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Influence of temperature and time during malaxation on fatty acid profile and oxidation of centrifuged avocado oil

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Abstract

Virgin oil from avocados (*Persea americana* Mill.) is obtained by mechanical processes after pulp malaxation at temperatures that minimize oxidation and improve separation. The objective of this study was to assess the effect of time (0, 20, 30, 40, 60, 120 and 180 min) and temperature (40 and 50 °C) conditions during pulp malaxation on extraction yield, nutritional value (normalized fatty acid profile) and specific extintion (K232 and K270) of virgin oil extracted under laboratory conditions from avocados cultivated in southern Jalisco, Mexico. When pulp was malaxated for 120 min at 40 and 50 °C, a larger proportion of oil was extracted (82.9 ± 0.3% and 80.2 ± 0.8%, respectively). We observed that the normalized percentage of the fatty acids linoleic (18 ± 2%) and linolenic (1.2 ± 0.2%) decreased with mixing time, while that of palmitoleic (9 ± 1%), oleic (51.6 ± 1.2%) and stearic (0.5 ± 0.1%) remained without change. The ω-6:ω-3 ratio (15 ± 1) was higher than the recommended values but similar to those reported as favorable for health. Specific extinction ($K_{232,}$ 2.2 ± 0.3 and $K_{270,}$ 0.20 ± 0.03) indicate that the oxidation level remained low. Malaxation at 40 or 50 °C did not significantly alter the characteristics of the oil, but time significantly affected yield.

Keywords: essential fatty acid; fatty acids; linoleic:palmitic ratio; oil yield; oxidation.

Practical Application: Describe the malaxation conditions for avocado pulp that give higher extraction of good quality oil.

1 Introduction

Mexico is the largest producer of avocado (*Persea americana* Mill.) worldwide. Today, around 70 species of the genus *Persea* are distributed in the temperate regions of America, while in East and Southeast Asia there are 80 species (Ding et al., 2007). "Hass" is the variety most cultivated because it is highly productive and its fruit has favorable characteristics; for example, its oil contents can reach 55% (Oliveira et al., 2013). Most of the production is sold for fresh consumption and only 10% is used as raw material in the production of processed food. Avocado oil is one of its main products promoted as a gourmet food. Its fatty acid (FA) profile exhibits 80 to 85% unsaturated FA (mainly oleic and linoleic). This lipid profile has been associated with technological characteristics such as stability at frying temperature (180 °C), which makes it appropriate for cooking, for making dressings or for fabrication of margarines (Meyer & Terry, 2008; Restrepo et al., 2012).

This oil has been classified as "healthy", together with almond, hazelnut and macadamia oil, which have a lipid profile rich in monounsaturated fat like that of olive oil (Kochhar & Henry, 2009). The polyunsaturated/saturated FA ratio and that of ω-3:ω-6 in avocado oil is even higher than olive oil (Berasategi et al., 2012). Because of its lipid profile, it has preventive effects against cardiovascular accidents and antiatherogenic effects and corrects dyslipidemia by reducing levels of low density lipoproteins (LDL) while increasing high density lipoproteins (HDL) (Alvizouri & Rodríguez, 2009). Its composition includes dietary antioxidants such as carotenes, tocopherols and polyphenols, which inhibit growth of prostate cancer cell lines, and sterols, mainly β-sitosterol, which reduce absorption of cholesterol in the intestine; it is an immune potentiator and has anti-inflammatory effects (Ding et al., 2007).

Cold-pressed avocado oil is that which has been extracted mechanically at temperatures below 50 °C with no solvents (Woolf et al., 2009). The process begins with peeling and de-stoning the fruit, followed by pulp malaxation, mixing for no more than 90 min to rupture the sub-cellular compartments that contain the oil. Avocado malaxation conditions are superior to those used in other cases since the oil is more finely dispersed inside the cells. Lipoprotein membranes or lipophilic solids of the paste, which can absorb part of the oil, surround the emulsions formed. For this reason, the process is often facilitated by adding different proportions of water, calcium salts, talcum and acidifiers (hydrochloric or phosphoric acid) with discrete temperature increments, or cellulase, hemicellulase and pectinase enzymes available on an industrial scale (Buelvas et al., 2012; Schwartz et al., 2007). In some cases, the paste is dehydrated before extracting the oil by pressing, centrifuging or decanting (Costagli & Betti, 2015). With the procedures of centrifuging/pressing, yields are often low (Schwartz et al., 2007) but oil quality is higher since its characteristic color is maintained, the formation of free

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and trans FA is kept to a minimum, and oxidation of unsaturated FA is low. These standards are important for prevention or reduction to a minimum of lipid oxidation in edible oils since production and culinary consumption of cold-pressed avocado oil is increasing worldwide (Costagli & Betti, 2015).

This study assesses the effect of time and temperature conditions during pulp malaxation on extraction yield, FA profile and specific extinctions at 232 and 270 nm (K_{232} and K_{270}) of virgin oil from avocados (*Persea americana* Var. Hass) cultivated in southern Jalisco, Mexico.

