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EVALUATION OF PHENOLIC CONTENT, ANTIRADICAL AND ANTIBACTERIAL ACTIVITIES OF ORANGE AND CARROT POMACE EXTRACTS

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Abstract

Orange and carrot pomace are considered as food wastes, despite their high content in beneficial health compounds. The comparison of phenolic extracts from orange and carrot pomace, showed higher values for Orange pomace, with a polyphenols concentration (130 mg/L), flavonoids (8.67 mg/L) and tannins (2.5 mg/L). A higher antiradical activity was also noted for orange pomace. However, carrot pomace presented a higher anti-bacterial activity. The beneficial activities of these extracts were owed to their high content in phenolic acids. Our study exhibited that orange and carrot pomace might be utilized as natural preservatives for many industrial applications.

Keywords

Orange, Carrots, Pomace, Antiradical, Antibacterial

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ABSTRACT: *Orange and carrot pomace are considered as food wastes, despite their high content in beneficial health compounds. The comparison of phenolic extracts from orange and carrot pomace, showed higher values for Orange pomace, with a polyphenols concentration (130 mg/L), flavonoids (8.67 mg/L) and tannins (2.5 mg/L). A higher antiradical activity was also noted for orange pomace. However, carrot pomace presented a higher anti-bacterial activity. The beneficial activities of these extracts were owed to their high content in phenolic acids. Our study exhibited that orange and carrot pomace might be utilized as natural preservatives for many industrial applications.*

KEYWORDS: *Orange, Carrots, Pomace, Antiradical, Antibacterial*

1. INTRODUCTION

In the recent years, the valorization of fruits and vegetables byproducts is gaining increasing interest because of their content in many potential bioactive compounds and due to environmental and economic interests. These byproducts can be formed at any stage in the food supply chain (during production, postharvest and especially during industrial juice extraction process) (Sagar, Pareek, Sharma, Yahia, & Lobo, 2018). Several vegetables and fruits like carrots, oranges, apple, apricot were demonstrated to be an important source of dietary fibers, carotenoids, polyphenols and vitamins which can be used in the food industry as preservatives, additives (Cui, Gu, Zhang, Ou, & Wang, 2015; Figuerola, Hurtado, Estevez, Chiffelle, & Asenjo, 2005; Hernández-Ortega, Kissangou, Necoechea-Mondragón, Sánchez-Pardo, & Ortiz-Moreno, 2013; Sudha, Baskaran, & Leelavathi, 2007).

Citrus fruits are considered as a major crop worldwide, in which orange accounts for about 65% of this production. Orange fruit was found to be rich in bioactive molecules like polyphenols (mainly flavonoids), vitamin C as well as carotenoids (Ivanova, Khomich, & Perova, 2017; Luengo, Álvarez, & Raso, 2013). Around 80% of orange production is destined to juice extraction process (FAO, 2012).

Regarding vegetables, carrot is one of the highly nutritious crops containing several essentials bioactive molecules such as carotenoids, fibers and phenolic compounds. Carrot pomace is considered as the major byproduct remaining after industrial juice extraction (Hernández-Ortega et al., 2013).

During the last decade, polyphenolic compounds are attributed to different biological activities (antioxidant, anti-inflammatory, anticarcinogenic, and antimicrobial) as well as to the nutritional quality of processed fresh vegetables and fruits. For this reason, they are considered of great interest to the food industry encouraging their utilization as natural potential substances or food ingredients (Kim, Jeong, & Lee, 2003; Lapornik, Wondra, & Prošek, 2007).

Several studies showed that fruits and vegetables pomace possess different biological activities (Gowe, 2015). Cheaib *et al* (2018) showed that apricot pomace is rich in polyphenolic compounds and possess different biological activities which can be utilized as natural additives in different applications in the food industry such as (preservatives or antioxidants) (Cheaib et al., 2018)

In the literature, the evaluation of polyphenolic content in orange and carrot pomace has not been well explored.

The objective of this study is to compare the polyphenolic yield of orange as well as carrot pomace obtained from industrial juice extraction processes and to evaluate their biological properties (antioxidant and antimicrobial).

