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BIOACTIVE COMPOUNDS PROFILE OF 'DEKOPON' (*Citrus reticulata* Blanco Shiranuhi) PEEL

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RESUMO – 'Dekopon' peels are rich in phytochemicals that contribute to health, such as carotenoids, flavonoids and terpenes (essential oils), which makes 'Dekopon' waste an interesting by-product. In this context, the aim of this study was identify the carotenoids and fatty acids ester profile present in 'Dekopon' peel. The results demonstrated that a wide variety of carotenoids and fatty acids were identified and quantified in 'Dekopon' peel. Thus, the 'Dekopon' peels demonstrates great potential to be used in the food industry.

Palavras-chave: Dekopon, carotenoids, oil profile, peel.

1 INTRODUCTION

'Dekopon' tangerine (*Citrus reticulata* Blanco Shiranuhi) results of a crossing between 'Kiyomi' tangor (*Citrus unshiu* Marcov × *Citrus sinensis* Osbeck) and 'Ponkan' (*Citrus reticulata* Blanco) done in 1972 by Japanese Department of Agriculture. Therefore, 'Dekopon' tangerines belongs to genus *Citrus*, from family Rutaceae that includes about 17 species cultivated on tropical and temperate regions (KUMAR e BHASKAR, 2012). These tangerines show a parthenocarpic habit and produces almost seedless fruits, around 400 g of weight, very sweet and juicy. This variety is very well adapted to the climate in the highlands of São Paulo, where is also named as 'Kinsei' (LIM, 2012; MATSUMOTO, 2001).

Among these bioactive compounds, β-cryptoxanthin stands out as the main carotenoid present in citrus fruit and was found in the flesh and peel of 'Dekopon' (HEO et al., 2005). Higher concentration of this carotenoid is presented in 'Dekopon' peel, what provide to this residue a potential as functional food ingredient (LIM, 2012).

'Dekopon' peels are rich in phytochemicals that contribute to health, such as carotenoids, flavonoids and terpenes (essential oils) (CHEDEA et al., 2010; WANG et al., 2008), which makes 'Dekopon' waste an interesting by-product. Citrus fruit presents differences in the essential oils composition. It is important to highlight that these oils are the most widely used essential oils in the world, and they can be used to add aroma to products such as soft drinks, ice creams, cakes, and even perfumes. Furthermore, it plays functions as germicidal, antioxidant, and anticarcinogenic compounds (FERHAT et al., 2006; SONG et al., 2006).

Therefore, beyond the fruit consuming or its juice, there are possible applications of citrus peels, which allow industry finding ways to increase its incomes by recovering bioactive molecules and, consequently, reducing the environmental problem of this residue.

Although Brazil is the largest producer of citrus fruit, 'Dekopon' tangerine is still not widely known in the country, as well as its features. Thus, this study aimed to extract and identify the carotenoids and fatty acids ester profile present in 'Dekopon' peel.

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2 MATERIAL AND METHODS

Sampling

Fruits of 'Dekopon' tangerine (*Citrus reticulata* Blanco Shiranuhi) were acquired at São Paulo Supply Center (CEAGESP). Fruits were grown in Turvolândia (21° 52' 33" S, 45° 47' 13" W; MG/Brazil) at 840 m height, with humid subtropical climate. The fruits (≈ 3 kg) were freezed and transported to Bioactive Compounds Laboratory of Food Science and Technology Institute at Federal University of Rio Grande do Sul (UFRGS, Porto Alegre/RS/Brazil). Fruit were peeled and the peels were kept under vacuum at -20ºC to further analysis.

Carotenoids and Oil Profile of 'Dekopon' Peel

'Dekopon' fruit peels were processed with the purpose of homogenization. Then, the processed peel was used to extract carotenoids of exhaustive form (using acetone and ethers) and ethanolic (GRAS solvent) and to extract oil.

