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Characteristic Analysis, Antioxidant Components, and Benefits of Pomegranate Peel and Red Onion Skin as Natural Antioxidants on Sunflower Oil Stability

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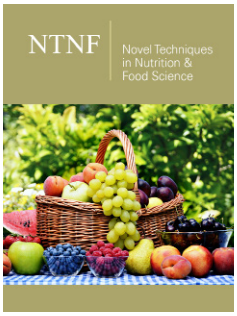

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Abstract

One of the most significant advancements in food technology recently is the management of natural antioxidants produced from plant waste to address issues with its sustainable production. The aim of the present study is to determine the chemical compositions, total tannin, phenolic, α-tocopherol, and antioxidant yields of red onion skin and pomegranate peel. Polyphenols compounds were identified by HPLC. Moreover, Sunflower oil was treated with aqueous and ethanolic extracts of red onion skin, and pomegranate peel as natural antioxidants at various concentrations compared with synthetic antioxidants (BHT and Catachin) at 80 °C for five hours each day for seven days. According to the findings, pomegranate peel and red onion skin had higher antioxidant yields from ethanol than from aqueous solution; also, pomegranate peel has a higher content of total tannin, and phenolic, while, red onion skin has a higher level of α-tocopherol. In comparison to these chemicals from red onion skin extract, the pomegranate peel extract utilizing HPLC contained the highest amounts of Syringic acid, Ferulic acid, Protocattechuic acid, Salicylic acid, and Resorcinol, respectively. On other hand, red onion skin extract contained the highest amounts of Coumarin, Caffic acid, P-Coumaric acid, Vanilne, Cinnamic acid, Quercetin, and Kaempferol. With time, lower acid values, peroxide values, and TBARS, also, higher iodine numbers were noticed than without it when sunflower oil was treated with increasing concentrations of extracts (ethanol and aqueous). The best results were noticed at 500ppm concentration. Pomegranate peel and red onion skin antioxidant extracts gave the best or the same effects recorded by synthetic antioxidants. It is therefore advised to use these edible extracts (natural antioxidants) extracted from onion skin and pomegranate peel instead of these toxic synthetic antioxidants.

Keywords: Red onion skin; Pomegranate peel; Antioxidants; Sunflower oil; HPLC; BHT; TBABS; Peroxide value

Abbreviations: BHT: Butylated Hydroxyl Toluene; TBARS: Thiobarbituric Reactive Substances; TBHQ: Tert-Butyl Hydroquinone

Introduction

The loss of a vital source of nutrients and phytochemicals makes food waste a serious issue for food processing businesses. The challenge of avoiding waste by turning processed food into usable items is brought on by rising customer demand for them [1]. One of the earliest consumed fruits, the pomegranate (Punica granatum) is a member of the Punicaceae family. Pomegranate peel has high concentrations of phenolics, flavonoids, complex polysaccharides, and minerals like potassium, calcium, phosphorus, magnesium, and salt [2]. Pomegranate fruit is recognized as a potent nutritional anticancer agent, according to numerous recent research [3]. According to Howell & D'Souza [4], pomegranate fruit (Punica granatum L.) has been found to have medicinal properties that include antioxidants, anti-carcinogens, anti-inflammatory, antibacterial, and anti-parasite properties. When compared to other recognized antioxidants like ascorbic acid, vitamin E, and beta-carotene, polyphenols in pomegranate fruit have a wider variety of effects on numerous types of free radicals. Because of their antioxidant properties, phytochemicals (such as phenolic compounds, anthocyanins, tannins, and others) found in plants have medicinal effects [5]. Recently, there has been a lot of focus on the inclusion of polyphenols in meals, possibly because of their potent antioxidant properties (scavenger of free radicals). The pomegranate peel has the strongest antioxidant capacity, according to recent studies [6]. Onion is a crop that is grown all over the world for its medicinal and functional benefits in addition to being used for basic human consumption [7]. Due to behavioral changes that increased the demand for freshly cut and cooked veggies (onion), more waste has been produced globally, which has led to a 25% increase in onion output. According to Sagar et al. [8], the majority of onion processing waste is skin (peel), which consists of two outermost leathery layers, undersized roots, and malformed bulbs. is still an underutilized commodity while having the potential to be a source of biologically active substances like phenols and flavonoids [9]. Peel also has a variety of medicinal effects and has antioxidant, antibacterial, and antimutagenic properties [10]. The majority of the by-products produced during the processing of onions are not used, despite the fact that they are packed with phytochemicals and bioactive substances. Due to a rising interest in sustainable resource utilization and the circular economy, nutritional supplements and medicinal treatments can still be further reformulated even though the mechanism of action of the compounds contained in onions has been investigated. A wide range of pharmacological pathways are used by the different onion chemical components to prevent disease [11]. Since the onion skin is not edible, it is quickly removed before processing and selling, but due to its composition and smell, it cannot be used as animal feed or disposed of in a landfill. As a result, the onion processing process generates a significant amount of waste. In order to produce allium sustainably, onion peel must be turned into a "food by-product" by valorizing it. In the food, cosmetics, and pharmaceutical industries, by-products of food processing frequently contain useful compounds that can be employed as functional additives [12]. Therefore, the current research is carried out to shed light on the pomegranate peel and red onion skin chemical composition, identification of polyphenolic, quercetin, kaempferol, and ᶐ-tocopherol by HPLC, and utilization of these compounds to enhance the shelf life of the oil by way of adding natural antioxidants which extracted from pomegranate peel and red onion skin to the sunflower oil.