2 Materials and methods

2.1 Samples

Fully ripened *Persea Americana* Mill var. Hass fruits produced in southern Jalisco were acquired in local commercial establishments in Ciudad Guzmán, Jalisco, Mexico, from May to June 2014. All reagents used were analytical grade (Sigma®, Missouri, USA).

2.2 Oil extraction conditions

The fruits were washed, dried, peeled and de-stoned manually, and pulp weight was recorded. For each trial, the oil was obtained in the laboratory from the pulp of six ripe avocados following an adaptation of the cold extraction procedure described by Ariza et al. (2011) in which the pulp is homogenized with a domestic roller. The pulp was then malaxated manually at 90 rpm in covered glass recipients in a water bath. The recipients were maintained at fixed temperatures (40 and 50 °C) during six different mixing times (0, 20, 30. 40, 60, 120, and 180 min). Pulp aliquots (35 g) were centrifuged twice at 15,557 g for 10 min (Eppendorf, USA) at ambient temperature. Oil from both centrifugations was combined in dark brown glass bottles, weighed and stored at -18 °C until analysis.

2.3 Fat content

The fat content of the fresh pulp was determined using the AOAC method 930.09 (Association of Official Analytical Chemists, 2007) and expressed as percentage (%).

2.4 Oil yields

Oil yields obtained during the laboratory extraction treatments described above were expressed as percentage of total fat (% total fat).

2.5 Fatty acid profiles

The fatty acid (FA) normalized composition of crude oil was determined by gas chromatography (GC) after double methylation (Guardiola et al., 1994) of the sample with a 0.5 N sodium methoxide solution (Sigma, Missouri, USA) followed by 14% boron trifluoride in methanol (Sigma, Missouri, USA). FA methyl esters (FAME) were recovered in hexane (Sigma, Missouri, USA). FAME was analyzed in a 6820 gas chromatograph (Agilent Technologies, China) equipped with a 50 m × 0.25 mm id coating cp-sil 88 tailor made FAME capillary column (Varian, California, USA), and a flame ionization detector. The column temperature was programmed from 60 °C (3 min) at 5 °C·min^{-1} to 170 °C (9 min), at 10 °C·min^{-1} to 230 (15 min) (the injector and detector temperatures were 230 °C and 280 °C, respectively). Nitrogen was the carrier gas at a flow rate of 1 mL·min^{-1}; the split ratio was 1:5. Fatty acids in the oil samples were identified and qualitatively analyzed using the thirty-seven FAME external standard mixture solutions (Supelco FAME mix 37) (Sigma, Bellefonte, Pennsylvania, USA). Results were expressed as percent (%). Linoleic:linolenic (Massafera et al., 2010) and linoleic:palmitic ratios (Navas, 2007) were calculated from the FA profiles to evaluate, respectively, the lipid nutritional and oxidative quality.

2.6 Specific extinction

Avocado oil samples were diluted in cyclohexane UV-Vis (Sigma, Missouri, USA) using the method outlined in COI/T.20/Doc. No 19/Rev. 3 (International Olive Council, 2015). Samples were measured in the UV region at wavelengths of 232 and 270 nm on a Jenway UV-Vis spectrophotometer (Dunmow, UK) using 1 cm path length quartz cuvettes with solvent as a blank. Specific extinction (extinction coefficient) was calculated from absorbance, concentration and path length data.

2.7 Statistical analyses

All experiments and analyses of the samples were performed in triplicate. The results were expressed as mean ± standard deviation. The means obtained by the different treatments were compared using ANOVA and the multiple comparisons of means test. The statistical significance level of the study was 0.05. Data analysis was carried out using SPSS for Windows v. 19.

3 Results and discussion

3.1 Fat content

Initial content of fat in the fresh pulp used in the experiments had values ranging from 17.3 ± 2.4 to 19.6 ± 3.2% (Table 1). These percentages agree with those reported for Hass avocado pulp (16%) by Villa-Rodríguez et al. (2011) and those cited by Oliveira et al. (2013) for the same variety (21.1%), and they surpass those quantified by the latter author in other regional varieties such as Ouro Verde (8.5%), Margarida (8.8%), Quintal (10.9%) and Fortuna (6.4%). The content of oil in avocado pulp is affected by diverse factors, such as variety and climate; however, of these parameters, the degree of ripeness is that of greatest impact. Thus, oil yield improves as the fruit ripens; once the fruits are harvested, the amount of oil does not change (Meyer & Terry, 2008).

3.2 Oil yields

Extraction yield expressed as the proportion of total fat (Table 1) ranged from 0.1% (control with no malaxation) to 82.9% (40 °C, 120 min). These values coincide with those obtained by Schwartz et al. (2007), who reported cold press extraction yields from avocado pulp (Fuerte variety) of 66 to 70%. Our data reveal an increase in this variable in response to temperature

Table 1. Fat content and oil yield during cold extraction of avocado oil (*Persea americana* var. Hass) using different malaxation times and temperatures.