2. MATERIALS AND METHODS

2.1 Raw Materials

Carrot and orange pomaces were received from Lebanese market (juice shop). The pomace consists of pressed pulp and skin residues. The fresh raw materials were stored at -20°C until utilization.

2.2 Solid-liquid Extraction Method

The orange and carrot pomace extracts were equally cut to obtain the same particle size. Afterwards, they were subjected to extraction. It was done using solid-liquid ratio (w/v) of 1:10 phenolic compounds' extraction was done at 50°C (in water) for 120 minutes in order to determine the maximal phenolic concentration. The extracts were then stored at -20°C for further analysis.

2.3 Quantification of Polyphenols Content

The polyphenolic content quantification was performed according to the Folin-Ciocalteu assay. In brief, 0.2 mL of each extract, 0.1 mL of Folin-Ciocalteu reagent as well as 0.8 mL of Na2CO3 (75 mg/L) solution were mixed and then kept for 10 min at 60°C (Slinkard & Singleton, 1977). The absorbance (at 750 nm) was measured using a UV-VIS spectrophotometer (Gold S54T UV-VIS, China). The measurements were calculated by comparing them to a standard curve of gallic acid solution and then expressed in milligrams per liter (L).

2.4 Determination of Tannin Content

This assay was performed by preparing two tubes, each one containing 1 mL of the pomace or orange extract, 0.5 mL of water as well as 1.5 mL of hydrogen chloride HCl (12N). The first one was incubated at room temperature while the other tube was heated at 100°C for 30 min for the same duration. After a fast cooling of the heated tubes, 0.25 mL of ethanol was added (Ribéreau-Gayon, Dubourdieu, Donèche, & Lonvaud, 2012). Then, the absorbance was measured at 520 nm with a spectrophotometer, the tannin concentration is given by:

$$\text{Eq. (1)} \quad Tannin\ concentration\ \left(\frac{mg}{L}\right) = 19.33 \times \Delta\ optical\ densities$$

2.5 Determination of Total Flavonoids

This assay was performed by adding 1 mL of each extract to 4 mL of water. 5 min later, 0.3 mL of $NaNO_2$ (5 %) as well as 1.5 mL of $AlCl_3$ (2%) were added. Then, 2 mL of NaOH (1M) were added to the solution. The absorbance was measured at 510 nm. The flavonoids concentrations were expressed as mg per liter (L) (Michel, Destandau, & Elfakir, 2011).

2.6 Estimation of the Antiradical Activity

The radical scavenging activity was carried out using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method (Zhang & Hamauzu, 2003). In brief, 4 mL of 0.1 mM DPPH (diluted with 80 % methanol) were added to 0.2 mL of each extract and left at room temperature for 30 min. Methanol was used as a blank the decrease of the DPPH absorbance was read at 517 nm. The inhibition percentage was calculated by the following formula:

$$\text{Eq. (2)} \ \%\ inhibition = \left[\frac{(absorbance\ of\ control - absorbance\ of\ test\ sample)}{absorbance\ of\ control}\right] * 100$$

2.7 Microbiological Procedure

The bacterial strains used in this assay were: 2 resistant strains of *Staphylococcus aureus*: *Methicillin-resistant staphylococcus aureus* (MRSA 1) and *Methicillin-resistant staphylococcus aureus* (MRSA 3); 1 strain of *Staphylococcus aureus* (1512); 1 strain of *Enterococci* 44 (HLAR-VRE); 1 strain of *Pseudomonas* (30); 2 strains of *Escherichia coli* amongst which, one is extended spectrum beta lactamase (*E.coli* 365) and the other strain was *Klebsiella*.

These strains were cultured using fresh blood agar. Each bacterial strain was inoculated in 3 mL of cation adjusted Mueller Hinton agar. When the turbidity reached 0.5 McFarland, a dilution of 1/100 was done into the tubes containing the adjusted Mueller Hinton (CLSI, 2013).

