HASS AVOCADO OIL (Persea Americana Mill.)
FRACTIONATION BY NATURAL CRYSTALLIZATION

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RESUMO – O objetivo deste trabalho foi promover o fracionamento dos óleos de abacate Hass, verde e maduro, extraídos por prensagem contínua da polpa e casca do fruto, desidratadas. A acidez, estabilidade oxidativa, capacidade antioxidante e ponto de fusão foram determinados no óleo e nas frações obtidas após cristalização natural – oleína (fase líquida) e estearina (fase sólida). O óleo do fruto maduro apresentou maior teor de ácidos graxos livres e menor estabilidade oxidativa que o do fruto verde. Entretanto, a capacidade antioxidante das amostras íntegras foi mantida. As condições operacionais da cristalização foram satisfatórias para promover o fracionamento e separação das fases em equilíbrio. A estearina apresentou maior estabilidade oxidativa, 27 a 43 horas, que a oleína12 a 21 horas. O ponto de fusão da estearina, em ambos os casos, foi inferior ao da oleína. Os resultados obtidos para o óleo de abacate verde foram mais promissores para o fracionamento.

ABSTRACT – The objective of this work was to promote the fractionation of Hass avocado oils extracted by continuous pressing, from dehydrated unripe and ripe fruit pulps with peel. Acid value, oxidative stability, antioxidant capacity and melting point were determined for the oils and its fractions obtained by natural crystallization – olein (liquid phase) and stearin (solid phase). Oil extracted from ripe fruit presented higher free fatty acids content and smaller oxidative stability in relation to oil obtained from unripe avocado. However, the antioxidant capacity of raw-fruit oils was maintained. The selected temperature range for crystallization proved satisfactory to promote the fractionation and separation of the phases in equilibrium. As expected, the stearin presented higher oxidative stability, 27 to 43 hours, than olein, 12 to 21 hours. Stearin melting point was inferior, in both cases, as compared to olein. Results obtained from unripe avocado oil were the most promising for fractionation process.

PALAVRAS-CHAVE: fracionamento de óleos graxos; cristalização natural; estádio de maturação; estabilidade oxidativa; capacidade antioxidante.

KEYWORDS: fatty oil fractionation; natural crystallization; maturity stage; oxidative stability; antioxidant capacity.
1. INTRODUCTION

Avocado (*Persea americana* Mill.) is a tropical fruit of high economic and nutritional value. It originates from the American continent, in an area between Mexico and Panama, and in Brazil is grown in almost all states of the country. Lipid content of some cultivars exceeds 15%, in wet basis, making it an important raw material for vegetable oils production. Besides the high oil content, avocado oil marketing is favored by avocado production during all the year. Despite this, its world production is still very low, being mainly destined for pharmaceutical and cosmetics products given its physical and chemical characteristics (MASSAFERA, et al., 2010; TANGO, et al., 2004; GALVÃO, et al., 2013).

Oils melting point is influenced by the fatty acid carbon chain. The triacylglycerol’s containing polyunsaturated fatty acids in their structure are usually liquid at 25 °C due to the difficulty of intermolecular interaction, while those containing saturated fatty acids are usually solid or viscous at this temperature, because there is a greater interaction between the carbon chains, resulting in greater attraction force (VIANNI & BRAZ-FILHO, 1996).

The behavior of lipids crystallization have important implications for the industrial processing involving products whose physical characteristics are largely dependent on the fat content in the crystals form. The amount of crystallized lipid and the type of crystals in the lipid matrix has a strong correlation with the rheological behavior of the medium (GAMBOA & GIOIELLI, 2006; RODRIGUES et al., 2007).

The dry fractionation process is based on differences in melting points of the triglycerides, diglycerides and monoglycerides. It consists in a thermomechanical separation process in which lipids are fractionated by partial crystallization and separated in the next step. Traditionally, it is applied in the palm oil processing and fractionation of fats such as beef tallow, lard and milk fat. The dry crystallization is simpler and lower cost, being recognized as “natural” technology. The natural oil fractionation without agitation was observed in recent research from macaúba and pequi oils with very promising results for small pilot plants (MARIANO, 2014). Brazil, one of the major world avocado producers, with the public institutions, national and private research centers support has dedicated the past 30 years to the growth of varieties rich in lipids and more suitable for processing.