Malaxation conditions		Yield parameters[A]	
Temperature (°C)	Time (min)	Fat content (%)	Extraction yield (% total fat)
40	**0**	18.9 ± 0.1^{a}	0.10 ± 0.01^{a}
	20	19.2 ± 0.7^{a}	63.9 ± 1.6^{b}
	30	19.1 ± 0.9^{a}	69.5 ± 1.3^{c}
	40	19.2 ± 0.7^{a}	68.1 ± 0.5^{bc}
	60	19.1 ± 0.9^{a}	74.3 ± 0.4^{cde}
	120	17.3 ± 2.4^{a}	82.9 ± 0.3^{g}
	180	19.6 ± 3.2^{a}	74.4 ± 9.6^{de}
50	**0**	18.9 ± 0.1^{a}	0.10 ± 0.01^{a}
	20	19.2 ± 0.7^{a}	72.6 ± 1.7^{cde}
	30	19.2 ± 0.7^{a}	75.6 ± 0.3^{ef}
	40	19.2 ± 0.7^{a}	75.1 ± 0.1^{de}
	60	19.1 ± 0.9^{a}	77.4 ± 0.4^{efg}
	120	18.3 ± 2.4^{a}	80.2 ± 0.8^{fg}
	180	18.3 ± 2.4^{a}	70.7 ± 0.5^{cd}
Mean		19.4 ± 1.3	63.2 ± 27.2

f.w.: Fresh weight; [A] Values are the means ± SD (n = 3); [a-e] Means with different letters within the same column are significantly different ($p < 0.05$).

and mixing time. In addition, the analysis of variance found statistically significant differences ($p \leq 0.05$) among treatments. The results found for the control treatments separated them into an independent group with values of almost 0%; in control treatments, the small quantities obtained during centrifugation were distributed in thin layers over the pulp surfaces and of the walls of the tubes, making it difficult to recover the oil. For the rest of the tested conditions, intermediate yields were found. The highest values obtained with the procedures at 40 and 50 °C for 120 min were significantly superior to the other combinations of mixing times and temperatures. Equally long treatments have been recommended before, even with auxiliary technology; such is the case of Buelvas et al. (2012), who applied two hours of malaxation in the presence of enzymes and obtained oil extraction of 84.5% with "Lorena" variety avocados. Our results are similar and even superior. In our experiments, the extraction yield at 50 °C for 180 min malaxation decreased significantly compared with all the other treatments except the control. In contrast, at 40 °C for 180 min, it was below the treatment with 120 min. This reduction is due to a higher loss of moisture with 180 min of malaxation making extraction difficult. This was observed by Santana et al. (2011) during oil press extraction from avocado pulp subjected to malaxation and dehydration; they related a lower proportion of extracted oil to lower moisture contents and, thus, a reduction in porosity of the matrix and oil fluidization.

3.3 Fatty acid profiles

The normalized composition of fatty acids of virgin avocado oil obtained by applying diverse mixing times and temperatures is shown in Table 2. The most abundant FAs were oleic and palmitic, followed in descending order by linoleic, palmitoleic, linolenic and stearic acids. These data suggest that the broad variation in percentage in function of the treatment depended on the compound studied. Thus, the acids with variations above 10%, in increasing order were linolenic (12.8%), palmitoleic (13.1%) and stearic (15.2%), while the most stable were oleic, palmitic and linoleic (1.5, 5 and 8.9%, respectively). Relative to other edible oils, the experimental avocado oil included in this study is characterized by high percentages of monounsaturated fatty acids (oleic and palmitoleic), elevated of palmitic acid and a lower of stearic (saturated) acid (Table 3). According to our results, the normalized lipid profile described defined the obtained oils as high in oleic even though the percentages of palmitic acid are higher than in other oils, such as olive oil (Meyer & Terry, 2008). Because of its high levels of oleic acid, avocado oil is considered to be very similar to olive oil.

According to normalized composition, percentages of saturated FAs, such as stearic acid, remained without change ($p \geq 0.05$). But in the case of palmitic acid the differences relative to the control were statistically significant ($p \leq 0.05$) in the longest treatments (120 and 180 min), for which the highest percentages were quantified, regardless of the temperature used. The monounsaturated FAs studied had slight changes only in palmitoleic acid with significant, but unclear differences ($p \leq 0.05$) between the control, treatment at 40 °C for 40 min and the rest of treatments with percentages that oscillated between the treatments at 0 min and 40 °C during 60 min. Thus, oleic acid remained without significant differences ($p \geq 0.05$). In the polyunsaturated FA studied such as linoleic acid (Table 2), there was no significant effect of temperature on the normalized percentage ($p \geq 0.05$), but the oil normalized profiles obtained with the longest malaxation times (120 and 180 min) had lower ($p \leq 0.05$) percentages than the other mixing times tested. The normalized percentages of linolenic acid were higher in the oils extracted after malaxation at 40 °C for 60 min, while extraction after malaxation at 40 °C for 120 min yielded the lowest percentage of this fatty acid. However, only this pair of treatments showed clear significant differences ($p \leq 0.05$) in the normalized linolenic acid percentage of the oil. The effect of time and temperature was the same as in the case of linoleic acid ($p \leq 0.05$). Malaxation temperature and time have been reported as physical factors that influence oil oxidation. Temperature is the basic factor, while a longer malaxation time increases exposure to factors such as air and light (Kochhar & Henry, 2009). Prolonged exposure to air and light produces deteriorative changes in the oil since oxidation occurs even at temperatures very close to ambient temperature (30 °C). Light and heat trigger the autoxidation chain reaction of unsaturated FA by supplying the energy required to activate their reaction with O_2 and to generate the first radicals necessary for their propagation, which, without light and heat and despite the presence of air, is thermodynamically difficult (Naz et al., 2005).