Materials and Methods

Materials

The pomegranate specimen needed for the planned study was bought from an Egyptian fruit store. The needed fruit rind was cut and removed from the pomegranate fruits after they had been easily peeled. The fruit rind was then dried in an oven at 40 °C for 24 hours. The fruit rind (pomegranate peel) was mechanically ground into a fine powder and stored at -18 °C until used [13]. The skin of a red onion Allium cepa L was obtained as a waste (by-product) from the Dehydro-Foods Manufactory for Onion Dried Production (Olam Food Manufactory Co.), Sixth Industrial Area of 6th October City, Giza Governorate, Egypt, and was then washed, hand peeled, naturally dried, as well as before being ground and sieved powder according to Marotti & Piccaglia [14]. El-Nasr Pharmaceutical Chemical, El-Ameriea, Cairo, Egypt provided the ethanol, as well as Butylated Hydroxyl Toluene (BHT), and catachin as synthetic antioxidants were bought from Sigma Chemical Co. in St. Louis, Missouri, the United States, including flavonoids and phenolic compounds. Sunflower oil was purchased from Tanta Company for Oils and Soaps in Tanta, Egypt.

Methods

Determination of the chemical analysis of raw materials: In the raw materials, proximate analysis including crude protein, crude lipids, ash, and crude fibers was determined in accordance with AOAC [15], total carbohydrates were calculated by deference.

Antioxidant extraction: Red onion skin and pomegranate peel that had been weighed out (10g each) were extracted for 24 hours with 50ml each of water, and ethanol. The antioxidant extracts were filtered and dried out at 40 °C in a rotary evaporator (RE 300/MS).

Determination of total phenolic, tannins, and α-tocopherol: Using the Folin-Ciocalteu reagent and the method outlined by Muscolo et al. [16], the total phenolic content of the raw materials was assessed. The results are reported as Gallic Acid Equivalents (GAE) mg/100g of dry weight. Additionally, the method of Çam & Hişil [17] was used to determine the total tannins content. Vitamin E (α-Tocopherol) was extracted separately from raw materials with 10mL of a 3:2 v/v hexane: isopropanol solution stirred for 5h and centrifuged at 3000rpm for 10 minutes. According to Prieto et al. [18], the supernatant was utilized to determine the amount of vitamin E.

Determination of antioxidants by HPLC: Polyphenolic compounds were determined by HPLC (HP 1050) according to the method described by Hajnos et al. [19].

Addition of Antioxidant to Sunflower Oil

Studies on oxidations were conducted using sunflower oil as a substrate. According to Buford [20], in order to examine the antioxidant potency of natural antioxidants extracted (aqueous and ethanolic) from pomegranate peel and red onion skin powder as well as synthetic antioxidants (BHT and catachin), antioxidants were added at concentrations of 100, 200, and 500ppm on a dry weight basis. The same procedures were used to prepare an additive-free control sample. In a 500ml glass beaker, sunflower oil with and without antioxidants (natural or synthetic) was heated intermittently for five hours per day for seven days at a temperature of 80±2 oC.