All stocks were sterilized by using a disposable syringe filters (0.4 µm). Five serial dilutions of each extracts were done (from 1.25 to 20 µg/mL). After adding the same volumes (100 µL) of each concentration of the two extracts to the bacterial strains (100 µL) in a 96 well microtiter plates (U shape), the final concentrations of the different polyphenolic extracts were diminished to: 10 µg/mL up to 0.62 µg/mL. The inoculated plates were then incubated overnight at 37°C. The Minimum inhibitory concentration (MIC) of each extract was measured and noted (CLSI, 2013).

2.8 RP-HPLC Standardization

Agilent HPLC (Japan) attached to C-18 stationary phase column and an Agilent online degasser (Japan). The mobile phase was methanol and phosphate buffer 34.1 mM (pH 2.1) in a ratio (43:57) respectively. The flow rate used was 1 ml/min at 40 °C. Standardization and quantification were utilized by using the steeping method utilizing Sigma standards (Germany) calibration curves and the UV absorbance ranged between 214 and 600 nm.

2.9 Statistical Analysis

Each assay was performed twice. The means and standard deviations of the results were determined. Variance analyses (ANOVA) as well as a Least Significant Difference test (LSD) were calculated using STATGRAPHICS® Centurion XV (StatPoint Technologies, Inc., Warrenton, VA, USA)

3. RESULTS AND DISCUSSION

3.1 Kinetic Model for Polyphenols Extraction of Orange and Carrot Pomace

Figure 1 represents the kinetic model for the solid-liquid extraction of phenolic compounds from orange and carrot pomace during 120 minutes. The phenolic yield for both byproducts reached its maximum after 60 minutes and remained stable until 120 minutes. This time (120 minutes) was chosen to perform the rest of the analysis in this study. Orange pomace gave higher polyphenolic yield of 130 mg/L compared to carrot pomace (30 mg/L). Those results are in agreement with the study of Faller and Fialho, who showed the orange pulp had higher polyphenolic content than carrot pulp (Faller & Fialho, 2010).

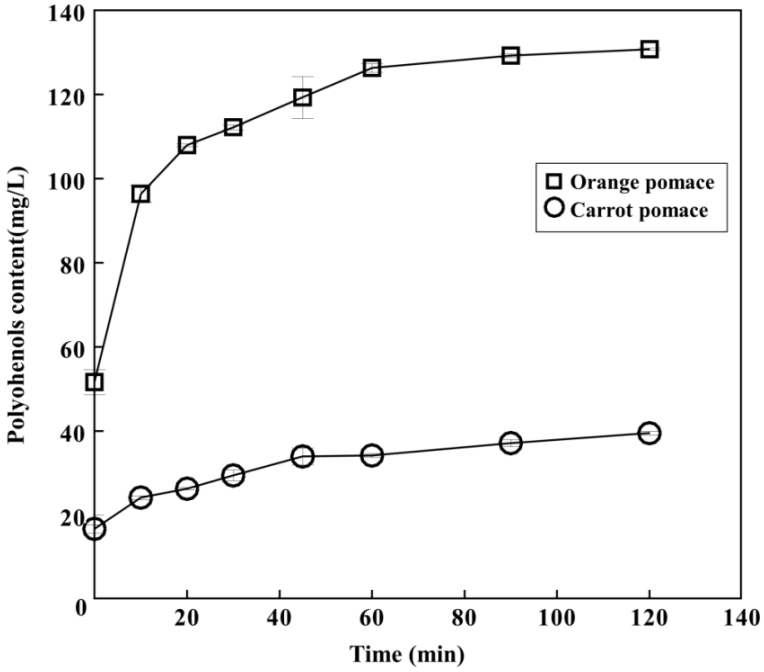

Fig. 1 Kinetic model for the solid-liquid extraction of polyphenolic compounds from orange and carrot pomace during 120 minutes**.**

3.2 Quantification of Flavonoids and Tannins Yield of Orange and Carrot Pomace

Table 1: Flavonoids and tannins content of orange and carrots pomace. Different alphabets indicate significant statistical difference ($p < 0.05$)

	Flavonoids (mg/L)	Tannins (mg/mL)
Orange pomace	8.67 [a]	2.5[c]
Carrots pomace	3.98 [b]	2 [d]

Table 1 represents the flavonoids and tannins yield of orange and carrot pomace. In concordance with the polyphenolic results orange pomace showed higher flavonoids and tannins content then carrot pomace. Many studies assessing the flavonoids content of citrus fruits, showed the orange had the highest yield compared to other citrus fruits (mandarin, lemon and grapefruits) (Fadlinizal et al., 2010; Gattuso, Barreca, Gargiulli, Leuzzi, & Caristi, 2007; Wang et al., 2017). A study done by Macagnan *et al.* (2015) also showed that orange pomace gave the highest tannin content compared to apple and passion fruit pomace (Macagnan et al., 2015).