Overall, the changes in the normalized percentage of FA of oils obtained under the different malaxation conditions studied, consisted of an increase in unsaturated fatty acids with mixing time and temperature ($p \leq 0.05$). The percentage of monounsaturated acids remained without alteration and there were slight increases ($p \geq 0.05$) in palmitoleic acid. The percentage of polyunsaturated fats decreased ($p \leq 0.05$) with mixing time. Linolenic acid was that which most decreased, followed by linoleic acid. The changes in the normalized fatty acid profile in our study coincide with

Table 2. Fatty acid normalized profile in cold extracted avocado oil (*Persea americana* var. Hass) using different malaxation times and temperatures.

Malaxation conditions		Fatty acid percentage[A] (%)					
Temperature (°C)	Time (min)	Palmitic (C16)	Stearic (C18)	Palmitoleic (C16:1)	Oleic (C18:1)	Linoleic (C18:2n-6)	Linolenic (C18:3n-6)
	0	18.5 ± 0.1^{a}	0.5 ± 0.1^{a}	8.10 ± 0.04^{a}	50.6 ± 0.1^{a}	20.9 ± 0.1^{e}	1.3 ± 0.1^{bcd}
	20	19 ± 1^{a}	0.6 ± 0.1^{a}	9 ± 1^{bc}	51.4 ± 0.7^{a}	18.8 ± 1.1^{cde}	1.3 ± 0.2^{bcd}
	30	18.8 ± 0.8^{ab}	0.4 ± 0.1^{a}	9.6 ± 0.8^{c}	51.4 ± 0.4^{a}	19 ± 1^{bcde}	1.2 ± 0.2^{abc}
40	**40**	18.4 ± 0.8^{a}	0.60 ± 0.04^{a}	8.5 ± 0.8^{ab}	51.6 ± 0.4^{a}	20 ± 1de	1.4 ± 0.1^{bcd}
	60	19.6 ± 1.6^{abc}	0.5 ± 0.2^{a}	9.2 ± 0.5^{bc}	53.1 ± 3.1^{a}	20.0 ± 0.4^{cde}	1.5 ± 0.2^{d}
	120	21.3 ± 1.3^{c}	0.5 ± 0.1^{a}	10 ± 1^{c}	52.6 ± 2.6^{a}	14.9 ± 1.4^{a}	0.9 ± 0.2^{a}
	180	19.5 ± 1.6^{abc}	0.6 ± 0.1^{a}	9.0 ± 0.2^{abc}	52.4 ± 1.7^{a}	17.5 ± 3.3^{abcd}	1.1 ± 0.2^{abcd}
	0	18.5 ± 0.1^{a}	0.5 ± 0.1^{a}	8.10 ± 0.04^{a}	50.6 ± 0.1^{a}	20.9 ± 0.1^{e}	1.3 ± 0.1^{bcd}
	20	19.5 ± 0.2^{abc}	0.6 ± 0.1^{a}	9.8 ± 0.1^{c}	50.7 ± 0.5^{a}	18.0 ± 0.1^{bcd}	1.3 ± 0.2^{bcd}
	30	19.4 ± 0.1^{a}	0.5 ± 0.1^{a}	9.7 ± 0.1^{c}	51.2 ± 0.5^{a}	18.1 ± 0.1^{bcde}	1.2 ± 0.1^{bcd}
50	**40**	19.8 ± 0.4^{ab}	0.6 ± 0.1^{a}	9.6 ± 0.3^{c}	51.3 ± 0.6^{a}	18.1 ± 0.2	1.10 ± 0.04^{abc}
	60	18.9 ± 1.1^{a}	0.5 ± 0.1^{a}	9.3 ± 1.1^{bc}	51.2 ± 1.1^{a}	19 ± 1^{bcde}	1.3 ± 0.1^{bcd}
	120	21.3 ± 2.3^{c}	0.50 ± 0.03^{a}	9 ± 1^{bc}	51 ± 3^{a}	17.1 ± 3.4^{abc}	1.1 ± 0.2^{ab}
	180	20.6 ± 1.1^{bc}	0.6 ± 0.1^{a}	8.9 ± 0.4^{bc}	52.2 ± 2.2^{a}	16.5 ± 2.4^{ab}	1.1 ± 0.2^{ab}
Mean		19.4 ± 1.3	0.5 ± 0.1	9 ± 1	51.6 ± 1.2	18 ± 2	1.2 ± 0.2

[A] Values are expressed as normalized percentages and are the means ± SD (n = 3); [a-c] Means with different letters within the same column are significantly different ($p < 0.05$).

