NUTRITIONAL CHARACTERIZATION AND THE ANTIOXIDANT PROPERTIES OF SWEET ORANGE AND RED GRAPEFRUIT PEELS

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Abstract

Sweet oranges (Citrus sinensis) and red grapefruit (Citrus paradisi) are fruits consumed frequently in the world, being part of the human diet for decades due to high nutritional and medicinal values. These are excellent sources of vitamin C, a powerful natural antioxidant that builds the body's immune system. Their consumption (natural or processed) generates more quantities of shells and wastes that cause pollution of the environment under incorrect handling conditions. The study was conducted to analyze the potential of orange and grapefruit peels, from a chemical point of view, to determine the nutritional value for inclusion in broiler feed. The orange peels were characterized by a 5.42% crude protein and 1.04% crude fat vs. 5.78% and 0.80% grapefruit. The total content of polyphenols (mg acid galic /g) determined was higher with 36.01% in grapefruit peels compared to those of oranges. Antioxidant capacity was determined by two in vitro methods. The grapefruit peels were characterized by a high antioxidant capacity (288.49 mmol /kg equivalent ascorbic acid) with 51.39% higher than the values of the orange peels, in the first method. For the second method, the determined antioxidant capacity was with 61.20% higher than the orange peels (95.46 mmol/ kg equivalent vitamin E). Based on these results, the analyzed by-products fulfill the necessary conditions to be integrated into the animal feed, and they will be tested in an experiment on broiler chickens in order to improve the nutritional quality of the meat.

Key words: *Citrus*; orange peel; grapefruit; nutritional characterization; antioxidant properties

INTRODUCTION

Many studies have shown that fruits and vegetables contain high amounts of dietary fiber, which helps people's physiological activities by lowering cholesterol levels, reducing hyperlipidemia and high blood pressure, and maintaining gastrointestinal health [1;2]. Furthermore, dietary fibers from vegetables and fruits have a higher ratio than those of cereal bran [3]. Fruits and vegetables contain significant levels of biologically active components that confer health benefits beyond basic nutrition [4]. They are a major source of dietary antioxidants that increase the capacity of plasma antioxidants, leading to the inhibition of atherosclerosis-related diseases in humans.Although the *Citrus* taxonomy remains controversial, the most important citrus fruits consumed worldwide are sweet oranges *(Citrus sinensis)* being the main fruit of this group, accounting for 70% of the total citrus production and consumption, mandarin *(Citrus reticulata)*, grape fruit *(Citrus paradisi)*, lime *(Citrus aurantifulia*) and lemon *(Citrus limonum)*. Due to the pleasing aromas and taste, *Citrus* species are widely consumed fresh or used as raw materials for juice. It is well known that *Citrus* fruits are not only an extremely rich source of vitamin C. Their products are abundant sources of nutrients and rich in vitamins, minerals and dietary fiber essential for nutrition, growth and in general human body development [5]. In addition to high nutritional value, secondary metabolites of *Citrus* fruits, including flavonoids, limonoids and coumarins, are also known to have many

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health benefits, such as antioxidant, anti-inflammatory, anticancer and neuroprotective activities.Unlike other types of fruit, *Citrus* fruits have a small edible portion. Therefore, large quantities of waste, such as shells and seeds, are discarded during processing of juices and foods. The residues of citrus juice production are a source of several compounds, mainly water, soluble sugars, fibers, organic acids, amino acids and proteins, minerals, oils and lipids and also contains flavonoids and vitamins [6].

Grapefruit *(Citrus paradisi)* are subtropical fruit trees of medium size, which belong to the *Rutaceae* family, being a hybrid formed by the cross between pomelos and sweet orange [7]. According to the data of the United Nations Food and Agriculture Organization, China and the United States are the major producers of grapefruit worldwide. Due to many bioactive phytochemicals, such as flavonoids, carotenoids, coumarins and organic acids, grapefruit has many health promoting properties, such as anti-inflammatory, cancer and obesity [8, 9]. Flavonoids are considered the most important bioactive components present in grapefruit. Major flavonoids found in grapefruit, including hesperetin, naringenin, narirutine, and didimine, have been extensively studied both *in vitro* and *in vivo* to confirm their role for the benefit of human health [10,11].

