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Quality Assessment of Avocado Pulp Oils during Storage †

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Abstract: Recently, consumers' awareness is becoming a crucial aspect driving the food industry to develop new products with high nutritional value. Oil industry explores the use of less known plant materials such as avocado fruit which is a rich source of bioactive compounds. The objective of this study was to assess quality and oxidative stability of avocado pulp oils during a 2-month storage period. Two avocado varieties, Hass and Reed were selected and oil extraction was performed with the use of hexane. The extent of oxidative deterioration and oils stability were tested by measuring the acid value and peroxide value. The PDSC method was applied to evaluate the oxidative induction time. The composition of fatty acids and their distribution in internal (sn-2) and external (sn-1 and sn-3) positions in triacylglycerols were also analysed. The acid value and the peroxide value of fresh extracted avocado oils reached approximately 0,6 mg KOH g^{-1} fat and 5 meq O2 kg^{-1} fat, respectively. Generally, during avocado oils storage both the acid values and the peroxide values were in accordance with Codex Alimentarius requirements (limit for acid value is 4 mg KOH g^{-1}, while for peroxide value is 15 meq O2 kg^{-1}). The Hass avocado pulp oil was characterized by higher value of the oxidative induction time, about 111 min, compared to the oil extracted from Reed avocado pulp (61 min). The GC analysis revealed that avocado pulp oil could be considered as a source of monounsaturated fatty acids. The dominant fatty acid found in this group was oleic acid with percentage share of above 60%. In accordance with the results of fatty acids distribution in triacylglycerols molecules, the main fatty acids in the sn-2 position were linoleic acid and oleic acid and their percentage share in this item was up to 59% and 34%, respectively. It was also noticed that after a 2-month storage period, the acid value and the peroxide value increased about 7-fold and 2-fold, the oxidative induction time decreased about 2-fold and the percentage shares of fatty acids groups changed. In conclusion, the results obtained in this research indicate that storage period has significant impact on avocado pulp oils quality.

Keywords: avocado oil, acid value, peroxide value, fatty acids composition, oxidative stability

1. Introduction

In recent years the demand for unprocessed, "healthful" foods and beverages has increased rapidly [1]. Functional foods are edible products with additional health benefits beyond the essential nutrients, which are enhanced by the addition of bioactive compounds, or by the changes in production processes. A plethora of functional foods, throughout the market, diminish the disparity between pharmaceuticals and nutrition [2].

Vegetable oils are an original source of fat-soluble compounds such as free fatty acids, vitamins or phenolics. Due to the abundance of bioactive compounds, vegetable oils have numerous functional

properties mainly antioxidant, antibacterial, anti-inflammatory and anticancer capacities [3]. Nowadays manufacturers seek out new sources of vegetable oils.

Avocado oil is a rich source of unsaturated fatty acids which are invaluable in cancer prevention and in cardiovascular diseases prophylaxis. Also the unsaponifiable fraction has high concentration of compounds such as phytosterols and policosanols which can decrease the low-density lipoprotein cholesterol in blood. Oils containing high monounsaturated fatty acids are less susceptible to oxidation, which is a main cause of deteriorative changes in sensory, chemical and nutritional properties during storage [4].

Taking above into the account, the aim of this research was to monitor the quality of avocado pulp oils during a two-month storage period.

2. Materials and methods

2.1. Preparation of avocado oil samples

To obtain avocado oil samples, Hass and Red cultivars pulps were selected. The oil samples were extracted with hexane in 6:10 ratio (*w*/*v*; homogenized pulp sample:hexane). Subsequently mixtures were put in laboratory shaker Elpin Plus type 357 (Lubawa, Poland) for one hour. After shaking process the samples were filtrated, dried with anhydrous magnesium sulfate, evaporated under vacuum in BÜCHI laboratory evaporator and finally dried under nitrogen atmosphere.

2.2. Analytical methods

2.2.1. Determination of acid value

Acid value was determined with regard to the procedure described in PN-EN ISO 660:2010 [5]. It was calculated as the mg of potassium hydroxide needed to neutralize the organic acids present in 1 g of oil samples, with the use of phenolophthalein as an indicator.

2.2.2. Determination of peroxide value

Peroxide value was computed as meq O_2/kg (miliequivalents of oxygen per kg of oil samples). It was determined in pursuance of PN-EN ISO 3960:2012 [6], after dissolving the oil samples in acetic acid-chloroform mixture (3:2; *v*/*v*) in the presence of a potassium iodide solution. The mixture was then titrated with sodium thiosulfate using starch as an indicator.

2.2.3. Determination of oxidative stability by means of PDSC method

A differential scanning calorimeter DSC Q20, TA Instruments coupled with a high-pressure cell was used. Oil samples of 3–4 mg were put into the aluminum pan and placed in the sample chamber. The analyses were performed with an initial pressure of 1400 kPa, under oxygen atmosphere, with the temperature set to 120 °C (isothermal process). Oxidative induction time was determined based on the PDSC diagrams.