Table 3. Normalized saturated and unsaturated fatty acids percentages in cold extracted avocado oil (*Persea americana* var. Hass) using different malaxation times and temperatures.

Malaxation conditions		Fatty acid percentage[A] (%)			
Temperature (°C)	Time (min)	Saturated	Monounsaturated	Polyunsaturated	Unsaturated
	0	19.1 ± 0.1^{a}	58.7 ± 0.1^{a}	22.2 ± 0.1^{a}	80.9 ± 0.1^{c}
	20	19.2 ± 1.1^{a}	60.7 ± 0.5^{a}	20.1 ± 1.5^{a}	80.8 ± 1.1^{c}
	30	19.2 ± 0.9^{a}	61 ± 1^{a}	19.7 ± 1.2^{a}	80.8 ± 0.9^{c}
40	**40**	18.9 ± 0.8^{a}	60.2 ± 0.7^{a}	20.9 ± 1.1^{a}	81.1 ± 0.8^{c}
	60	20.1 ± 1.3^{ab}	62.3 ± 1.8^{a}	21.5 ± 0.2^{a}	83.8 ± 1.6^{d}
	120	21.7 ± 1.3^{c}	62.4 ± 2.9^{a}	15.8 ± 1.5^{a}	78.2 ± 1.3^{a}
	180	20.1 ± 1.2^{ab}	61.3 ± 1.3^{a}	18.6 ± 2.5^{a}	80 ± 1^{bc}
	0	19.1 ± 0.1^{a}	58.7 ± 0.1^{a}	22.2 ± 0.1^{a}	80.9 ± 0.1^{c}
	20	20.1 ± 0.1^{ab}	60.5 ± 0.4^{a}	19.3 ± 0.1^{a}	79.7 ± 0.3^{abc}
	30	19.9 ± 0.1^{a}	60.9 ± 0.2^{a}	19.3 ± 0.1^{a}	80.1 ± 0.1^{bc}
50	**40**	20.3 ± 0.1^{abc}	60.9 ± 0.3^{a}	19.2 ± 0.2^{a}	80.1 ± 0.4^{bc}
	60	19.4 ± 1.2^{a}	60.4 ± 0.2^{a}	20.2 ± 1.1^{a}	80.6 ± 1.2^{c}
	120	21.8 ± 2.3^{c}	60.1 ± 2.9^{a}	18.2 ± 3.6^{a}	78.2 ± 2.3^{a}
	180	21.2 ± 1.1^{bc}	61.2 ± 2.3^{a}	17.6 ± 2.6^{a}	78.8 ± 1.1^{ab}
Mean		20 ± 1	60.7 ± 1.1	19.6 ± 1.8	80.3 ± 1.4

[A] Values are expressed as normalized percentages and are the means ± SD (n = 3); [a-c] Means with different letters within the same column are significantly different ($p < 0.05$).

those reported by Bešter et al. (2008), who used more severe treatment conditions (frying temperatures) in their study on stability of extra virgin olive oils. Although the normalized analytical data is a way of expression frequently used in a large number of papers, the disadvantage is that in oils and fats heated at frying temperatures the relative fatty acid methyl ester (FAME) composition given is that of the non-altered FAME fraction, which is the only one eluted in the GC analysis, and oxidized FAME are not quantified because they are adsorbed in the column because of their high polarity (Dobarganes & Márquez-Ruiz, 2007). Table 4 shows the adjusted percentages on the eluted fractions of non-oxidized FAME in experimental avocado virgin oils. These percentages were calculated using the procedure proposed by Berdeaux et al. (2012), which considers the major saturated fatty acid (palmitic) unchanged in order to measure indirectly the thermoxidative FAME alteration.

There were variations of more than 10% in the adjusted data for linoleic (16.1%) and linolenic (13.4%) acids. However, oleic and palmitoleic acids were more stable (2 and 6.3%, respectively). The variations in percentages of stearic acid on the eluted fractions of non-oxidized FAME in the different combinations of malaxation time and temperature studied were not significant ($p \geq 0.05$). Regarding the monounsaturated fatty acids, we confirmed a reduction in the percentage of oleic acid in the eluted fraction of the oil from longer treatments (120 and