3.3 Antiradical Activity of Orange and Carrot Pomace

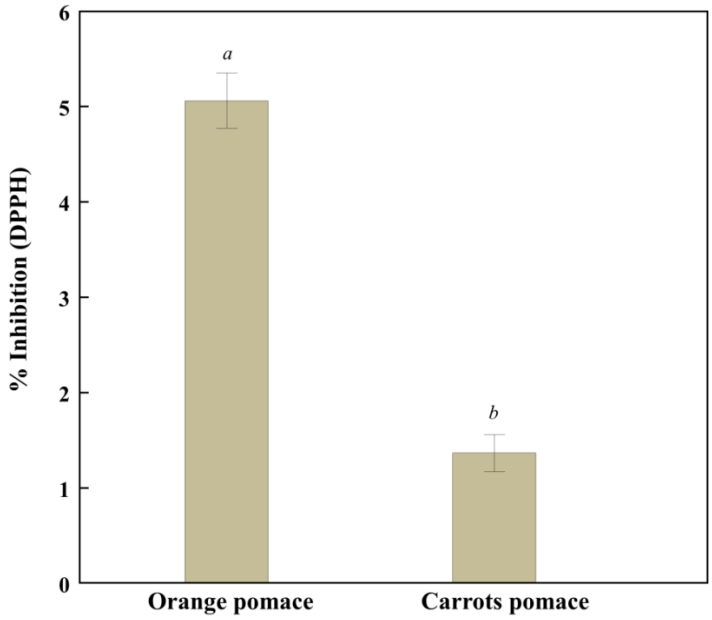

Fig. 2 Antiradical activity of orange and carrot pomace using DPPH assays. Different alphabets indicate significant statistical difference ($p < 0.05$).

The DPPH test was carried out in order to assess the antiradical activity of orange and carrot byproducts. Orange pomace showed higher percentage of inhibition than carrot pomace. These results are in agreement with the polyphenols, flavonoids and tannins results (figures 1). Several studies have correlated the free antiradical activity to the phenolic compounds (Soobrattee, Neergheen, Luximon-Ramma, Aruoma, & Bahorun, 2005; Yilmaz & Toledo, 2004). For example, Hasmida *et al.* (2014) showed that flavonoids might be responsible for the improvement of the antiradical capacity (Hasmida, Nur Syukriah, Liza, & Mohd Azizi, 2014). Thus, the higher content of flavonoids for the orange pomace (8.67 mg/L) could explain the higher antiradical activity, compared to carrots pomace.

3.4 Antibacterial Activity of Orange and Carrot Pomace

The antimicrobial activities of orange and carrot pomace were evaluated against different gram-positive and gram-negative bacterial strains (table 2) at different concentrations. Carrot pomace extracts exhibited an inhibitory activity against two *Methicillin-resistant Staphylococcus aureus* gram-positive strains and *Enterococci* (HLAR-VRE) strain, while orange pomace showed an antimicrobial activity against only two *Methicillin-resistant Staphylococcus aureus* gram-positive strains (with the same inhibitory concentration of carrot pomace). Table 2 shows that the effectiveness of phenolic compounds was only against gram-positive ones. Those findings could be due to the fact that phenolic compounds have better activity against gram-positive bacteria compared to gram-negative ones. Since the latter possess in their cell wall an outer membrane acting as a barrier consequently reducing their uptake (Nakamura et al., 2015; Naz, Ahmad, Ajaz Rasool, Asad Sayeed, & Siddiqi, 2006)

Table 2: Minimum inhibitory concentration (µg/mL) of gram-positive and gram- negative bacteria of orange and carrots pomace. (-) indicates absence of inhibitory effects.