Oranges *(Citrus sinensis)* are very attractive and nutritious fruits. They have a unique color, smell and taste. From a nutritional point of view, they are one of the most abundant sources of vitamin C among fruits and vegetables. However, vitamin C is not its only nutritional benefit; it is also a good source of carotenoids, flavonoids, essential oils, sugar, fiber and some minerals [12]. Oranges are generally consumed as such, either in the form of freshly squeezed orange juice, concentrated juice or pasteurized juice. 85% of oranges are processed in the form of orange juice, leaving behind tons of by-product after production. Usually, these "wastes" are administered in animal feed or eliminated at producer costs [13;14].

Regarding all of the above, the present study was carried out to explore the potential components of orange peel and red grapefruit in order to establish nutritional characteristics and antioxidant properties as raw materials for animal feed, especially broiler chickens.

MATERIAL AND METHOD

Fruit material

The sweet orange peels (SOP) and the red grapefruit peels (RGP) that were analyzed in order to determine the nutritional characteristics and the antioxidant capacity, were obtained after the extraction of the fruit juice. The fruits were purchased from a local supermarket (Carrefour), washed with hot water, then the juice was extracted with a manual press. All the fruits were of eating quality, and without blemishes, or damages. The peels resulting from the juice extraction were dried in the oven for 48 hours, at 103^0C temperature until they became crispy, then ground and stored in bags with hermetic closure until their use in experiments. The basic chemical composition analyses dry matter, protein, fat, ash minerals, polyphenols and antioxidant capacity were determined on samples dried at 65^0C.

Determination of nutrient composition

Standardized methods complying with Regulation (CE) 152/2009 (Sampling and analytical methods for the official inspection of feeds) and ISO standards were used to determine the nutrient concentration. The dry matter (DM) was determined with the gravimetric method according to Regulation (CE) nr. 152/2009 and standard SR ISO 6496:2001; the crude protein (CP) was determined by the Kjeldahl method according to Regulation (CE) nr. 152/2009 and standard SR EN ISO 5983-2:2009; the crude fat (EE) was determined by extraction in organic solvents - the method complies with Regulation (CE) nr. 152/2009 and standard SR EN ISO 6492:2001; the crude fibre (CF) was determined by the method with intermediary filtration, according to Regulation (CE) nr. 152/2009 and standard SR EN ISO 6865:2002, the ash (Ash) was determined by the gravimetric method using a Caloris CL 1206 furnace.

Determination of trance minerals

Trace mineral concentrations were determined in SOP and RGP samples applying flame atomic absorption spectrometry (FAAS) as described by [15] after microwave digestion. The used equipment was as follows: Atomic absorption spectrometer Thermo Electron – SOLAAR M6 Dual Zeeman Comfort (Cambridge, UK), with deuterium lamp for background correction and air-acetylene flame and microwave digestion system with remote temperature measurement, BERGHOF, Speed wave MWS-2 Comfort (Eningen, Germany). Stock solutions of Cu, Fe, Mn, Zn, 1000 ppm traceable to SRM from NIST, were used to standardize the flame atomic absorption spectrometer. Class A glassware was used for transvasation, dilution and storage.

Determination of polyphenols

The methanol extract was obtained by mixing 1 g of sample in 20 mL methanol (80%), which was kept on a rotary shaker for 24 h, in the dark. The extract was centrifuged at 1500xg for 10 min and the supernatant was considered for analysis (total phenol content, total antioxidant capacity). The total phenol content of SOPandRGP was measured spectrophotometrically according to the Folin-Ciocalteu's method, described by [16]. The absorbance was measured at 732nm against a blank (solution with no extract added). Calibration curve of Gallic acid was used to determine total phenol compounds, and the results were reported as mg gallic acid equivalents per gram sample (mg GAE/g).

Determination of antioxidant capacity

The total antioxidant capacity of the SOP and RGP extract was evaluated by phosphomolybdenum method described by [17]. The absorbance of the green phosphate/Mo(V) complex was measured at 695 nm against a blank (solution with no extract added). The results were expressed as mmol equivalent ascorbic acid /kg sample and as mmol equivalent vitamin E/kg sample.

Statistical analysis

The analytical results have been compared with the variance analysis (ANOVA), with WINDOWS Stat View (SAS, version 6.0).

