these mayonnaise formulas was in the antioxidant source: controls mayonnaise without antioxidants, mayonnaise contained 0.02% (0.2 mg/g) butyl-hydroxy toluene BHT ragher of mayonnaise 0.125% (1.25 mg/g) of immature *OFI* peel extract (this last was added after microwave extraction, the solvent was removed by rotavapor (Rotavapor® R-300, Buchi), and the extract was lyophilized).

The oxidative stability of mayonnaises was determined using the rancimat test according to the ISO International Standard [21] method.

2.7. Evaluation of the in vitro anticoccidial activity

The *in vitro* anticoccidial activity of immature *OFI* peels was determined according to the protocol reported by Amrane-Abider, Imre, Herman, Debbou-Iouknane, Zemouri-Alioui, Khaled, Bouiche, Nerín, Acaroz and Ayad [13] . The results were *expressed as a* percentage of *Eimeria* oocysts destroyed (Table 2).

2.8. Statistical Analyses

All the experiments were performed in triplicate, and the results were expressed as the mean value ± standard deviation (SD). Statistical analysis was performed using STATISTICA. 12

3. Results and discussion

3.1. Phenolic compounds and antioxidant activities

Phenolic compounds, particularly flavonoids, are excellent potential substitutes for active pharmaceutical ingredients [22]. According to the current study, liposoluble pigments and polyphenols are abundant in immature *OFI* peels. Total phenolic, flavonoids, and tannin values were 21.01±0.33, 1.12 ±0.28, and 10.25±0.10 mg of standard equivalent (GAE) per g of dry weight (DW). Total phenolic content results are not far from Algerian and South African varieties (28.68 and 17.59 mg GAE /g DW, respectively) [17,23]. However, it is higher than Egypt OFI peel (8.48 mg GAE /g DW) [24]. According to Table 1, the flavonoid content value in immature OFI peels was 1.12 ±0.28 mg quercetin equivalents (QE) per g dry weight (DW). This value was lower than Amrane-Abider, Nerín, Tamendjari and Serralheiro [17] (5.05± 0.12 mg QE/g DW), which may be due to the degree of maturity of the fruits. Several studies show that flavonoids are concentrated in the mature stage rather than the immature stage [5,25]. Some of the major flavonoids included in OFI peel are quercetin derivatives, isorhamnetin derivatives, and dihydrokaempferol [17,26]. In addition, immature OFI peel contains tannins 10.25±0.10 mg cyaniding equivalent (GAE) per g of dry weight (DW). Cardador-Martínez, Jiménez-Martínez and Sandoval [5] investigation reported that OFI peel contains 15–52% tannins. Carotenoid compounds are natural pigments that are usually known to exhibit antioxidant activities. In the present study, our sample contains 5.62±0.53 mg β-carotene equivalent /100g. That result was higher than that of OFI fuit (1.77±0.04 -2.65±0.04 mg/100 g) [27]. Cano, *et al.* [28] investigation shows that β-carotene, lutein, lycopene, violaxanthin, and neoxanthin are the primary carotenoids in OFI peel.

Table 1. Bioactive compounds and antioxidant activities of immature *Opuntia ficus-indica* peel.

Bioactive compounds	Contents	antioxidant activities	%
Total phenolic (mg /g DW)	21.01±0.33	DPPH radical,	90
Flavonoids (mg /g DW)	1.12 ±0.28	iron chelation power	88
Tannins(mg /g DW)	10.25±0.10	β-carotene bleaching	94
Carotenoids (mg/100g DW)	5.62±0.53		

Bioactive compounds are often linked to biological activities such as antioxidant and anti-pathogenic activities. Given the richness of OFI peel in bioactive molecules (total phenolics, flavonoids, tannins, and carotenoids), the good results shown in Tables 1 and 2 are not surprising. As shown in Table 1 OFI peel extract exhibit a strong antioxidant activities including DPPH radical (90%), iron chelation power (88%), and β-carotene bleaching (94%). It's in line with other investigations that confirm that OFI peel and seed have strong antioxidant activities, including DPPH radical scavenger and iron chelation power [17,29,30]. Moreover, in the β-carotene bleaching inhibition assay, Morales, Ramírez-

Moreno, Sanchez-Mata, Carvalho and Ferreira [20] show a high capacity of *Opuntia* peel in inhibiting lipid peroxidation.

3.2. Anticoccidial activity and mayonnaise oxidative stability

In view of the damage caused by avian coccidiosis and the parasites' resistance to synthetic treatments, a number of studies have focused on the ability of natural extracts to destroy parasites, in particular *Eimeria. As shown* in Table 2, immature *OFI* peel extract destroys the *Eimeria* oocysts by 22.25±0.38%. This result is not far from that found by Debbou-Iouknane*, et al.* [31] pulp olive extract (25.36%). However, they are four times higher than leaf olive extracts [32]. Our results are inferior to those found by Amrane-Abider, Imre, Herman, Debbou-Iouknane, Zemouri-Alioui, Khaled, Bouiche, Nerín, Acaroz and Ayad [13], where the OFI flowers show a great capacity to destroy the *Eimeria* oocysts (44.89%).

Table 2. Anticoccidial activity and mayonnaise oxidative stability of immature *Opuntia ficus- indica* peel extract.

Anticoccidial activity (oocyste number %)	
Control	100
Immature OFI peel	22.25±0.38
	Rancimat (hours)
Mayonnaise control	5.68±0.58[a]
Mayonnaise BHT	9.98±0.40[b]
Mayonnaise en-riched (OFI)	8.18±0.69[c]

Mayonnaise is an oil-water emulsion. The potential risk of oxidation is particularly significant due to the food's high fat content of over 70%. Therefore, the addition of antioxidants is necessary; natural antioxidants are even more valuable [33]. According to Table 2, immature OFI peel extract as a natural antioxidant increases the mayonnaise's oxidative stability by 2.5 h, with the induction time equal to 8.18±0.69 hours. In a similar vein, Raikos*, et al.* [34] discovered that natural beetroot (*Beta vulgaris L.*) extract has higher mayonnaise oxidative stability than commercial control. However, the oxidative stability of mayonnaise containing OFI peel extract was lower than that of mayonnaise containing BHT as an antioxidant. The synthetic antioxidant usually shows high oxidative stability, such as TBHQ (essential oil has been used as a natural antioxidant in mayonnaise) [35]. In *Opuntia ficus- indica* oxidative stability, Amrane-Abider, Nerin, Cannelas, Zeroual, Hadjal and Louaileche [8] investigation shows that OFI seed is a good antioxidant source in margarine.

4. Conclusion

Immature *Opuntia ficus-indica* peel is a source of bioactive molecules, including phenolic compounds (total phenolic compounds, flavonoids, and tannins) and terpenoids (carotenoids). The three antioxidant tests (DPPH radical, iron chelation power, and β-carotene bleaching) show that immature OFI peels have a good antioxidant capacity. The results of anticoccidial activities show that our extract has a good capacity to reduce *Eimeria* oocysts. Moreover, it has shown promise as a natural antioxidant in mayonnaise.

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