Determination of oxidative status in sunflower oil

The Thio Barbituric Reactive Substances (TBARS) were determined in oils. Malonaldehyde (MA) is an aldehyde produced during the breakdown of unsaturated fatty acids which gives a pink color and can be measured using spectrophotometers Jung et al. [21] and Zeb & Ullah [22]. The peroxide value, and the acid value as well as iodine number, was determined in accordance with AOAC [15].

Statistical analysis

The SPSS10 program was used to conduct statistical analysis. Data were presented as means and Standard Error of the Means (SEM), and statistical analysis was carried out using a one-way analysis of variance and Duncan's tests [23].

Results and Discussion

Nutritional composition of raw material

(Table 1) lists the chemical compositions of the raw materials. The analysis revealed that the ash, protein, oil, crude fiber content, and total carbs for pomegranate peels were 4.21, 2.00, 0.78, 16.89, and 76.12%, respectively, and for red onion skin they were 8.19, 3.77, 1.78, 30.99, and 55.27%. In comparison to pomegranate peel, the red onion skin was subsequently thought to be a better supplier of chemical components. [24] indicated that the protein level of pomegranate peel and onion skin was higher than that of garlic peel, although the latter's carbohydrate amount (93.26%) was higher. [25] found that the best source of dietary fiber, polyphenols, and antioxidants is onion skin. In contrast, pomegranate fruit peel powder should be used to fortify foodstuffs. These findings concur with those of Essa & Mohamed [26], who discovered that pomegranate peels contain a healthy amount of crude fiber (12.17%).

Table 1: Chemical composition of pomegranate peel, and red onion skin on a dry weight basis. Values are mean±SD, n=3.

Chemical Analysis	Pomegranate Peel	Red Onion Skin
Ash	4.21±0.012	8.19±0.009
Crude Proteins	2.00±0.007	3.77±0.025
Crude Oils	0.78±0.021	1.78±0.011
Crude Fiber	16.89±0.0668	30.99±0.049
Carbohydrates Hydrolysable	76.12±0.405	55.27±0.321

Yields of antioxidant extracts and phytochemical content in raw materials

Pomegranate peel, and red onion skin yields in aqueous and ethanol extracts, as well as total phenols, tannins, and α-Tocopherol contents were determined. The results are shown in (Table 2). The results showed that the yield extract in pomegranate peel and red onion skin from ethanol was higher than aqueous, which may be because the solvent's polarity was increased. According to the findings, pomegranate peel had a higher concentration of ethanol extract (18.02mg/100g) than red onion skin (4.51mg/100g). The ethanol extract from pomegranate peel may have produced a larger quantity of components with potent antioxidant activity than the water extract, which may account for these results. The choice of solvent is important in extraction operations since selectivity is directly impacted, which influences the chemical makeup and functional characteristics of the final extract. The desired compound's solubility in the chosen solvent generally determines this choice. It is generally accepted that a polar solvent would encourage the solubilization and extraction of polar compounds, whilst a less polar solvent would fit for less polar molecules since solubilization involves electrostatic repulsions and attractions between the solvent and the solute [27]. The results from the same Table revealed that the pomegranate peel's ethanol extract had the highest levels of total phenolic and total tannins at 55.37 and 1838.66mg/100g, respectively. Red onion skin's phytochemical levels were reported to be 40.19 and 1089.20mg/100g, while vitamin E (α-Tocopherol) was higher at 27.137µg/100g in red onion skin than pomegranate peel was 22.095µg/100g, respectively. These findings demonstrated that when compared to red onion skin, which contained the highest levels of vitamin E, as well as the pomegranate peel enhanced the highest yield in phytochemicals, indicating that it contained a potent antioxidant. Pomegranate, or Punica granatum L, is a fruit that has long been used as a traditional micronutrient medicine and is a significant source of vitamins, phytochemicals, and bioactive components (such as phenolic acids, flavonoids, and tannins) [28]. In addition, the onion skin is also a rich source of total phenolics (9.4-52.7mg Gallic acid equivalent (GAE) /g dm) [11].