The objective of this study was to promote the fractionation by natural crystallization of Hass avocado oils, pressed from pulp and peel at unripe and ripe stages, and evaluate the influence of this process on olein and stearin physicochemical parameters.

2. MATERIAL AND METHODS

Hass avocado oils, extracted from dried, unpeeled using unripe and ripe avocados, by continuous pressing (Santana et al., 2015), were subjected to natural crystallization procedure.

2.1 Fractionation of Oils

Oils fractionation was carried out by slow natural crystallization (dry fractionation by cooling) in a temperature controlled chamber. The temperature ranged from 25 to 11 °C,
during the day, and remained at 11 °C, overnight. The two obtained phases, liquid phase (olein) and solid phase (stearin), were separated by decantation and stored in amber vials for subsequent analysis.

The diagram in Figure 1 shows the simplified scheme of Hass avocado processing to obtain the olein and stearin phases from unripe and ripe avocado. Physicochemical characterization of fruits, oils and fractions obtained from natural crystallization was carried out.

Figure 1 – Avocado processing flowchart

![Avocado processing flowchart](image)

Source: elaborate by the authors

### 2.2 Physicochemical characterization

**Acid value (% free fatty acids–FFA):** determined in triplicate, by acid-base titration using as titrant NaOH 0.01 mol.L\(^{-1}\) (AOCS, 2004). The FFA distribution coefficients after separating the phases were calculated by Equation 1.

\[
K_{FFA} = \frac{FFA_{olein}}{FFA_{stearin}}
\]

(Equation 1)

**Oxidative stability:** determined in triplicate by Rancimat 743 (Metrohm, Switzerland) and the results expressed as Induction Period (IP). Samples were subjected to induced oxidation at 110 °C using air flow of 20 L.h\(^{-1}\) (Martínez Nieto, Hodaifa, Peña, 2010).

**Antioxidant capacity:** antioxidant capacity of pressed oils, olein and stearin were determined by TEAC methodology described by Re et al. (1999) and Tuberoso et al. (2007). The antioxidant capacity was evaluated for raw samples and their hydrophilic and lipophilic fractions, after extraction with methanol.

Antioxidant capacity of the extracts (expressed as μmols.kg\(^{-1}\)) was determined by reaction of 30.0μL of sample with 3.0 mL of ABTS solution. Measure of absorbance was taken after 6 minutes of reaction at 734 nm.

**Melting point:** measured by differential scanning calorimetry (DSC - TA Instruments, model DSC Q 200), with temperature range from -40 °C to 60 °C, heating rate of 5 °C/min, cooling rate of 10
°C/min and reading at second heating. Samples were placed in aluminum hermetic pans and analysis conducted in duplicate.

Statistical analysis: experimental data was evaluated by variance analysis (ANOVA) using the software Statistica (Statsoft, Tulsa, EUA) and Fischer LSD post-hoc test with significance level of 5%.

3. RESULTS AND DISCUSSION

The melting point of the fatty acids in the triacylglycerol molecule present in greater quantities in the avocado oil is very distinct and therefore it is possible to promote their separation by reducing the oil temperature. Thus, the process of dry crystallization was suitable to separate the fractions of unripe and ripe avocado oil, and the unripe avocado oil phases showed more defined characteristics in the crystallization temperature. In both separation processes, olein phase was obtained in greater amounts that the stearin phase (ratio 2:1, approximately). Mariano (2014) found different proportions of these phases in the crystallization of macauba pulp oil, which may be due to the relevant differences in fatty acid composition between both oils.

The stearin phase of ripe avocado oil presented the worst appearance of the fractions obtained after crystallization, represented for its darker color and sandy texture. Olein phase of unripe avocado oil presented the best appearance, with color similar to commercial oils and low viscosity.

Physicochemical analyses results are shown in Table 1. As expected, the acid value of unripe avocado oil and its fractions is smaller than its corresponding ripe avocado oil. This may be a consequence of endogenous enzymes during storage and/or thermal hydrolysis. The acid values obtained for the pressed oils are higher than those found by Santana et al. (2014), but all are below the maximum allowed by Brazilian law to pressed edible and unrefined oils.

