Physico-chemical Characterization of Seed Oils Extracted from Oranges (*Citrus sinensis*)

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This study aimed to characterize the seeds of oranges, varieties Hamlin, Natal, Pera-rio and Valencia, on the composition and physico-chemical properties of its oil through the determination of free fatty acids, peroxide value, refraction, iodine value, saponification value, unsaponifiable matter, oxidative stability and fatty acid profile. The results were submitted to analysis of variance and differences between the average results were tested at 5% probability by Tukey test. The orange seed oils showed high levels of lipids, about 40%, and showed a low degree of degradation, when analyzing the free fatty acids and peroxide value. It was found through the fatty acid profile and refraction and iodine indexes seed oil, Pera-rio orange was the most unsaturated. The seed oil, Valencia orange had a higher oxidative stability in Rancimat at 100°C.

Keywords: agro-industrial residues, physico-chemical, oxidative stability

Introduction

The citrus industry has great growth potential, being one of the most competitive agricultural sectors among the countries with tropical and subtropical climate. Annually, 105 million tons of citrus fruit are produced worldwide. The area planted to citrus in the world is approximately 7.62 million hectares. Among the citrus fruits, the orange acreage represents about 55% of the total, which confirms this as the main crop in citrus (Hernández-Montoya et al., 2009).

Thus, may the above cited oranges they may be cited oranges presenting a high potential for use primarily as industrial products, such as juice. For this reason, daily, a large volume of waste is generated by this agribusiness industry, and in most cases, its use has been limited only to the animal feed industry, or disposing into the environment causing serious environmental damage. However, these wastes have high potential, as well as having sugars, vitamins and minerals, are rich in fiber, oils and other compounds with functional properties. The investigation of essential oils in the diet and its role in food quality and consumer health is an emerging research area still unexplored (Laufenberg, 2003).

The importance of further studies related to industrial waste as a source of bioactive, is related to the recovery of these raw materials and their waste to obtain new products with high added value. Orange is extensively processed by juice manufacturing industries, however, the use of these residues, when present, almost does not add value. The incorporation of waste for the production of high quality essential oils can increase the availability of products to meet the emerging needs of new sources of oil. Moreover, also favor a better utilization of waste generated by industry, thus avoiding wasting (Bampidis and Robinson, 2006; Moraes et al., 2009). In this context, this study aims to characterize the oil extracted from orange seeds, and use of industrial wastes, through physico-chemical properties and fatty acid profile, in order to verify the use of these seeds as alternative sources for obtaining special oils.

Material and Methods

Material

In the present study we used a variety of orange seeds, such as Hamlin, Natal, Pera-rio and Valencia, as they are mostly used in juice production in the state of São Paulo. Three batches of waste of each variety were purchased on different days between the months of June-August 2010 at the harvester industry Cutrale Citrus Juice Inc. – Uchôa/SP/Brazil. Immediately after receiving the waste, the seeds were manually separated from husks and bagasse and dried for approximately 96 h on trays at room temperature to reduce the moisture content (< 10%). The plots of each variety
of orange seeds, weighing 800 – 850 g, were homogenized, packed in plastic containers, sealed with screw caps and appropriately labeled for subsequent analyzes.

**Centesimal seed composition** At the time of analysis, the seeds were crushed in a grinder knife, Marconi brand, model MA340. Moisture analysis were executed, by drying in a vacuum oven at 70°C until constant weight is obtained, the quantification of lipids using petroleum ether 40 – 60°C in Soxhlet extractor; the determination of ash content by calcination in a muffle presenting 550°C in accordance with AOCS official methods (2009). The gross protein concentration was determined by quantification of total nitrogen contained in the sample, still using the micro distiller Kjeldahl method according to AOAC (1995). The percentage of total dietary fiber was determined by AOAC 985.29 (2005) enzymatic-gravimetric method. The total carbohydrate content was obtained by the difference of the total sample (100%) moisture, lipids, proteins, ash and total dietary fiber.

**Extraction of the oils of seeds** Oils were obtained in extraction by pressing of vegetable oil, Brazil Metal Wilhelms brand at room temperature, with initial rotation of 1500 rpm and the end of approximately 3600 rpm. After extraction, the oils were placed in amber color glass bottles, inertised with gaseous nitrogen and stored at −18°C for further analysis.