Table 2: Antioxidant of aqueous and ethanolic yields extract, total phenols, tannins, and α-tocopherol contents in pomegranate peel, and red onion skin. Values are mean±SD, n=3.

Extracts	Pomegranate Peel	Red Onion Skin
Aqueous extract (mg/100g)	11.58±0.009	1.33±0.007
Ethanolic extract (mg/100g)	18.02±0.058	4.51±0.066
Total phenols (mg/100g)	55.37±0.057	40.19±0.047
Total tannins (mg/100g)	1838.66±1.110	1089.20±4.990
Tocopherol (µg/100g)	22.095±0.054	27.137±0.076

Identification of polyphenols for pomegranate peel, and red onion skin by HPLC

Using HPLC, the polyphenolic content of pomegranate peel and red onion skin extracts was determined. The results are shown in (Table 3). According to the findings, syringic acid, kaempferol, ferulic acid, protocatechuic acid, salicylic acid, and cinnamic acid were the polyphenolic components found in the red onion skin at the greatest concentrations (17.574, 12.019,11.039, 9.487, 8.801, and 6.610ppm, respectively). In terms of polyphenolic quantities, coumarin and p-coumaric acid were the two medium sources (2.589 and 2.869ppm). The lowest concentrations of polyphenolics were caffeine acid, resorcinol, vanillin, and quercetin, all of which were less than one. Onion plant skin is a biodegradable by-product.

By removing the dyestuffs from these wastes, it is feasible to assess their usage in the manufacturing of pigments and natural colors. There are some naturally occurring flavonoid (quercetin, for example) and anthocyanidin (pelargonidin, for example) colors in the skin that makes up this outermost section. The majority of the flavonols in plants are flavonoids called quercetin glycosides. From the interior to the exterior of the onion plant, the amount of quercetin may increase [29]. Syringic acid, ferulic acid, protocatechuic acid, Salicylic acid, kaempferol and cinnamic acid were the greatest concentrations of polyphenolics in the pomegranate peel extract, were present in concentrations of 19.301, 12.199, 11.468, 10.619, 7.969, and 5.829ppm, respectively. The moderate levels of polyphenolics were coumarin and p-coumaric acid (2.411 and 2.500ppm). The pomegranate peel extract had no Vanilne and contained the least quantities of Caffic acid, Resorcinol, and Quercetin of all the polyphenolics, this results agree with Soheir & Sara [30]. According to Saeed et al. [31], pomegranate peel (Punica granatum L.) extract by HPLC analysis revealed that the amount of ascorbic acid was higher than the phenolic acids (gallic acid and protocatechuic acid) and flavonoids (quercetin and kaempferol) in all extracts. The highest amount of ascorbic acid was discovered in the aqueous extract, followed by ethanolic. The amount of protocatechuic acid in the aqueous extract was larger than the number of other polyphenols, and the amount of quercetin was the lowest, despite the fact that phenolic acids were higher than flavonoids in all extracts. Gallic acid (3,4,5-trihydroxy benzoic acid), protocatechuic acid, and other phenolic acids are secondary metabolites that are naturally occurring molecules found in the plant kingdom with distinctive structural characteristics. Phenolic acids are one of the most prevalent classes of phytochemicals (2,4-dihydroxy benzoic acid). These substances are essential for growth and reproduction, protecting against infections, and sensory qualities may have a significant influence in determining the antioxidant capacity of meals, making them a possible natural source of antioxidants [32].

Table 3: Analysis of the HPLC polyphenols, quercetin,and kaempferol for pomegranate peel, and red onion skin.

Polyphenolic (ppm)	Pomegranate Peel	Red Onion Skin
Ferulic acid	12.199	11.039
Protocattechuic acid	11.468	9.487
Syringic acid	19.301	17.574
Coumarin	2.411	2.589
Caffic acid	0.799	0.973
ρ-Coumaric acid	2.5	2.869
Salicylic acid	10.619	8.801
Resorcinol	0.921	0.864
Vanilne	----	0.401
Cinnamic acid	5.829	6.61
Quercetin	0.223	0.271
Kaempferol	7.969	12.019

Adding aqueous and ethanolic pomegranate peel, red onion skin extracts, catachin and BHT to sunflower oil

Table 4: Effect of natural (red onion skin and pomegranate peel) and synthetic antioxidants on acid value, Peroxide value, TBARS, and iodine number during heating sunflower oil at 80 °C/5hrs daily for 7 days. Values are mean±SD, n=3 control sunflower oil free antioxidants.

