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Evaluation of total carotenoids content in some Venezuelan orange peel varieties

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Abstract

With the aim of evaluating the total carotenoids content in orange peels (Varieties: california, pineapple, creole and cajera) were determined a selection of fruits was made according to the following criteria: the apparent absence of chlorophyll and homogeneous ripeness. Peels were processed in order to obtain flour, which was submitted to independent exhaustive extraction processes, using as a solvent hexane and an ether mix petroleum/hexane (25:75) Significant differences were determined in relation to the quantification of the total evaluated carotenoids among the four evaluated varieties ($P<0.05$). The best treatment was the 75% hexane mix: 25% of petroleum ether, independently of the variety. The variety with the highest yield was the Creole, with a 140.0 mg content of carotenoids/kg of peels.

Key words: Oranges, carotenoids, extraction, organic solvents.

Introduction

Species of Citrus genus are from the Tropic and sub-tropic of the east of Asia and the Indo-Malayo archipelago (2). In relation to sweet orange, it is thought that it originated on the southeast Asiatic and was spread through Arabia and the south of Europe (15). Nowadays, it is in almost all the tropical and subtropical regions of the world. Venezuelan plantations are on the high valleys of Carabobo, (Bejumam Montalbán and Miranda municipalities) and Yaracuy, Táchira state, Monagas, Sucre, Aragua and Miranda (10). In Venezuela the following varieties of sweet oranges are cropped: Valencia, California (Washintong Navel), Pineaple, Parson, Brown, Mediterránea dulce, Luegin and Puerto Rico (11).

In Venezuela, orange (*Citrus sinensis* L.), is the citric fruit with highest popularity by the industrialization of its juice (13). Approximately 50% of the existent biomass is advantaged, the other 50% constituted by peels and seeds, among others, is sub-used (5, 6, 19). For 2010, it is estimated a national orange production of 907.840 TM. In relation

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to the rubbish sing and flavel production, it is estimated 67.882 TM and 7.006 TM, respectively (4).

Orange peels do not have and exploited potential. The use of this sub-product is limited to the cattle food and recently to the alimentation of the red tilapia (22). However, these rubbish sings are rich in essential oils and natural pigments that deserve to be used in order to achieve a better use of this product (19). Among the natural pigments present in the orange rubbish sings are carotenoids, which are very important since it has a good source of vitamin A (1 equal to retinol = 1μ of retinol = 6 μg de of β–carotene), these are not toxic and inside the cell an antioxidant activity (16, 17), participate in the deactivation of free radicals produced in the cellular metabolism, these provide yellow and red color to plants and animals (3, 7, 9, 23, 27). The evaluation and posterior purification of these metabolites will allow using them as natural colorings to fortify the color in juices and/or in any other food instead of using artificial colors which has toxic components (6, 19, 20, 21, 23). Colorings used in the agro-industry are mostly imported and has an artificial origin. Many of these compounds produce allergies in children at high concentrations (23).

On the contrary, epidemiologic researches have proved that there is an inverse relation between the incidence of Cancer and the consumption of natural sources of carotenoids (17). However, the purification of these compounds is expensive by the use of organic solvents on its extraction. Carotenoids are tetraterpenes composed by 40 atoms of carbon, derived from the phytoene, these are basically composed by eight isoprenic units, so their disposition of the isoprene units are inverted in the center of the molecule, the two central methyl groups are in 1.6 position, while the rest of the methyl groups are on 1.5 position (12, 27). These have a common biosynthetic origin with lots of isoprenic structures: monoterpenes, "ghibellino" and sterols, being the precursor the pyrophosphate isopentenyl, which forms after acetyl-CoA with a prior condensation of intermediates hydroximethyl glutamil and Mevalonate (27). The most used methods in the extraction of carotenoids are: removal with organic solvents, super critical fluids and oils (14, 20, 21, 24, 25, 29)

The aim of this research is to evaluate the total carotenoids content in peels of tour orange varieties cropped in the occident of Carabobo state, Venezuela, using two types of organic solvents.