2.2.4. Determination of fatty acid profiles

The fatty acids composition was determined in accordance to the method described in PN-EN ISO 5508:1996 [7]. To prepare the samples for GC analysis, esterification with potassium hydroxide in methanol (1 M) according to PN-EN ISO 5509:2001 [8] was performed. After esterification process samples were analyzed in YL6100 GC chromatograph coupled with flame ionization detector. A BPX-70 capillary column (0.2 mm i.d × 60 m length × 0.25 μm film thickness) was used. Samples were injected into the GC with and inlet temperature of 225 °C, and with a split ratio of 1:100. Initial oven temperature of 60 °C was maintained for 5 min, rising to 180 °C with an increment of 10 °C min^{-1}, from 180 °C to 230 °C at a rate of 3 °C min^{-1}, finally kept at 230 °C for 15 min. Temperature of the detector was set to 250 °C. Nitrogen was used as a carrier gas at a flow rate of 1 mL min^{-1}. The area under the curve for each peak was manually integrated and the results were expressed as a relative

percentage of each fatty acid. Identification of fatty acids was done by comparing the relative retention times of peaks within the sample with the chemical standard.

2.2.5. Distribution of fatty acids in the sn-2 and sn-1,3 positions of triacylglycerols

TAGs positions of oil samples were analyzed by the use of Brockerhoff method which involves partial hydrolysis of triacylglycerol carried out in the presence of the pancreatic lipase enzyme [9, 10]. The content of sn-1,3 fatty acids was calculated based on the information on the initial composition of TAGs and the composition of the sn-2 MAG:

$$\text{sn-1,3} = [3 \times (\text{FA in TAGs}) - (\text{FA in sn-2 MAG})] \times 2^{-1}, \quad (1)$$

where:
sn-1,3 – content of a given FA in the sn-1 and sn-3 positions [%],
FA in TAGs – content of a given FA in starting TAGs [%],
FA in sn-2 MAG – content of a given FA in sn-2 MAGs [%].
The percentage of selected fatty acids in sn-2 positions in relation to the total content of given fatty acid was calculated based on the formula:

$$\text{sn-2} = (\text{FA in sn-2 MAG}) \times [3 \times (\text{FA in TAGs})]^{-1} \quad (2)$$

2.3. Statistical Analysis

The results were analyzed using STATISTICA 13 software. Multivariate ANOVAs with Tukey's post hoc tests were conducted for significant differences between samples at $p<0.05$.

3. Results and Discussion

The acid values (AV) and peroxide values (PV) are important indicators to assess edible oils quality. In Table 1, the changes of AV and PV of avocado oil during storage are shown. The AV and PV of freshly extracted avocado oils did not exceed 0,6 mg KOH g^{-1} oil and 5 meq O_2 kg^{-1} oil, respectively. The examined oils were of good quality being in agreement with Codex Alimentarius [11] recommendations which states that AV and PV for cold pressed oils should not exceed 4 mg KOH g^{-1} oil and 15 meq O_2 kg^{-1} oil.

Table 1. Changes of acid value (AV), peroxide value (PV) and oxidation induction time (OIT) of oil samples during storage**.** Different letters indicate that the samples are significantly different during storage at p < 0,05.

	Oil Sample	**Storage Time**		
		0 month	1 month	2 months
AV	Hass	0,62±0,08^{A}	0,82±0,04^{A}	3,91±0,05^{C}
[mg KOH*g oil^{-1}]	Red	0,57±0,01^{A}	1,31±0,16^{B}	4,66±0,13^{D}
PV	Hass	3,99±0,08^{A}	7,01±0,06^{C}	9,13±0,33^{E}
[meq O_2*kg oil^{-1}]	Red	4,90±0,08^{B}	7,48±0,08^{C}	8,29±0,05^{D}
OIT	Hass	111,42±2,57^{B}	59,55±1,08^{A}	49,44±4,00^{A}
[min]	Red	60,63±8,24^{A}	50,62±1,94^{A}	48,06±0,21^{A}

After 2 months of storage time, AV and PV increased significantly - over 6 times for Hass oil and over 8 times for Red oil. After that time only oil extracted from Red cultivar exceeded the recommended limit for AV. This substantial increase of AV and PV was probably induced by the ambient temperature of the oils storage [12, 13].

According to the literature PV values of avocado oils might differ greatly, affected mostly by extraction process and source material used. Bora et al. [14] reported a PV of 1,4 meq O_2 kg^{-1} oil for Fuerte cultivar, while Salgado et al. [15] found PV at a level of 20,58 meq O_2 kg^{-1} oil for Margarida variety. Our results are in accordance with Ortega et al. [16] who reported a PV of 3,79 meq O_2 kg^{-1} oil for the oil extracted from Hass cultivars.

Freshly extracted avocado oils were characterized by relatively long OIT (111 min for Hass cultivar and 60 min for Red cultivar). This disparity might be the result of source fruits being in different ripening stages or higher SFA content in Hass oil. After 2 month period of storage OIT of Hass oil decreased significantly by about 56%.