**Oil physico-chemical properties** Oil physico-chemical analyzes of free fatty acids, peroxide value, refractive index, iodine value, saponification value and unsaponifiable matter were made by official AOCS methods (2009). The index oxidative stability was performed by the method proposed by AOCS (2009), using the rancimat at 100°C with airflow of 20 L/h. The fatty acid profile was determined by gas chromatography of samples from transesterified with methanolic potassium hydroxide and n-hexane, according to method AOCS Ce 2-66 (2009).

**Statistical analysis** The obtained results from the analytical determinations in triplicate were subjected to analysis of variance, and Tukey test for the 5% average were obtained using the software ESTAT 2.0 (Gacula and Singh, 1984).

## Results and Discussion

**Seeds centesimal composition** Table 1 shows orange seeds centesimal composition results. We observe a significant difference by Tukey test for moisture, lipid, protein, ash and dietary fiber.

The seed moisture ranged from 3.56 to 8.00%; which difference may be due to the drying process. However, all the seeds found to be below 10%, ideal value for extraction of vegetable oils. Hernández-Montoya et al. (2009) analyzing *Citrus sinensis* embryo and husk humidity, have found 3.83 and 9.09% respectively. Waheed et al. (2009) obtained 13.5% moisture for *Citrus sinensis* seeds.

Singh et al. (2002), in oil extraction by pressing studies on process optimization, reported the importance of defining an optimal range for moisture, since it was observed that very high values are friction reducers of the grain mass, causing a low yield extraction, whereas very low levels impair the functioning of the press.

The seeds lipids’ percentages ranged from 37.45 to 41.74%. Pera-rio seeds showed significantly higher amounts of lipids (41.74%), followed by Valencia (39.67%), Natal (39.03%) and Hamlin (37.45%). The varieties Natal and Valencia did not differ significantly. Other authors also found high levels of lipids in orange, tangerine, and lemon seeds (Ajewole and Adeyeye, 1993; Lazos and Servos, 1988; Reda et al., 2005). By analyzing the oranges families *Citrus sinensis, aurantium* and *paradisi*, Waheed et al. (2009) found levels of 36.52, 30.07 and 21.80%, respectively.

Obtained lipids values indicates that orange seeds are a good oil source, especially when compared to soybean seeds which have approximately 20% of lipid content.

As for protein content in orange seeds, Pera-rio was significantly higher than the other varieties, with protein content of 15.02%. Hamlin variety had lower protein content with 11.20% and the other varieties did not differ significantly with levels of 12.22 and 12.61% for Natal and Valencia, respectively.

Pera-rio seeds had the highest percentages of fat (41.85%)

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Moisture</th>
<th>Lipids</th>
<th>Proteins</th>
<th>Ash</th>
<th>Dietary fiber</th>
<th>Carbohydrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamlin</td>
<td>3.56 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.45 ± 0.28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.20 ± 0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.61 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.51 ± 0.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.67 ± 0.15&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Natal</td>
<td>8.00 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.03 ± 0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.22 ± 0.00&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.87 ± 0.02&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>34.92 ± 0.80&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.96 ± 0.27&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pera-Rio</td>
<td>5.88 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>41.74 ± 0.46&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.02 ± 0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.79 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>29.80 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.77 ± 0.68&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Valencia</td>
<td>3.66 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>39.67 ± 0.38&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.61 ± 0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.92 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>29.61 ± 0.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.53 ± 0.56&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The results represent the average ± standard deviation of the analyzes perfomed in triplicate. a,b... means followed by same letters in row do not differ by Tukey test (p < 0.05). *Analysis perfomed in duplicate. **Calculated by difference.
and protein (15.02%) among the seeds analyzed, while the Hamlin variety seeds had the lowest percentage of fat (37.56%).

According to Table 1, the ash contents found in the seeds were 2.61 to 2.92%. As the ash content of these minerals indicates the mineral amount the sample has, it can be concluded that oranges seeds, studied in this work, are important sources of these micronutrients. In a chemical Citrus sinensis seeds composition, Hernández-Montoya et al. (2009) found ash content ranging from 1.59 to 3.09%.

Dietary fiber has been identified as an important component of a healthy diet. Defined as components of plant cells that resist digestion by the human digestive enzymes. Its consumption has been associated with reduced risk of chronic diseases (Liu, 2007).