Materials and methods

Sample:

Two hundreds oranges were collected (*Citrus sinensis* L.) for each variety Criolla, California, Cajera and Pineapple. The sample for the first three varieties was done in Canoabo, Carabobo state, Venezuela, and Pineapple variety was collected at the

Montero farm located on Montalban municipality, Carabobo state. All samples belonged to the crop of May-June, 2002. The transfer of fruits was done in the Biomolecules laboratory in conventional sacks. The sample of fruits was done according to the criteria stablished by Moreno-Alvarez *et al.*, (21)

Physical-chemical characterization of the juice and peels

Oranges were processed with the aim of extracting juice to evaluate the following physical-chemical parameters: soluble solids expressed as ºBrix, determined using a refractometer Baush & Lomb ABBE-3L, pH determined using a Hanna instrument equipment, pHep1®. The acidity of the juice and the humidity of peels were determined following the AOAC methodology (1). The maturity index was calculated through the ºBrix/acidity relation. Averaged values of three replications were calculated.

Obtaining of the orange peels wholegrain flour

A sample was done in the laboratory to select approximately 100 oranges per variety with a homogeneous maturity level, discarding those fruits that had green areas. Fruits were put in plastic containers with enough clean water and were rubbed manually to eliminate residues of land, leafs and any other particles present in the surface of the fruit.

Peels were gotten rid of the flabel and were cut in pieces of 4 cm approximately, using for that a stainless steel knife. Subsequently, peels were cut and dried in a stove at a temperature of 45ºC until reaching humidity from 7% to 10%. A grinding was done with a grinder MLW brand model 214, with the aim of obtaining the desired granulometry (sieve Sardo brand 0.25 aperture) to facilitate the extraction process (22).

Carotenoids extraction

Once obtained the flour 10.000±0.001 g was weighted and was put on amber flasks and 100 ml of organic solvent was added. The independent treatments established were: 100% n-hexane (H) Mallinckrodt ChormaAR® HPLC brand, with a boiling interval of 60-70ºC and 75% hexane (H):25% of petroleum ether (E) (Riedel-de Haën® brand, with a boiling interval of 40-60ºC). Extractions were all in the dark for 48 hours. The extracted were filtered at vacuumed in porcelain funnels (Pyix USA, Nº 36060; 15 ml, ASTM 10-15 ml) rinsing with 100 ml of solvent. The obtained extracts were concentrated in a rotator equipment Heidloph brand model VV2011, at a temperature of 40ºC ± 0.1. The concentrated and previously rooted extracts with their respective organic solution were put in separation funnels, were mixed with 100 ml of a KOH methanolic solution at 1% p/v. Finally, it was proceeded to quantify the total carotenoids after the organic extracts present in orange peels.

Quantification of the carotenoids content present on each variety

The extracts were evaluated through a visible spectroscopy using a Spectronic 20, Bausch & Loumn, with 1 cm of thickness against their respective whites, the determination of the total carotenoids was done through the following calibration curve: Y = 0,0219 + 28,138 X; as a pure pattern was used beta-carotene (Sigma St Louis, Mo, USA.) at a waves longitude of 440nm.

Experimental design

A 4 blocks design at random was used: orange varieties (Criolla, Cajera, Pineapple and California) with 3 replications and 2 treatments with 2 solvents ("H" pure hexane and a mix of hexane-petroleum ether "H-E" at a relation of 75% H: 25%E). This was supported on a prior research where was determined that two mixes resulted more suitable for the extraction of the total corotenoids in oranges peels (18). Values obtained were submitted to a variance analysis (P<0.05) and a Tukey's mean comparison test using for that the statistical software SAS (26).

Results and discussion

In table 1 are shown the values obtained of the physical-chemical characterization of the raw matter for the juice (pH, acidity, ºBrix and maturity index). pH oscillated from 2.8 to 4.3 being the Criolla variety of 3.5 and Pineapple of 3.8 the ones with the most similar values of the Valencia variety (21).

Of the evaluated varieties, Cajera variety was the one with the highest assigned acidity value 1.34, which is directly related to the lowest pH value obtained, meanwhile, California variety was the one with the nearest value of 0.81 grams of citric acid/100 ml of juice than the mentioned for Valencia variety (21). Values of soluble solids are within the established limits for the different varieties of orange (18). California variety has a value of 11.4 ºBrix, a very close value to the established for Value (21) that was 11.2ºBrix.