180 min); the highest percentages were found in the time treatments of 20, 30, and 40 min at 40 °C ($p \leq 0.05$). The same trend was observed with palmitoleic acid, although in this case the temperature increase did not cause significant effects ($p \geq 0.05$). The polyunsaturated fatty acids followed the same trend; that is, significantly lower concentrations resulted at longer times (120 and 180 min), but with higher losses of linoleic acid. However, these fatty acids were not significantly affected by temperature ($p \geq 0.05$). Most of the changes observed in the adjusted data of virgin avocado oil (Table 4) are similar to those described for sunflower seed oil and olive oil at frying conditions (temperatures of 180 °C during 0, 5, 10 and 15 h) (Berdeaux et al., 2012). Is very well known that polyunsaturated fatty acids oxidize earlier than monounsaturated, and saturated fatty acids do not oxidize. Frankel (1985) showed rates of relative oxidation of stearic, oleic, linoleic and linolenic acids of 1, 100, 1200 and 2500, respectively, and the rate of oxidation increased with increases in the number of double bonds.

3.4 ω-6:ω-3 ratio (nutritional assessment)

In our study, we calculated the ratio of ω-6:ω-3 from the normalized FA percentages of the linoleic (ω-6) and linolenic (ω-3). The values for the experimental oils were found within a very narrow range that oscillated between 13.5:1 ± 1.3 and 17:1 ± 3.3 (Table 5), with no significant differences among the treatments, mixing times or temperatures studied ($p \geq 0.05$). Massafera et al. (2010), who studied the FA composition in oils from pulp of different avocado cultivars in a region of Brazil, reported similar low ratios of 7.7:1, 7.1:1 and 12:1 in oil obtained from the cultivars Fortuna, Oro Verde and Princesa, respectively. From a nutritional perspective, the balance between daily input of food sources of ω-6 and ω-3 fatty acids is important since a lower ratio of ω-6 to ω-3 is desirable to reduce the risk of diverse chronic diseases that are highly prevalent in western society and are becoming increasingly present worldwide. The ratios we found are above the near 1 values in the ratios of the diet with which man evolved (Simopoulos, 2004). They are also higher than those recommended for optimum transformation of linoleic acid into very long-chained polyunsaturated fatty acids (4:1 to 5:1) and above that which favors greater conversion of alpha-linolenic acid into ω-3 acids (2:1 to 3:1) (Martin et al., 2006). However, they are within the ratios reported for different countries, which recently have oscillated between 10:1 to 20:1, but with records of up to 50:1 (Simopoulos, 2004).

3.5 Linoleic:palmitic ratio (C18:2 n-6/C16:0)

The linoleic:palmitic ratios obtained in our study were between 0.70 ± 0.02 (40 °C for 120 min) and 1.10 ± 0.01 (control). The highest value (1.1) was calculated in the oil from pulp that was not exposed to any of the treatment conditions, while the lowest ratios were obtained in oils extracted with the longest malaxation times, 120 and 180 min ($p \leq 0.05$) (Table 5). We also observed that the treatments conducted at 50 °C tended to have ratios lower than those conducted at 40 °C, but variations were not statistically significant in any case ($p \geq 0.05$). When a fat or oil is exposed to heat, the linoleic acid/palmitic acid ratio decreases because oxidation decomposes linoleic acid. This ratio is considered an indicator of fat or oil deterioration. The lack of significant changes may be attributed to the lower temperatures assayed despite the long malaxation times. Other studies in edible oil conducted at higher temperatures have reported similar but significant changes associated to intense thermo-oxidative transformations produced in heated oil-containing food (Alireza et al., 2010), and in olive oil subjected to temperature increases in the frying range (Navas, 2007).

Table 4. Adjusted fatty acid methyl ester profile of the eluted fraction in cold extracted avocado oil (*Persea americana* var. Hass) using different malaxation times and temperatures.