RESULTS AND DISCUSSIONS

The nutrient composition of SOP and RGF (Table 1) shows that both feedstuffs are similar in terms of their dry matter (DM), crude protein (CP) contents but the RGF has higher levels of crude fiber (CF) and ash but lower levels of ether extract (EE) compared with SOP. The fat content from SOP may be advantageous not only as a dietary energy source but also in binding the powdery constituents of mash feed. The average CP content is ranging between 5.42% (SOP) and 5.78 (RGP). while the EE level of the citrus peels was 1.04% in SOP vs. 0.80% in RGP. Also, the CF, which is the limiting factor for the inclusion of these meals in compound feeds for poultry had close values between the two by-products, ranging from 10.13% (SOP) to 10.68% (RGP). Other researchers [18;19;20] reported similar values for SOP in the case of DM. Significant differences P <0.05, between the results obtained for us for SOP were observed in the case of CP (5.42% vs 10.76% respectively 10.96%). Noteworthy is the difference of values obtained by [18;19] in the two results from different years for EE and CF from the two types of peels analyzed. Contrary to these, [21] obtained results similar to ours in the case of SOP. These differences in the results obtained by the researchers are accounted by the fruit production and provenience. Growth microclimate, temperature and soil play an important role in the development of nutrients from fruits.

Table 1 Nutrient composition of sweet orange peels (SOP) and red grapefruit peels (RGP)

Specification	SOP	RGP	SOP*	SOP**	SOP***
DM, %	88.90	87.66	89.65	85.91	88.00
CP, %	5.42	5.78	10.76	7.44	5.46
EE,%	1.04	0.80	12.60	2.29	2.00
CF, %	10.13	10.68	7.86	12.87	10.00
Ash, %	2.93	3.29	11.90	3.85	7.00

Oluremi et al., 2006*; Oluremi et al., 2010**; Ebrahimi et al. 2013***

The SOP and RGP were evaluated for trace mineral content (table 2). From the screening of essential minerals, iron concentrations in RGP registered noticed values. Other minerals (Cu, Mn, Zn) presented comparable amounts in the two studied by-products. The results obtained in the present study are similar with others reported in the scientific literature. [22] reported in *Citrus jambhiri* concentrations of metal ions: Fe^{2+} - 4.49 ppm; Zn^{2+} -1.14 ppm and for Mn^{2+} -0.80 ppm. It is well observed that this result are in the same range with those obtained for SOP and RGP, except for iron concentration. Of all microelements evaluated, iron was detected in a higher concentration than Cu, Mn and Zn for both citrus peels. [23] also obtained the higher concentration of iron in grapefruit peels compared with oranges. In addition, many antioxidant defenses depend on micronutrients. Some minerals are components of antioxidants enzymes: superoxide dismutase depends on Mn, Cu and Zn; catalase depends on Fe [24].

Table 2 Trance mineral content of the studied *Citrus* peels

Specification	SOP	RGP
Copper, ppm	2.02	1.08
Iron, ppm	8.47	54.59
Manganese, ppm	6.20	5.01
Zinc, ppm	7.06	9.41

The extraction of polyphenols from fruit peels is a crucial step for the phytochemical analysis and in vitro evaluation of antioxidant properties. The data on the total polyphenols of the analysed *Citrus* peels (figure 1) show that the RGP has a more important level of polyphenols (15.45 mg equivalent acid galic/g) compared with that from the SOP (9.86 mg equivalent acid galic/g), the level being with 56% higher for RGP than SOP. [25] reported that the total extractable polyphenols in peel from SOP were 0.37 mg GAE/100 g DM while in the RGP was 0.30 mg GAE/100 g DM. Other, [26] reported different concentrations of polyphenols from different varieties of SOP, collected from Portuguese (14.94 mg GAE/100 g DM), Washington (9.61 mg GAE/100 g DM) compared with some peels from Bigarade (31.62 mg GAE/100 g DM). Other works, reported that the SOP contains 73.54 mg GAE/100 g DM, and the RGP contains 161.60 GAE/100 g DM, and also have been reported that orange peel contains 35.5 mg QE/100 g DM of flavonoids. Contrary to our results [23] stated that the highest content of total polyphenols was in peels of lemons and the lowest in peels of grapefruits, while the orange peels were in the middle as values. These values reported in scientific literature are smaller than the values obtained in our study, proving again that the growth conditions, genetic factors, geographical variations in the level of soil fertility, efficiency of mineral uptake, and the analytical procedure employed are crucial parameters for chemical composition of fruits and vegetables. These results also show that our samples have good levels of polyphenols for the peel of citrus analyzed and suggest that the RGP is very rich in phenolic compounds compared to SOP.