Carotenoids: Extracts were prepared mixing fruit peels (3 g) in cold acetone, followed by successive washes with distilled water, petroleum ether and alcohol ether. Then, it was submitted to the saponification process adding 10% KOH in methanol during overnight (dark at room temperature). After that, it was washed and the extracts were concentrated in a rotary evaporator (Fisatom Quimis 0214 M2) (T < 35 °C) (MERCADANTE e RODRIGUEZ-AMAYA, 1991). Methyl tert-butyl ether (MTBE) was utilized to recover the samples and apply them to high performance liquid chromatography (HPLC) for quantitation. The analysis were carried out in triplicates and carotenoids were identified using a C30 reversed phase polymeric column (250 mm × 4.6 mm ID, 3 µm) (YMC, Japan). The wavelength was adjusted to 450 nm. The elution gradient of mobile phase (consisting of MTBE/water/methanol) was set as follow: 5:90:5 to start, then 0:95:5 reached after 10 minutes, 0:89:11 at 20 minutes, 0:75:25 at 30 minutes and 0:50:50 at 40 minutes. The mobile phase flow rate was 1 mL min^{-1}, injection volume was 5 µL and the injector temperature was 33 °C (ZANATTA e MERCADANTE, 2007). Carotenoid quantitation was based on standard curves constructed with patterns acquired from Sigma-Aldrich: β-carotene (5−50 $mg.mL^{-1}$), α-carotene (2−25 $mg.mL^{-1}$), lutein (1−65 $mg.mL^{-1}$), β-cryptoxanthin (4−100 $mg.mL^{-1}$), and zeaxanthin (1−40 $mg.mL^{-1}$). The limits of quantitation (LOQ) and detection (LOD) were, respectively, for β-carotene, 10.89 $\times10^{-5}$ $g.kg^{-1}$ and 6.53 $\times10^{-5}$ $g.kg^{-1}$; for α-carotene, 3.28 $\times10^{-5}$ $g.kg^{-1}$ and 1.97 $\times10^{-5}$ $g.kg^{-1}$; for lutein, 1.15 $\times10^{-5}$ $g.kg^{-1}$and 6.9 $\times10^{-6}$ $g.kg^{-1}$; for β-cryptoxanthin, 3.51 $\times10^{-5}$ $g.kg^{-1}$ and 2.11 $\times10^{-5}$ $g.kg^{-1}$; and forzeaxanthin, 1.59 $\times10^{-5}$ $g.kg^{-1}$ and 9.56 $\times10^{-5}$ $g.kg^{-1}$. The results were expressed in µg of carotenoid per g of fruit peel. However, the carotenoids ethanolic extraction from the 'Dekopon' peel was carried out through the 'Dekopon' peel (10 g) and ethanol (50 mL), this mixture was macerated using the Turrax equipment. In sequence, it was kept in a water bath under stirring (30 minutes) and then vacuum filtered. This procedure was repeated four times in order to optimize the process in relation to the exhaustive extraction. The sample was rotoevaporated until 25 mL, these 25 mL were completely dried in nitrogen and frozen until HPLC analysis. In the HPLC analysis, the sample was recovered with 2 mL of MTBE and homogenized (3 minutes) in ultrasound, then filtered and put into a vial. It was used an injection volume of 5 µL, reverse polymer column, mobile phase of Milli-Q water, methanol and MTBE.

Oil extraction: The oil extraction method from the 'Dekopon' peel was the steam-dragging distillation through a Clevenger extractor. The flask with sample (250 g) and water (1 L) was placed on a heating blanket and coupled to the Clevenger apparatus. After boiling, the apparatus was left in operation (four hours) for complete extraction of the essential oil. The essential oil and water were then collected in a bottle. A separation funnel was used for the correct separation of the essential oil from the water. Finally, the oil was placed in a pre-weighed amber bottle and thus its yield was determined. This essential oil was used for fatty acids ester profile determination.

Fatty Acid profile of peels: The essential oil composition of 'Dekopon' fruit peel were determined by GC-FID (SHIMADZU, model GC- 2010 Plus, Japan), with fused silica capillary column SLB-IL 100, Supelconalytical (30 m 0.25 mm 0.2 mm), automatic injector and flame ionization detector coupled. With the increase rate of 3 ºC/min, the column temperature reached 240 ºC. The carrier gas was hydrogen (20 cm/s) at a flow rate of 1 mL/min, split ratio 1:50 and the volume injected was 1 mL. Before injection, the essential oil was saponified using a methanolic solution (NaOH 0.5 N). To determine the fatty acid methyl esters profile of the essential oil was used an established method (JOSEPH e ACKMAN, 1992). The fatty acid methyl esters were identified by comparison of the retention time with the standard (FAME MIX Supelco37, Sigma- Aldrich, St. Louis, MO, USA). The injections were carried out in triplicate and GC solution software program performed processing and results acquisition.