From Table 1 it was noted that the dietary fiber content of orange seeds ranged from 29.61 to 43.51%, indicating that they are good fiber sources. Hamlin variety seeds showed the highest fiber content with 43.51%, being so, these seeds can be used for food enrichment and also for animal feed formulation.

Rocha et al. (2008) found fiber content in the orange bark (Citrus aurantium) of 4.6%. Mendonça et al. (2006) while analyzing tahiti lime flavedo, albedo and pulp (Citrus latifolia) had a fiber content of 24.67, 24.71 and 17.28%, respectively.

The amount of carbohydrates in the seeds was calculated excluding the percentages of moisture, lipids, proteins, ash and dietary fiber of 100%. Valencia variety had the highest carbohydrates percentages and Hamlin had the lowest: 11.53 and 1.67% respectively.

Oil physico-chemical properties Table 2 shows oils of orange seeds physico-chemical characteristics results. It appears that rates of peroxide and saponification were that differed significantly by Tukey test (p < 0.05), for all varieties of oranges.

The percentage of free fatty acids and acid value are related to hydrolytic reactions development in the oil. Codex Alimentarium Commission (2009) sets to judge crude oil quality the maximum of 4.0 mg KOH/g. It is observed that the oils analyzed acid number varied from 0.36 to 0.54 mg KOH/g, with values within the limits allowed for crude oils.

There was a significant difference noted between free fatty acids levels found in orange seed oil, Valencia being the variety with the highest content, 0.27%, and Natal variety with less content, 0.18%. This difference may be due to factors that accelerate the formation of free fatty acids from the hydrolysis of glycerides, such as heat, light, injury, falls in fruit and enzymatic action.

Free fatty acids percentages found in this work for orange seeds’ oils were lower than those obtained by Holser (2003) in oils extracted by seed pressing Limanthes alba, ranging from 0.51 to 1.77%.

Ixtaina et al. (2011) studied chia seed oils (Salvia hispanica L.), from Guatemala and Argentina, and found acidity levels of 0.7 and 0.91 mg KOH/g when the oil was extracted by pressing and 1.64 and 2.05 mg KOH/g oil extracted by the solvent, respectively. When studying seed oil, osage orange (Maclura pomifera), Saloua et al. (2009) obtained acidity of 0.66 mg KOH/g.

The peroxide value is one of the commonly parameter used to determine the oil quality during its production and storage. This ratio determines the oxidation stage, since the peroxides are the primary products formed during the oil oxidative degradation. To crude and refined oil, the Codex Alimentarium Commission (2009) provides maximum peroxide value of 10 and 15 meq/kg, respectively. The oils analyzed in this work showed peroxide below 15 meq/kg, ranging from 0.44 meq / kg oil for the Natal variety, to 1.27 meq/kg for the Pera-rio.

Table 2. Physico-chemical characterization of seed oils oranges.

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Hamlin</th>
<th>Natal</th>
<th>Pera-Rio</th>
<th>Valencia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free fatty acid (%)</td>
<td>0.22 ± 0.01bc</td>
<td>0.18 ± 0.02c</td>
<td>0.23 ± 0.02ab</td>
<td>0.27 ± 0.03a</td>
</tr>
<tr>
<td>Acid index (mg KOH/g)</td>
<td>0.44 ± 0.02bc</td>
<td>0.36 ± 0.03c</td>
<td>0.46 ± 0.04ab</td>
<td>0.54 ± 0.05a</td>
</tr>
<tr>
<td>Peroxide value (meq/kg)</td>
<td>0.91 ± 0.05b</td>
<td>0.44 ± 0.06d</td>
<td>1.27 ± 0.05</td>
<td>0.63 ± 0.05</td>
</tr>
<tr>
<td>Refraction index (40°C)</td>
<td>1.4668 ± 0.0001b</td>
<td>1.4670 ± 0.0001b</td>
<td>1.4695 ± 0.0002a</td>
<td>1.4660 ± 0.0002a</td>
</tr>
<tr>
<td>Iodine index (g L/100 g)</td>
<td>108.89 ± 1.24a</td>
<td>109.48 ± 1.49b</td>
<td>122.58 ± 1.73c</td>
<td>98.70 ± 1.47c</td>
</tr>
<tr>
<td>Saponification index (mg KOH/g)</td>
<td>181.60 ± 0.47c</td>
<td>183.77 ± 0.36c</td>
<td>190.83 ± 0.16c</td>
<td>186.43 ± 0.29c</td>
</tr>
<tr>
<td>Unsaponifiable matter (%)</td>
<td>1.49 ± 0.07b</td>
<td>1.48 ± 0.01b</td>
<td>0.57 ± 0.03c</td>
<td>5.31 ± 0.07a</td>
</tr>
<tr>
<td>Oxidative stability (h)</td>
<td>12.32 ± 0.04b</td>
<td>11.67 ± 0.33c</td>
<td>11.55 ± 0.16c</td>
<td>15.81 ± 0.13c</td>
</tr>
<tr>
<td>Physical state at room temperature</td>
<td>liquid</td>
<td>liquid</td>
<td>liquid</td>
<td>liquid</td>
</tr>
</tbody>
</table>