	Minimum Inhibitory Concentration (µg/mL)	
	Orange pomace Extracts	**Carrots pomace Extracts**
Methicillin-resistant *Staphylococcus aureus* (MRSA 1) (gram +)	20	20
Methicillin-resistant *Staphylococcus aureus* (MRSA 3) (gram +)	10	10
Staphylococcus aureus 1512 (gram +)	-	-
Enterococci 44 (HLAR-VRE) (gram +)	-	20
Pseudomonas 30 (gram -)	-	-
Klebsiella (gram -)	-	-
Escherichia coli ESBL 365 (gram -)	-	-
Escherichia coli 2280 (gram -)	-	-

3.5 Quantification of Polyphenol Extracts in Orange and Carrots Pomace using HPLC

In order to understand better the antiradical and antibacterial activities of the orange and carrot pomace, an HPLC (Fig. 3) was performed to determine the different phenolic molecules in orange and carrot pomace. The main phenolic molecules detected in orange and orange pomace are phenolic acid.

RP-HPLC of carrots pomace has shown 8 major peaks: (1) Caffeoyl quinic acid (6.9%), (2) Cyanidin hexosyl pentosyl hexoside (4.6%), (3) Cyanidin caffeoyl hexosyl hexosyl hexoside (9.0%), (4) Cyanidin sinapoyl hexosyl pentosyl hexoside (18.3%), (5) Caffeoyl methyl quinic acid (34.2%), (6) Dicaffeoyl quinic acid (1.1%), (7) Caffeoyl methyl quinic acid (9.3%), and (8) Caffeoyl dimethyl quinic acid (5.7%) focusing on 320 nm and 520 nm. However, RP-HPLC orange fruit aqueous extract has shown 6 major peaks: (I) Oxalic acid (8.9%), (II) Tartaric acid (6.5%), (III) Malic acid (7.2%), (IV) Lactic acid (6.3%), (V) Ascorbic acid (21.5%), and (VI) Citric acid (35.8%).

Figure 3 shows that orange and carrot pomace exhibit both antibacterial activity. This could be due to their content in phenolic acids, known for its antibacterial activity (Junqueira-Gonçalves et al., 2015). A better antibacterial effect was noted for the carrot pomace, since it was able to inhibit a broader range of bacteria, this could be caused by the presence of Caffeoyl quinic acid and Caffeoyl methyl quinic acid since caffeic acid was shown to exhibit a potentiating antibacterial effect (Lima et al., 2016).