Statistical analysis

Results were analysed by ANOVA with Tukey's comparison test at 5 % of significance, using the software 10.0 (STATSOFT Inc., São Paulo, Brazil).

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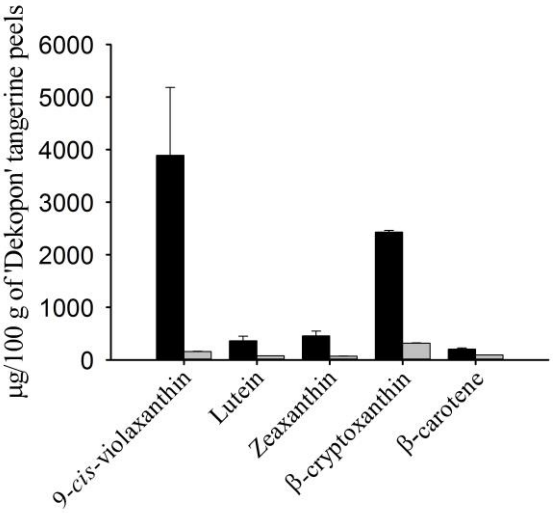
3 RESULTS AND DISCUSSION

Carotenoids and Oil profile of 'Dekopon' peel

Immediately after homogenization of 'Dekopon' tangerine peels was performed the identification and quantification of the carotenoids by exaustive and ethanolic method (Figure 1). In general, the carotenoids detected in 'Dekopon' tangerine peels were 9-*cis*-violaxanthin, lutein, zeaxanthin, cryptoxanthin and β-carotene. By analyzing the different extraction methods (exhaustive and ethanolic), it is possible to observe a higher carotenoid content when the exhaustive method was employed (9-*cis*-violaxanthin - 3888.82 ± 1292.99 µg/100g; lutein - 363.03 ± 85.22 µg/100g; zeaxanthin - 456.92 ± 91.45 µg/100g; cryptoxanthin - 2429.70 ± 30.79 µg/100g; β-carotene - 203.88 ± 20.98 µg/100g) and lower carotenoid content when the ethanol method was applied (9-*cis*-violaxanthin - 158.82 ± 5.95 µg/100g; lutein - 75.37 ± 1.59 µg/100g; zeaxanthin - 70.22 ± 3.19 µg/100g; cryptoxanthin - 318.24 ± 6.45 µg/100g; β-carotene - 92.26 ± 1.07 µg/100g). The carotenoids 9-*cis*-violaxanthin, lutein and cryptoxanthin were also observed in the peel profile of different citrus fruits (orange, mandarin, clementine, kumquat, grapefruit and lemon) (AGÓCS et al., 2007).

The total carotenoid content was 7342.35 µg/100g (exhaustive method) and 714.91 µg/100g (ethanolic method). In this context, the yield of the ethanolic extraction in relation to exhaustive was 9.14 %. Although this percentage of carotenoid recovery seems low, we should note the environmental issue, since the ethanol solvent is generally recognized as safe (GRAS), and also considered environmentally friendly and not toxic to human health (BUCIĆ-KOJIĆ et al., 2009).

Figure 1. Content of individual carotenoids (µg/g) in 'Dekopon' tangerine peel.



The major fatty acids found in 'Dekopon' peels were the saturated fatty acids such as palmitic acid (13.30 µg/g), stearic acid (8.20 µg/g), and unsaturated fatty acids such as oleic acid (6.46 µg/g), linoleic acid (7.71 µg/g), and γ-linolenic acid (3.76 µg/g). A research investigated the composition of fatty acids in different citrus fruits cultivated in Japan, and the main fatty acids found in 'Dekopon' peel were oleic acid, stearic acid, palmitic acid, myristic acid and lauric acid (WADA et al., 2013). This difference in the fatty acid profile between the work recently cited and the present study is probably due to edaphoclimatic factors. The steam-dragging distillation method (Clevenger extractor) allowed to achieve an essential oil extraction yield of 0.41 %, this value is significant when taking into account the extraction of essential oils.

4 CONCLUSION

The present study allowed to know the carotenoids profile and fatty acids ester of 'Dekopon' tangerine peel, and a possible use of this residue for extraction and purification of these compounds and their future application in food matrices.

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