The results represent the average ± standard deviation of the analyzes performed in triplicate. a,b... means followed by same letters in row do not differ by Tukey test (p < 0.05).
Kobori and Jorge (2005) analyzing the orange seed oil extracted by hot solvent, found peroxide value of 29.4 meq/kg. Reda et al. (2005) obtained rose lime seed oil ands siccative lime, peroxide value of 1.94 and 1.90 meq/kg, respectively. The difference between the values of peroxides may indicate that in some way, the oil underwent oxidation during the raw material preparation, extraction or storage.

The refractive index is related to the molecules unsaturation degree, with the ratio of double bonds cis/trans and can be influenced by the oil oxidation. The oils of orange seeds had refractive indexes from 1.4660 to 1.4695, with a significant difference. These values are consistent with those obtained by Nagy et al. (1977), in studies with different varieties of orange, 1.4608 to 1.4714, consistent with the value obtained by Romero et al. (1988), who found 1.4710 at 20°C, and the largest found by Kobori and Jorge (2005), 1.4651.

The iodine value is an unsaturation degree measure of fatty acids in oils and fats. In accordance with the amount of iodine, vegetable oils can be classified into siccative (> 130 g I2/100 g), semi-siccative (115 − 130 g I2/100g) and non-siccative (< 115 g I2/100 g) (Van De Mark and Sandefur, 2009). In the oils analyzed, except the Pera-rio, the iodine value ranged from 98.70 to 109.48 g I2/100 g classifying them as non-siccative oils, meaning, they have considerable amounts of saturated fatty acids. As for Pera-rio oil, with iodine value of 122.58 g I2/100 g, can be classified as semi-siccative.

Observing the analyzed iodine oil index we can verify that Valencia variety seed oil is the most saturated and varieties of Hamlin and Natal present intermediate saturation and Pera-rio as unsaturated. At room temperature, citrus seed oils were present in a liquid state.

As the refractive and iodine indexes increases along with the unsaturation degree, was performed correlation analysis and obtained value of 0.97, which shows a strong correlation between the two indexes for oils analyzed in this work.

The saponification number indicates the average molecular weight for fatty acids esterified to glycerol in the triacylglycerol molecule, in other words, the higher a saponification number suggests for fatty acids, the lower will be their molecular weights. Among the oils studied, the highest saponification rate was presented by Pera-rio orange seeds (190.83 mg KOH/g) and the lowest for Hamlin orange seeds (181.60 mg KOH/g). The values obtained are similar to Codex Alimentarium Commission (2009) for vegetable oils such as palm (180 – 205 mg KOH/g), rice (180 – 199 mg KOH/g) and canola (182 – 193 mg KOH/g).

The amount of unsaponifiable matter in oils analyzed ranged from 0.57 to 5.31%, presenting Valencia orange seed oil the highest value. Since the unsaponifiable material corresponds to the compounds present in oils that after saponification with alkali are insoluble in aqueous solution, including naturally occurring substances such as sterols, tocopherols, hydrocarbons and pigments, the oil of the seed should contain high quantities of chemicals. According to Codex Alimentarium Commission (2009), the maximum amount of unsaponifiable matter to soy and cotton oil is 1.5%, 2.8% for corn and 6.5% for rice. Based on these values, the seed oils of this study are consistent with the amount of unsaponifiable material for edible vegetable oils.

Table 2 shows the average rates of oxidative stability, in hours, seed oils obtained from the oranges. The oxidative stability is related to the nutritional and sensory quality of vegetable oils, and this measure is used to predict the shelf life of edible oils and fats, using as parameter the induction period, defined as the time to achieve detectable level of rancidity or rapid increase in the rate of oxidation.