The mass distribution coefficient values ($K_{FFA}$) of free fatty acids from unripe and ripe avocado oils were respectively 1.5 and 0.84. There is a predominance of polyunsaturated free fatty acids in unripe avocado, whose melting points ranges between -10 and -5 °C, in the range observed for olein fraction.

Regarding the oil extracted from ripe avocados, free fatty acids are distributed mostly in the highest melting point fraction, also due to the presence of free oleic acid whose melting point is about 13 °C, above the melting point of stearin. These results indicate that, as expected, the hydrolysis of monounsaturated fatty acids mainly occurs during fruit ripening.

As for oxidative stability, oil extracted from ripe avocados was less stable than the oil pressed from unripe fruits. In both oils, stearin presented higher IP when compared to olein, probably due to a superior amount of saturated fatty acids in the solid phase. Induction periods (Table 1) show that fractionation favored the stability of the solid phase. On the other hand, the olein phase of ripe avocado oil showed greater loss of stability when compared to its source oil. Similar results were reported by Mariano (2014) for pequi pulp oil, with IP of 51.5 hours for stearin and 5.4 hours for olein.
Table 1 – Physicochemical characterization of pressed oils from unripe and ripe Hass avocado and olein and stearin fractions after crystallization.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Acid value (% FFA)</th>
<th>Induction period (h)</th>
<th>TEAC (μmols.g⁻¹)</th>
<th>Melting point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unripe avocado</strong></td>
<td></td>
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<tr>
<td>Whole oil</td>
<td>0.70 ± 0.0001³⁴</td>
<td>22.7 ± 1.0142³⁴</td>
<td>1.61 ± 0.0936³⁴</td>
<td>5.73 ± 0.4066³⁴</td>
</tr>
<tr>
<td>Olein</td>
<td>1.04 ± 0.0001³⁴</td>
<td>21.5 ± 0.6751³⁴</td>
<td>0.87 ± 0.0264³⁴</td>
<td>6.08 ± 0.0212³⁴</td>
</tr>
<tr>
<td>Estearin</td>
<td>1.36 ± 0.0002³⁴</td>
<td>27.3 ± 1.0022³⁴</td>
<td>1.51 ± 0.0688³⁴</td>
<td>5.12 ± 0.2015³⁴</td>
</tr>
<tr>
<td><strong>Ripe avocado</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole oil</td>
<td>1.52 ± 0.0005³⁴</td>
<td>18.0 ± 0.1375³⁴</td>
<td>1.71 ± 0.1055³⁴</td>
<td>7.12 ± 0.5233³⁴</td>
</tr>
<tr>
<td>Olein</td>
<td>1.39 ± 0.0005³⁴</td>
<td>12.4 ± 0.2228³⁴</td>
<td>0.40 ± 0.0702³⁴</td>
<td>7.35 ± 0.2404³⁴</td>
</tr>
<tr>
<td>Estearin</td>
<td>3.18 ± 0.0011³⁴</td>
<td>43.2 ± 1.2736³⁴</td>
<td>1.55 ± 0.0943³⁴</td>
<td>5.73 ± 0.0212³⁴</td>
</tr>
</tbody>
</table>

* Lowercase letters corresponds to samples and fractions of the two analyzed oils; uppercase letters evaluates characteristics of the same oil; equal letters indicate no significant differences at a significance level of 5%; Different letters indicate significant differences at a significance level of 5%.

Antioxidant capacity did not differ (p < 0.05) between pressed oils from unripe and ripe avocados. This result indicates that antioxidant activity was not harmed by ripening. In all assays the antioxidant capacity of stearin was superior to olein, confirming the retention of lower molecular weight compounds during the crystals formation.

Stearin melting point was inferior, in both cases, as compared to olein. This probably occurs due to the metastable form of nucleation during dry crystallization, which will be confirmed by scanning electron microscopy (SEM).

4. CONCLUSIONS

It can be concluded that the fruit ripening promoted an increase in free fatty acids in the oil, reducing its oxidative stability. However, there was no significant reduction in antioxidant capacity of oils. Furthermore, the conditions selected for the fractionation were suitable for crystallization and separation of the olein and stearin phases. However, part of the antioxidants and free fatty acids was retained in the crystal structure.

5. REFERENCES