Acid Value								
Concentration of Extracts	Control at Zero Time	Control after 7 Days	Red Onion Skin		Pomegranate Peel		Synthetic Antioxidants	
			Aqueous	Ethanolic	Aqueous	Ethanolic	BHT	Catachin
100ppm	0.0080 ±0.0001	2.3850 ±0.0100	1.069 ±0.004	0.813 ±0.003	0.901 ±0.0042	0.740 ±0.0028	0.8180 ±0.0012	1.0980±0.0050
200ppm			0.859 ±0.002	0.788 ±0.004	0.653 ±0.0038	0.617 ±0.0031	0.7990 ±0.0021	0.9680±0.0038
500ppm			0.447 ±0.001	0.408 ±0.002	0.369 ±0.0011	0.221 ±0.0020	0.3220 ±0.0017	0.4340±0.0022
Peroxide Value								
Concentration of Extracts	Control at Zero Time	Control after 7 Days	Red Onion Skin		Pomegranate Peel		Synthetic Antioxidants	
			Aqueous	Ethanolic	Aqueous	Ethanolic	BHT	Catachin
100ppm	0.0140 ±0.0006	11.0990 ±0.0390	3.6014 ±0.0027	2.2185 ±0.0020	2.6182 ±0.0013	1.8953 ±0.0019	2.0786 ±0.0150	2.6390±0.0110
200ppm			2.4908 ±0.0030	1.6152 ±0.0040	2.3819 ±0.0030	1.1999 ±0.0050	1.5810 ±0.0070	2.3999±0.0116
500ppm			0.8199 ±0.0021	0.7816 ±0.0022	0.3928 ±0.0011	0.2900 ±0.0011	0.1270 ±0.0010	0.1280±0.0010
TBARS								

Concentration of Extracts	Control at Zero Time	Control after 7 Days	Red Onion Skin		Pomegranate Peel		Synthetic Antioxidants	
			Aqueous	Ethanolic	Aqueous	Ethanolic	BHT	Catachin
100ppm			0.1100 ±0.0030	0.0750 ±0.0019	0.0890 ±0.0026	0.0720 ±0.0011	0.0940 ±0.0002	0.1020±0.0003
200ppm	0.0070 ±0.0010	0.7900 ±0.0020	0.0830 ±0.0017	0.049 ±0.0023	0.0730 ±0.0018	0.0630 ±0.0025	0.0810 ±0.0003	0.0790±0.0004
500ppm			0.0140 ±0.0019	0.0110 ±0.0022	0.0190 ±0.0021	0.0110 ±0.0018	0.0120 ±0.0001	0.0370±0.0001
Iodine Number								
Concentration of Extracts	Control at Zero Time	Control after 7 Days	Red Onion Skin		Pomegranate Peel		Synthetic Antioxidants	
			Aqueous	Ethanolic	Aqueous	Ethanolic	BHT	Catachin
100ppm			103.87 ±0.5874	104.99 ±0.6100	104.33 ±0.4986	106.16 ±0.5002	109.17 ±0.4810	107.88±0.5710
200ppm	111.69 ±0.5378	97.14 ±0.4897	105.59 ±0.4895	107.19 ±0.5399	107.17 ±0.6010	110.86 ±0.5603	109.97 ±0.5027	109.36±0.5301
500ppm			110.01 ±0.5991	111.01 ±0.5783	111.79 ±0.5004	111.62 ±0.5611	111.07 ±0.5331	110.11±0.4209