Regarding oils analyzed in this study, oxidative stability indexes differed significantly, except for Natal and Pera-rio varieties. The stability values ranged from 11.55 h for Pera-rio and 15.81 h, for the oils from the seeds of Valencia variety.

Kobori and Jorge (2005) found for orange seed oil oxidative stability of 3.25 h, less than those observed in orange seed oils of this work. This difference may be due to the drying and storage conditions of seed.

Table 3 shows the results for orange seed oil fatty acid profile. In total we identified 11 fatty acids, being palmitic acids (21.72 to 37.18%), oleic (24.58 to 29.31%) and linoleic acid (21.32 to 31.86%) detected in larger amounts for the four varieties. Waheed et al. (2009) while studying the fatty acid composition of seed oil of Citrus sinensis also found as major acids, palmitic acid, 31.37%, oleic 22.89% and linoleic, 30.53%.

Among the major fatty acids found, the oils of varieties Hamlin, Natal and Valencia presented mostly as the main constituent of palmitic acid in amounts of 37.18, 37.02 and 36.70%, respectively. This characteristic was also found in citrus seed oils by other authors (Ajewole and Adeyeye, 1993; Saïdani et al., 2004; Anwar et al., 2008). As for Pera-rio seed oil obtained more linoleic acid, with 31.86%. This value is lower than that reported by El-Adawy et al. (1999) (38.4%) for orange seed oil.

According to Banni et al. (1995), Menendez et al. (2005), among the monounsaturated fatty acids, oleic acid was the main one, which has as main effects, reducing the fraction of total cholesterol and cholesterol low density lipoprotein (LDLc) without reducing the fraction of high lipoprotein cholesterol density (HDLc). Furthermore, it causes changes in the platelet membrane producing an anti-thrombotic action.
and 13.2%, respectively. Reda gerine seed oils, obtained amounts of α-linolenic acid of 6.7 served that the amount of unsaturated fatty acids is greater rates were the polyunsaturated (34.61%).

unsaturated fatty acids (64.98%), whereas the major unsaturated fatty acids, 35.02%, and consequently higher amounts of the other oils studied, with a lower percentage of satu-

rated and polyunsaturated. It is ob-

served that the amount of unsaturated fatty acids is greater

fatty acids. These results agree well with those reported by Malac-

erida et al. (2012) for orange, lemon and tangerine seed oils, 23.04, 20.80 and 27.78%, respectively.

Regarding the fatty acid α-linolenic acid, oils from orange seeds showed percentages of 2.10% for Valencia variety, 2.30% for Hamlin, 2.75% for Pera-rio and 2.76% for variety oil had 47.66%, followed by Natal (47.21%), Valencia (46.84%) and Pera-rio (35.02%). As for the monounsaturated and polyunsaturated ranged from 25.26 to 30.37% and 25.82 to 34.61%, respectively.

Seed oils all shown to be composed predominantly unsaturated fatty acids, comprising more than 50% of the total. Knowing the consumption of unsaturated fatty acids provides a lower risk of cardiovascular diseases, it can be argued that among the oils studied, Pera-rio, which had 64.98% of unsaturation, is the one that can provide the greatest health benefits. Figure 1 shows the chromatogram of the fatty acid profile of Pera-rio seed oil.

Waheed et al. (2009) found in Citrus sinensis seed oils saturated fatty acid content of 41.2% and 58.8% of unsaturated, these values are consistent with those obtained in this work.

Comparing saturated and unsaturated total ratio for fatty acids in table 3, it appears that Pera-rio seed oil has the best value (1/1.85), and is therefore a salad oil.

Correlation analysis was performed between the refractive and iodine indexes with saturated fatty acids, monounsaturated and polyunsaturated oils studied. The correlations are shown in Table 4.

Accordingly, there was a significant correlation between saturated fatty acids, mono and polyunsaturated and refractive index, seen as negative with the saturated fatty acids, and positive with the unsaturated. Thus, increase of the oil saturation causes a decrease in refractive index.

The iodine index was significantly correlated with the
than saturated. The Valencia orange seed oil stands out for the amount of unsaponifiable material and the increased oxidative stability, as being the most suitable for thermal processes.

Regarding the composition of fatty acids, palmitic, oleic and linoleic were the major fatty acids detected in the oils analyzed. The amount found α-linolenic acid in the seed oil of orange are comparable to some commercial oils.

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References