Malaxation conditions		Fatty acid percentage on the eluted fraction[A] (%)					
Temperature (°C)	Time (min)	Palmitic (C16)	Stearic (C18)	Palmitoleic (C16:1)	Oleic (C18:1)	Linoleic (C18:2n-6)	Linolenic (C18:3n-6)
40	**0**	18.5 ± 0.1^{a}	0.5 ± 0.1^{a}	8.10 ± 0.04^{a}	50.6 ± 0.1^{c}	20.9 ± 0.1^{d}	1.3 ± 0.1^{def}
	20	18.5 ± 0.1^{a}	0.6 ± 0.1^{a}	9.3 ± 0.7^{bc}	51.2 ± 3.5^{c}	18.8 ± 2.3^{cd}	1.3 ± 0.3^{cdef}
	30	18.5 ± 0.1^{a}	0.4 ± 0.1^{a}	9.5 ± 0.9^{c}	50.7 ± 2.5^{c}	18.3 ± 1.6^{c}	1.2 ± 0.3^{bcdef}
	40	18.5 ± 0.1^{a}	0.6 ± 0.1^{a}	8.6 ± 0.7^{ab}	52.1 ± 2.7^{c}	19.7 ± 1.7^{cd}	1.4 ± 0.1^{ef}
	60	18.5 ± 0.1^{a}	0.5 ± 0.1^{a}	8.7 ± 0.9^{abc}	50.2 ± 0.8^{bc}	18.9 ± 1.4^{cd}	1.40 ± 0.03^{f}
	120	18.5 ± 0.1^{a}	0.4 ± 0.1^{a}	8.6 ± 1.2^{ab}	46 ± 5^{ab}	13.0 ± 0.4^{a}	0.8 ± 0.2^{a}
	180	18.5 ± 0.1^{a}	0.5 ± 0.1^{a}	8.5 ± 0.3^{ab}	49.8 ± 1.8^{bc}	16.8 ± 3.2^{bc}	1.1 ± 0.2^{abcd}
50	**0**	18.5 ± 0.1^{a}	0.5 ± 0.1^{a}	8.10 ± 0.04^{a}	50.6 ± 0.1^{c}	20.9 ± 0.1^{d}	1.3 ± 0.1^{def}
	20	18.5 ± 0.1^{a}	0.5 ± 0.1^{a}	9.30 ± 0.03^{bc}	48.1 ± 0.8^{abc}	17.1 ± 0.1^{bc}	1.2 ± 0.2^{bcdef}
	30	18.5 ± 0.1^{a}	0.5 ± 0.1^{a}	9.3 ± 0.1^{bc}	48.9 ± 0.5^{abc}	17.3 ± 0.1^{bc}	1.1 ± 0.1^{bcde}
	40	18.5 ± 0.1^{a}	0.5 ± 0.1^{a}	9.0 ± 0.3^{bc}	48.1 ± 0.8^{abc}	17.0 ± 0.1^{bc}	1.10 ± 0.04^{abc}
	60	18.5 ± 0.1^{a}	0.5 ± 0.1^{a}	9.1 ± 0.6^{bc}	50.2 ± 4.1^{bc}	18.6 ± 2.2^{cd}	1.3 ± 0.2^{cdef}
	120	18.5 ± 0.1^{a}	0.5 ± 0.1^{a}	7.9 ± 0.1^{a}	44.9 ± 6.3^{a}	15.2 ± 4.5^{ab}	0.9 ± 0.2^{ab}
	180	18.5 ± 0.1^{a}	0.6 ± 0.1^{a}	8.0 ± 0.1^{a}	47 ± 3^{ab}	14.9 ± 2.8^{ab}	1.0 ± 0.3^{ab}
Mean		18.5 ± 0.1	0.5 ± 0.1	8.7 ± 0.7	49.2 ± 3.2	17.6 ± 2.7	1.2 ± 0.2

[A] Values are expressed as adjusted percentages on non-oxidized FAME and are the means ± SD (n = 3); [a-f] Means with different letters within the same column are significantly different ($p < 0.05$).

Table 5. Nutritional and oxidative parameters in cold extracted avocado oil (*Persea americana* var. Hass) using different malaxation times and temperatures.

Malaxation conditions		Nutritional and oxidative parameters			
Temperature (°C)	Time (min)	ω-6:ω-3 ratio	Linoleic/ palmitic acid ratio	Specific extintion (K_{232})	Specific extintion (K_{270})
	0	15.7 ± 1.2^{a}	1.10 ± 0.01^{a}	2.80 ± 0.01^{c}	0.8 ± 0.1^{c}
	20	15.7 ± 1.6^{a}	1.0 ± 0.1^{a}	1.9 ± 0.1^{bcd}	0.10 ± 0.01^{a}
	30	15.8 ± 1.8^{a}	1.0 ± 0.1^{a}	1.8 ± 0.4^{b}	0.10 ± 0.01^{a}
40	**40**	13.9 ± 0.5^{a}	1.1 ± 0.1^{a}	2.40 ± 0.02^{de}	0.10 ± 0.01^{ab}
	60	13.5 ± 1.3^{a}	1.0 ± 0.1^{a}	2.10 ± 0.01^{bcde}	0.100 ± 0.003^{ab}
	120	17 ± 3^{a}	0.70 ± 0.02^{a}	2.3 ± 0.5^{bcde}	0.20 ± 0.02^{ab}
	180	15.4 ± 0.1^{a}	0.9 ± 0.2^{a}	2.3 ± 0.2^{bcd}	0.20 ± 0.02^{a}
	0	15.7 ± 1.2^{a}	1.10 ± 0.01^{a}	2.80 ± 0.01^{c}	0.8 ± 0.1^{c}
	20	14.1 ± 1.8^{a}	0.90 ± 0.01 ^{a}	2.2 ± 0.1^{bcde}	0.10 ± 0.04^{ab}
	30	15.2 ± 0.7^{a}	0.90 ± 0.01^{a}	2.2 ± 0.1^{bcde}	0.20 ± 0.04^{ab}
50	**40**	16 ± 1^{a}	0.90 ± 0.01^{a}	2.2 ± 0.1^{bcde}	0.10 ± 0.20^{ab}
	60	14.9 ± 0.5^{a}	1.0 ± 0.1^{a}	1.9 ± 0.3^{bc}	0.100 ± 0.004^{ab}
	120	16.4 ± 1.2^{a}	0.8 ± 0.2^{a}	2.4 ± 0.2^{cde}	0.20 ± 0.02^{ab}
	180	15.8 ± 1.6^{a}	0.8 ± 0.2^{a}	2.20 ± 0.03^{bcde}	0.20 ± 0.03^{b}
Mean		15 ± 1	1.0 ± 0.1	2.2 ± 0.3	0.20 ± 0.03

Values are the means ± SD (n = 3); $^{a-e}$ Means with different letters within the same column are significantly different ($p < 0.05$).