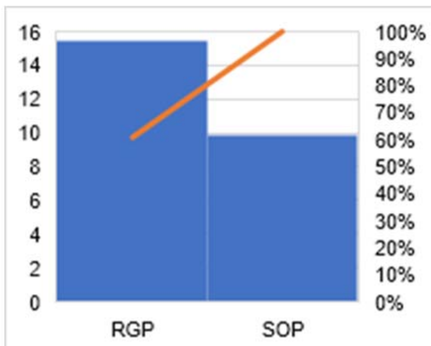

Figure 1 Total concentration of polyphenols from *Citrus* peels (SOP and RGP)

Previous studies have shown that they do not fit one-dimensional methods to evaluate food antioxidants such as fruits and vegetables, then they can have a wide variety of natural antioxidants. Therefore, multiple tests for antioxidant activity are preferred or addressed [27]. Total antioxidant capacity determined by phosphomolybdenum method expressed antioxidant activity of hydrophilic and lipophilic compounds. The hydrophilic compounds antioxidant capacity was measured by ascorbic acid equivalents and RGP registered a concentration with 47.60%

higher than SOP. The lipophilic compounds antioxidant capacity was measured by vitamin E equivalents and RGP registered more than double concentrations, being with 61,20% higher than in SOP. Also, [23] determined a higher concentration of ascorbic acid in lemons and orange peels, while grapefruit had the lower concentration (43.80 mg/100g).

According to [28], all *Citrus* flavonoids have an antioxidant action in a hydrophilic environment while, in a lipophilic environment, some molecules show a reduced antioxidant capacity, and others invert their behavior, becoming prooxidants. Other authors reported antioxidant capacity measurement determined using different methods and the results are related to: method used; type of citrus family; origin, harvesting time and others [28;29] which act by affecting the level of phytochemicals.

It is well to observe that the total polyphenol content and the antioxidant capacity was higher in both cases for RGP. The correlation between total phenol contents and antioxidant activity has been widely studied in different foodstuffs such as fruit and vegetables [30;31].

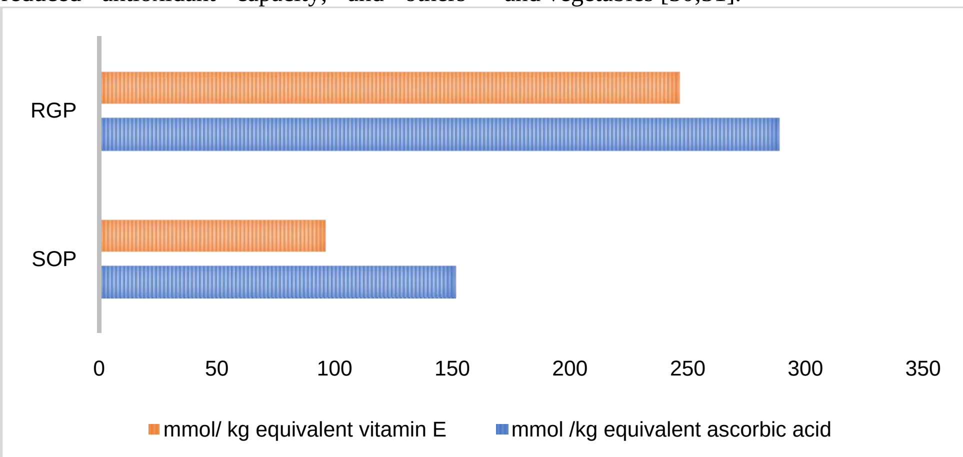

Figure 2 Antioxidant capacity from sweet orange peels and red grapefruit peels expressed as a) mmol/kg equivalent ascorbic acid b) mmol/kg equivalent vitamin E

CONCLUSIONS

Due to the low cost and easy availability of fruit residues which otherwise would be discarded as waste in the environment should be regarded as potential resources, capable of offering significant low-cost, nutritional dietary supplements. Rich in bioactive compounds, these unwanted cast-offs of manufacturing could be recycled as value added food supplements, that provide advantageous dietary fiber, minerals, antioxidant capacity and polyphenols. Along with the rapid development in purification and identification technologies of plant bioactive compounds, studies concerning Citrus bioactive compounds and antioxidant activities will attract more and more attention in the future.

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