Table 1. Physical-chemical characteristics of orange juice (*Citrus sinensis* L.)[1]

	Varieties			
Analysis	California	Pineapple	Criolla	Cajera
pH	4.3 ± 0.1	3.8 ± 0.1	3.5 ± 0.1	2.8 ± 0.1
Soluble solids (ºBrix)	11.4 ± 0.1	12. 1± 0.1	11.0 ± 0.1	12.5 ± 0.1
Total acidity (*)	0.81 ± 0.01	0.90 ± 0.01	1.21 ± 0.01	1.34 ± 0.01
Maturity index (**)	13.3 ± 0.9	13.22± 0.2	9.1 ± 0.1	9.3 ± 0.1

*Grams of citric acid/100 mL of juice

**ºBrix/acidity relation

1: Averaged value of three determination ± standard deviation

In relation to the maturity index, California and Pineapple varieties had the highest maturity level of 13.84 and 13.22 respectively, values that might be since samples were selected without any apparent chlorophyll, because these metabolites interfere with the carotenoids analysis (21). California variety was the one with the closest maturity level to Valencia, which is 13.8 (4). The variation of the obtained values of pH, acidity, soluble solids and maturity indexes among the varieties might be due to the climatic conditions, soil conditions and the seasons of the year where the collection is made.

The results of the physical-chemical characterization of orange peels are shown on table 2. The humidity values obtained for the four studied varieties are from 72.83 and 59.62%, being the California variety the one with the closest value (66.50%) to the Valencia variety (21).

In table 3 are shown the averaged results obtained of the total carotenoids for the four studied variables. The variance analysis indicates that significant differences were not found among the studied variables, independently of the used solvent ($P<0.05$), which means that statistically Criolla, Pineapple, California and Cajera varieties have the same carotenoids content.

In relation to the effect of solvents on the carotenoids extraction on each studied variety, the variance analysis indicates that there are significant differences among these, therefore, a mean test was done and it determined that the highest concentration of CCT/kg was obtained extracting the carotenoids with the 75% hexane mix with 25% ether ($P<0.05$). These results agree to those mentioned in the other research (4) with Valencia variety using the same solvent relation. Criolla variety had the highest content of total carotenoids in the mix (75% hexane: 25% of petroleum ether) and California variety on the hexane solvent; these differences might be due to an intrinsic composition of the compounds (8).

Comparing the carotenoids values obtained from orange peels (*Citrus sinensis* L. var criolla) to the pericarp of *Cyphomandra betacea* Sendth var. Roja (8), *Citrus reticulata* Dancy and Sapa (King) varieties (28) and *Citrus sinensis* L. var.Valencia (4) in the treatment with ether-hexane, was observed that *Cyphomandra* has a high pigmentation power, but at the

Table 2. Humidity percentage of orange peels (*Citrus sinensis* L.)[1]

	Varieties			
Analysis	California	Pineapple	Criolla	Cajera
Humidity (%)	66. 50± 0.38	59.62± 0.46	72.83± 0.56	69.13± 0.53

1: averaged values of three determinations ± standard deviation

Table 3. Concentration of total carotenoids (CCT) in peels of four orange varieties (*Citrus sinensis* L) studied using different mixtures of solvents.

	Concentratiom (mg CCT/kg peel)	
Variety	75% Hexane- 25% petroleum ether	100% Hexane
California	120.0[a]	70.0[b]
Criolla	140.0[a]	60.0[b]
Cajera	103.3[a]	53.3[b]
Pineapple	110.0[a]	60.0[b]

Note: different letters in a same row and column indicate that there are significant differences ($P<0.05$)

same time, orange of Criolla variety has a higher content of carotenoids than Valencia variety and *Citrus reticulate,* which makes of it an important pigment source

Conclusions

The physical-chemical parameters evaluated for the four varieties are inside the intervals mentioned for varieties *Citrus sinensis* L. The extraction and quantification of total carotenoids for the four varieties resulted to be suitable. The highest extraction of pigments was obtained from the hexane-petroleum ether mix (75:25) independently of the used sample. Criolla variety presented the highest concentration of total carotenoids (140 mg CCT/kg) for the hexane – petroleum ether mix. Significant differences were not determined between the content of total carotenoids for the studied variables and using a same mix of solvent.

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