3.6 Specific extinction (K_{270} Y K_{232})

The analysis of variance of specific extinction values at 232 nm found statistically significant differences ($p \geq 0.05$) among the tested treatments (Table 5). The lowest, quantified in oils from the treatments at 40 °C for 30 min and 50 °C for 60 min, were clearly differentiated from the highest, which corresponded to the treatments with no malaxation, while the rest of the oils were grouped in a single set. Analysis of the specific extinctions at 270 nm showed lower estimations for the treatments at 40 °C for 20 and 30 min, which separated from those quantified in the treatments without malaxation. Pastore et al. (2014) conducted a study on extra virgin olive oil obtained by centrifugation applying different levels of environmental oxygen during malaxation and reported low specific extinctions at 232 nm and 270 nm (1.7 and 1.9, respectively). In our experiments, the index at 232 nm of all the treatments surpassed those obtained by the above mentioned study, except in the oils obtained at 40 °C with 20, 30, 40 and 60 min mixing times and at 50 °C with 20 and 40 min mixing times. In the case of the treatments with no malaxation (0 min), it was possible to obtain oil only from fruits over-ripened in postharvest. This condition of the raw material originated the elevated K_{232} and K_{270} calculated in these oils. The same results were observed in the case of oil extracted from olives in this state of maturation (Fuentes de Mendoza et al., 2013). Our experimental avocado oils showed a low index of conjugated hidroperoxides (K232) according to the European Union standards for extra virgin olive oil quality (Unión Europea, 2011). The exception was the oil from the control treatments (Table 5). According to the estimates of specific extinction at 270 nm, all the coefficients calculated were low. The K_{232} and K_{270} estimates (Table 5) showed no differences among malaxation temperatures, but there were differences among malaxation times ($p \geq 0.05$). These results indicate that in the experimental oils the oxidation levels remained low and that the shortest malaxation times resulted in lower estimations ($p \leq 0.05$). Again, the exceptions were the control treatments.

3.7 Oil yield and quality analysis

From the results discussed above, we can confirm that the highest proportion of oil was extracted with the treatments that included malaxation at 50 °C for 120 min. However, the treatments whose malaxation conditions were 50 °C for 30 and 60 min had equivalent yields, lower but not statistically different ($p \geq 0.05$). The use of shorter malaxation times would be an improvement for the process since it would speed up extraction. However, the treatments with 30 and 60 min malaxation were also equivalent to those treatments in which the lowest proportion of oil was recovered ($p \geq 0.05$). Moreover, the treatments with higher extraction percentages preserved high proportions of monounsaturated FA (oleic and palmitoleic) ($p \leq 0.05$), the smallest proportions of linoleic acid and, in the case of the treatment at 40 °C for 120 min, of linolenic acid ($p \leq 0.05$). Although there were small proportions of ω-6:ω-3 FA, there were no statistical differences in the nutritional quality index of the oils studied ($p \geq 0.05$), which were within a narrow range of values and, overall, were low and equivalent to those reported in other studies (Massafera et al., 2010). For this reason, we consider the proportion of linoleic acid, relative to that of linolenic acid, is favorable for health in all the cases analyzed regardless of the malaxation temperature used. The oxidative effect on the normalized FA profile, measured as the proportion of linoleic acid to palmitic acid, was a more marked reduction with increased malaxation times ($p \geq 0.05$), but not with the tested temperatures. However, the coefficients of absorption in the ultraviolet region (K_{232} and K_{270}) indicated that the oxidation level of avocado virgin oil remained low. Thus, the 120 min malaxation time improved oil extraction yields from Mexican Hass avocados and using temperatures of 40 or 50 °C equally contribute to an adequate nutritional profile.

4 Conclusions

Malaxation under the conditions studied induced changes in yield of virgin avocado (*Persea americana* Mill) oil extracted by centrifugation from Mexican Hass avocados, in its normalized fatty acid profile, nutritional quality and oxidation state. Despite the variations, all the oils obtained conserved the fatty acid profile characteristic of high oleic acid oils. The ω-6:ω-3 ratio was low, and corresponded to the values of mean intake worldwide. The specific extinctions indicated that the oxidation level of avocado virgin oil remained low. However, the malaxation treatments at 40 and 50 °C for 120 min were those that obtained the highest extraction yield as well as adequate nutritional quality associated to its lipid profile.

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