The beneficial influence on human health of avocado oil could be attributed to its fatty acids profiles. The fatty acids composition of Hass and Red avocado pulp oil were shown in Table 2. It can be seen that tested avocado pulp oli were characterized by a high content of monounsaturated fatty acids (63-70%). Among MUFA, the oleic acid is dominant fatty acid and its percentage share accounted for approximately 60%. The results agree with the findings of Krumreich et al. [17].

Table 2. Changes of fatty acids profiles of tested avocado oils during storage. Different letters indicate that the samples are significantly different during storage at $p < 0,05$.

		Hass			**Red**		
		0 month	1 month	2 months	0 month	1 month	2 months
SFA	C 16:0	$17,12\pm0,01^{B}$	$18,01\pm0,01^{C}$	$17,86\pm0,46^{BC}$	$14,39\pm0,04^{A}$	$17,25\pm0,03^{BC}$	$17,29\pm0,11^{BC}$
	C 18:0	$0,63\pm0,01^{A}$	$0,65\pm0,01^{A}$	$0,64\pm0,06^{A}$	$0,92\pm0,03^{B}$	$0,93\pm0,08^{B}$	$0,91\pm0,03^{B}$
	C 20:0	$0,13\pm0,02^{A}$	$0,12\pm0,02^{A}$	$0,12\pm0,04^{A}$	$0,15\pm0,08^{B}$	$0,18\pm0,01^{D}$	$0,17\pm0,01^{C}$
ΣSFA		17,88	18,78	18,62	15,46	18,36	18,37
MUFA	C 16:1	$7,34\pm0,05^{C}$	$7,40\pm0,01^{C}$	$7,41\pm0,22^{C}$	$3,88\pm0,05^{A}$	$5,48\pm0,02^{B}$	$5,58\pm0,01^{B}$
	C 17:1	$0,14\pm0,01^{A}$	$0,13\pm0,02^{A}$	$0,12\pm0,01^{A}$	$0,14\pm0,01^{A}$	$0,14\pm0,01^{A}$	$0,11\pm0,01^{A}$
	C 18:1	$62,14\pm0,02^{B}$	$61,88\pm0,01^{B}$	$61,92\pm0,66^{B}$	$64,39\pm0,11^{C}$	$56,97\pm0,15^{A}$	$56,81\pm0,20^{A}$
	C 20:1	$0,26\pm0,02^{A}$	$0,25\pm0,01^{A}$	$0,26\pm0,02^{A}$	$0,28\pm0,01^{A}$	$0,28\pm0,01^{A}$	$0,29\pm0,05^{A}$
ΣMUFA		69,87	69,66	69,70	68,69	62,87	62,78
PUFA	C 18:2	$11,11\pm0,01^{C}$	$10,59\pm0,02^{A}$	$10,73\pm0,01^{B}$	$14,82\pm0,05^{D}$	$17,30\pm0,02^{E}$	$17,40\pm0,03^{F}$
	C 18:3	$0,84\pm0,02^{B}$	$0,72\pm0,02^{A}$	$0,73\pm0,07^{A}$	$0,77\pm0,01^{A}$	$1,17\pm0,03^{C}$	$1,14\pm0,03^{C}$
	C 20:5	$0,12\pm0,05^{A}$	$0,06\pm0,03^{A}$	$0,06\pm0,01^{A}$	$0,08\pm0,01^{A}$	$0,12\pm0,01^{A}$	$0,12\pm0,02^{A}$
ΣPUFA		12,06	11,37	11,51	15,67	18,58	18,66
ΣUFA		81,93	81,03	81,21	84,36	81,45	81,43
Unidentified		0,19	0,19	0,18	0,18	0,20	0,21

The quality of oils depends on the distribution of fatty acids in the triacylglycerols. Saturated fatty acids occupying the sn-2 position positively affect the absorption of fats [18]. According to the results presented in Table 3, it can be seen that sn-2 position is occupied mainly by oleic acid. The content of oleic acid in the sn-2 position was about 63% for Hass and 55,3% for Red cultivar and its share in this position reached 34% and 29% for Hass and Red respectively.

Table 3. Positional distribution of selected fatty acids in avocado oil samples.

Fatty Acids	**Oil Sample**	**Fatty Acid Content in TAG [%]**	**Fatty Acid Content in Position [%]**		**Percentage Distribution of Fatty Acid in sn-2 [%]**
			sn-2	sn-1,3	
C 16:0	Hass	17,12	7,77	21,8	15%
	Red	14,39	11,8	15,69	27%
C 16:1	Hass	7,34	5,95	8,03	27%
	Red	3,89	3,85	3,91	33%
C 18:1	Hass	62,14	62,95	61,73	34%
	Red	64,39	55,29	68,94	29%
C 18:2	Hass	11,11	19,49	6,91	59%
	Red	14,82	24,64	9,91	55%

4. Conclusions

Our results indicate that avocado oil could be considered as a rich source of unsaturated fatty acids, mainly monounsaturated fatty acids. Two month period of storage at ambient temperature had significant effect on the quality and composition of researched samples. Acid and peroxide values increased after storage but these parameters in most cases did not exceed the limits recommended by Codex Alimentarius. The oxidative induction time had decreased significantly in case of Hass avocado oil which suggests oil oxidation processes.

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