Sunflower oil was heated at 80 oC. for five hours each day for seven days with various concentrations (100, 200, and 500ppm) of pomegranate peel, and red onion skin extract, and compared to BHT and Catachin as synthetic antioxidants. The stability of all samples was assessed by measuring changes in acid value, peroxide value, Thiobarbituric Acid Reactive Substances (TBARS), and iodine number. (Table 4) demonstrated that pomegranate peel, red onion skin, and synthetic antioxidants had the best outcomes when sunflower oil was treated with extracts (ethanol and aqueous) at 500ppm concentration. With the passage of time, lower acid value, peroxide value, and TBARS were detected than without it, meanwhile, the iodine number increased gradually during seven days. This suggests that red onion skin, pomegranate peel extracts, and synthetic antioxidants like BHT and Catachin were all behaving as strong antioxidants. Additionally, there were noticeable changes in the three concentrations utilized in this study 100, 200, and 500ppm, thus the effect of natural and synthetic antioxidant extracts on the acid value, peroxide value, TBARS, and iodine number in sunflower oil depended on how much extract was added. In the presence of pomegranate peel, red onion skin, and synthetic antioxidants at a greater concentration of 500ppm, the autoxidation of sunflower oil was gradually suppressed. These results are consistent with data reported recently by Sarojini et al. [33] who found that Pomegranate Peel Extract (PPE) contains a considerable number of phenolic compounds that have the ability to scavenge free radicals during the oxidation process explaining the slow-down formation of peroxide in sardine fish oil compared to control. Antioxidants are considered a group of compounds that can counteract oxidation by acting as reducing agents, free radical scavengers, and quenchers of radical species, such as metals [34]. In comparison to synthetic antioxidants, the Peroxide Value (PV) and ThioBarbituric Acid (TBA) at 0.05% and 0.1% of pomegranate peel aqueous extract (POP-Ax) produced the best results [30]. Plants contain many natural antioxidant compounds, including polyphenols (anthocyanins, flavonols, and flavones), carotenoids, tannins, lignin, phenolic acids, and vitamins. However, these antioxidants can lose their activity under adverse conditions, such as high temperatures, extreme pH, and strong lights [35].

The antioxidant capacity of certain compounds can be measured directly or indirectly. The direct method determines the ability of antioxidants to intervene or stop the lipid oxidation process in meat homogenates, meat products, oil emulsion, and liposome systems in the presence of antioxidant compounds. Numerous studies have demonstrated that adding commercially available synthetic or natural antioxidants can slow down or prevent lipid and protein oxidation in processed beef products [36]. Because of their toxicity and potential health dangers, synthetic antioxidants including Butylated Hydroxyl Anisole (BHA), 2,6-di-tert-butyl-4-methyl phenol (BHT), and Tert-Butyl Hydroquinone (TBHQ) have had their use in industrial processing prohibited. As a result, using naturally occurring compounds instead of manufactured additions is becoming more and more common. For instance, the food sector has made substantial use of tea polyphenols as antioxidants. It has also been demonstrated that their antioxidant impact is comparable to that of synthetic antioxidants. Many studies have shown that certain natural substances can effectively prevent the lipid oxidation of foods that are consumed for muscle growth [37]. Numerous natural substances, like α-tocopherol and certain polyphenols, have shown benefits in delaying protein oxidation during the processing of meat and meat products [38]. An antioxidant inhibitor is added to the system to stop or detect the chain of oxidation, stabilize the radical that is produced, and lessen the amount of oxidative damage that occurs in the body. Antioxidant molecules play a function in exerting a protective impact against oxidative damage by interacting with reactive radicals to create non-radical products inactively. The majority of these tests are carried out by accelerating the chain reaction's induction phase by raising the temperature or adding more oxygen. A large number of pure chemicals' and plant extracts' antioxidative properties have been identified thanks to these experiments. These techniques for evaluating oxidation stability speed up oxidation under too severe circumstances and are too general and insensitive to be useful [39].

Conclusion

From the current investigation, it can be inferred that the skins of red onions and pomegranate peels may effectively stabilize sunflower oil at all concentrations. Pomegranate peel extract exhibits stabilizing effectiveness comparable to traditional synthetic antioxidants, such as BHT, and catachin at its legal limit, at a concentration of 500ppm. It increases sunflower oil's resilience to thermally destructive changes. Pomegranate peel and red onion skin can be suggested as powerful sources of antioxidants for the stability of food systems, particularly vegetable oils.

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