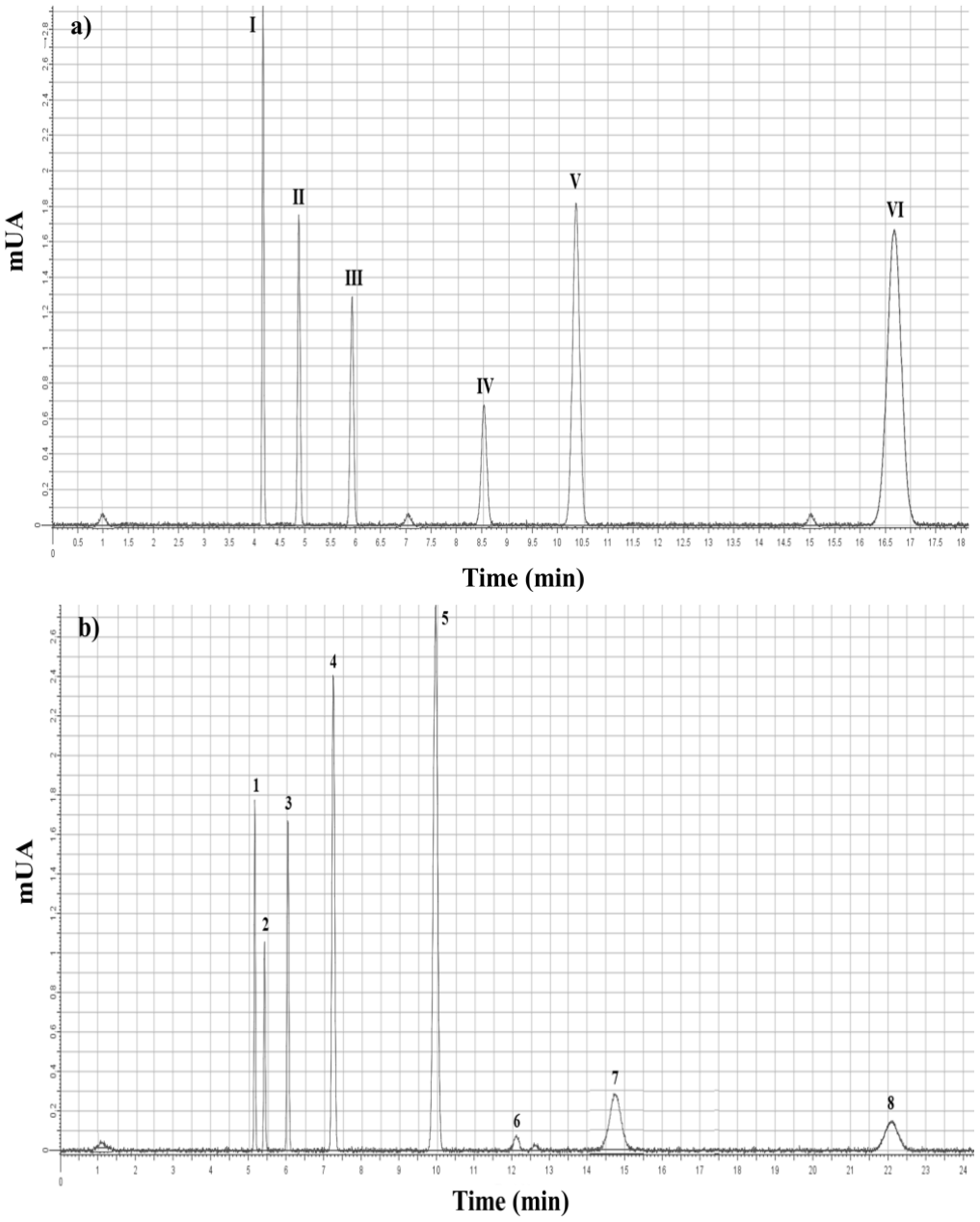

Figure 3. (a) RP-HPLC orange fruit aqueous extract major peaks: (I) Oxalic acid (8.9%), (II) Tartaric acid (6.5%), (III) Malic acid (7.2%), (IV) Lactic acid (6.3%), (V) Ascorbic acid (21.5%), and (VI) Citric acid (35.8%). (b) RP-HPLC Carrot aqueous extract major peaks: (1) Caffeoyl quinic acid (6.9%), (2) Cyanidin hexosyl pentosyl hexoside (4.6%), (3) Cyanidin caffeoyl hexosyl hexosyl hexoside (9.0%), (4) Cyanidin sinapoyl hexosyl pentosyl hexoside (18.3%), (5) Caffeoyl methyl quinic acid (34.2%), (6) Dicaffeoyl quinic acid (1.1%), (7) Cyanidin coumaoryl hexosyl pentosyl hexoside (9.3%), and (8) Caffeoyl dimethyl quinic acid (5.7%).

Furthermore, our results showed that both extracts presented different phenolic components. A better antiradical activity was noted for the orange pomace extract. However, the carrot pomace extract presented a better antibacterial activity.

4. CONCLUSIONS

This study compared the quantity and quality of polyphenols present in orange and carrot pomace. The findings showed that the orange pomace contains a higher quantity of polyphenols, flavonoids and tannins than carrot pomace. These results were as well correlated with a better scavenging activity for orange pomace extract than

carrot extract. With respect to their antibacterial activities, both extracts inhibited different bacterial strains, with a better effect for carrot pomace, owed to its content in caffeic acid. This study demonstrates that orange and carrot pomace by-products should be considered as valuable and natural sources of bioactive molecules, which can be utilized in several industrial applications such as (food preservatives, cosmetics etc.).

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