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RESEARCH ARTICLE

EXTRACTION, OPTIMIZATION AND QUALITY CHARACTERIZATION OF OIL FROM HASS AVOCADO (PERSEA AMERICANA) VARIETY GROWN IN ETHIOPIA (WONDOGENET)

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Abstract

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The purpose of this research was to extract, optimize and characterizethe qualityof oil from Hass avocado variety which is grown in Wondogenet, Ethiopia. Oil was extracted from Hass avocado pulp using soxhlet and ultrasonic extraction and some process parameter for optimization were carried out. Hass avocado is rich in fat (22.80±0.00%). The effects of three factors (particle size, solvent to sample ratio and extraction time) on the oil yield were considered. The optimum extraction conditions for soxhlet extraction were found to be at 8 h reaction time, particle size of 1.4mm and solvent to solid ratio of 20:1 with a maximum yield of 69.7%, while the optimum extraction conditions for ultrasonic extraction were found at 1.5 h reaction time, particle size of 2mm and solvent to solid ratio of 15:1 with a maximum percentage yield of 67.2%. The analyses of the oil characteristics demonstrated that the oil extracted by soxhlet extraction shows lower PV, FFA, MVM and Alkalinity than oil extracted using Ultrasonic extraction. The results of fatty acid profile displayed that the oleic and linoleic acid contents of the soxhlet extracted oil were higher than that of the Ultrasonic extracted oil while palmitic and butanoic acid were higher in ultrasound extracted oil. Generally, ultrasound assisted extraction showed a promising oil yield regarding short extraction time and low solvent consumption; but its oil quality is lower than that of soxhlet extracted oil.

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Introduction:-

Avocado is one of the climacteric fruits with high nutritious quality and low sugar content, making it a highly recommended source of high energy food for diabetics. It is widely utilized around the world because of the presence of unsaturated lipids and its importance in maintaining and improving circulatory and heart system health (Di Stefano et al., 2017) and. Furthermore, lipids contain linoleic acid, a polyunsaturated fatty acid that, along with alpha linoleic acid (Omega-3 fatty acid), forms important components of body systems, plays important functions in the immune system and vision, produces hormone like substances known as eicocasnoids and aids in the formation of cell membranes (Wong et al., 2010)and (Braide et al., 2010). The oil content of the fruit depends upon its

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ecological origin and on the cultivar, as for example, in Guatemalan and Mexican cultivars, the oil content varies from 10 to 13% and from 15 to 25%, respectively (Bora et al., 2001)

Avocados are classified into three horticultural species or races based on their origin and distinguishing characteristics: Guatemalan (Persea nubigena var. guatamalensis L. Wms.), Mexican (P. americana var. drymifolia Blake-), West Indian (P. americana Mill. var. americana). There are hybrid forms of all three types that are now more widely cultivated (Requejo-tapia & Bailey, 1999). About six cultivars of avocado, which are crossbreeds of the original races, are registered for production in Ethiopia. Fuerte, Hass, Nabal, Pinkerton, Ettinger and Bacon, are among them(FAO, 2010).

Avocado pulp is enriched with oil that can be extracted using a variety of techniques. In recent years, several novel techniques for oil extraction have been developed including microwave extraction (Kumaran & Joel Karunakaran, 2007), mechanical compression (Karaj & Müller, 2011), ultrasonic extraction (Özkan & Kuyumcu, 2007), and supercritical fluid extraction (Louli et al., 2004).In comparison to conventional soxhlet extraction, ultrasonic extraction offers greater selectivity, uses less energy, requires less time, and produces fewer emissions. The equipment is also affordable, the majority of the extraction solvent can be reused, and it is also environmentally safe (Li et al., 2016). So, application of ultrasonic extraction for extraction of oil from avocado and optimization of its extraction condition should be done.

The most commercially valuable cultivars of avocado are “Hass” and “Fuerte” which together contribute up to two thirds of all avocados grown worldwide. Therefore, the majority of research on avocado quality traits uses these two cultivars (Villa-Rodríguez et al., 2011). These cultivars have different physicochemical properties, proximate, mineral and phytochemical composition. However, no similar studies on avocado varieties grown in Ethiopia have been published to date. Their oil extraction conditions and the oils quality were not studied and characterized yet which were investigated in this study. The main objective of this research was extraction, optimization and quality characterization of oil from Hass avocado cultivars grown in Ethiopia (Wondogenet). The specific objectives of the research were to: extract Hass avocado oil, to optimize some process parameters of Soxhlet and ultrasound assisted extraction and evaluate their effects on the yield of oil, characterize the extracted oil and analyses fatty acid composition of extracted oils.

Materials and Methods:-

Materials:-

Raw Material collection, transportation, storage and sample preparation

Avocado Fruit:

Avocado fruit of Hass cultivar were picked and collected at the mature green stage of development on a farm of WARC (Wondogenet Agricultural Research Center), SNNP regional State of Ethiopia. These fruits were packed by carton box and transported to Food Engineering Laboratory, Addis Ababa Institute of Technology, and some of them were transferred to Food and Human Nutrition laboratory, Addis Ababa University and other food laboratories. Some of the fruits were stored at room temperature for about a week and ripened. The unripe and ripe avocado fruits were wiped off any dust, washed with distilled water, dried and weighed. The pulp of the fruit was cut into halves from the stem to the tip end. The skin and seed were removed; care was being taken to free the skin from adhering to the pulp. The pulp was oven dried, crushed and ground for oil extraction.

Processing Methods

Extraction of oil using Soxhlet Extraction

The extraction was carried out according to (Rybak-Chmielewska, 2003) AOAC (2005) with some modifications. The dried and ground avocado pulp (ten gram per sample) was packed inside a thimble bag and placed inside the thimble chamber of the 250 ml Soxhlet extractor. The extractor itself was placed inside a thermostatic water bath. A round bottom flask containing n-hexane solvent as well as a condenser was fixed to the extractor. The flask was heated and the solvent then vaporized and passed through the prepared sample to remove its oil. The process was allowed to continue for the specified time, as obtained from the experimental design. Thereafter, the oil extracted was recovered by distilling the solvent using rotary evaporator. At the end of each experiment, the yield of the oil was obtained using the following relationship.

$$\text{Oil Yield} = \frac{\text{W3} - \text{w1}}{\text{W2}} * 100\%$$

Where, W_1 is the weight of flask, W2 is the weight of sample, W_3 is the weight of flask and extracted oil

Extraction of oil using Ultrasound Assisted Extraction

The extraction was carried out according to (Cravotto et al., 2008) and (Lou et al., 2010) with some modifications. Fresh avocado pulp were dried in an oven at 60°C until their weights were constant, and they were then ground into powders and separated by particle size of 1.4mm (0.5–1.4), 2mm (1.4–2) and 2.6mm (2–2.6mm) mesh. The general method for extraction involved weighing avocado powder (10 g) into a 250 ml flask. Then the extraction solvent was added depending on the solvent to sample ratio and the flask was placed in the ultrasonic bath. The following extraction conditions were investigated for optimization: particle size of avocado pulp powder, extraction time and solvent–solid ratio. After the extraction, the solution was reduced on a rotary evaporator and the solvent was recovered. The avocado oil was weighed and the extraction yield was calculated as above stated.

Quality characterization of extracted avocado oil

Determination of oil properties were carried out PV: AOAC 965.33, %FFA: AOAC 940.28, Moisture and volatile matters AOAC 926.12, Soap Content: ISO 10539

Fatty Acid Analysis of extracted avocado oil

Conversion of Triglycerides to Fatty Acid Methyl Ester (FAME): Fatty acids were changed to their methyl esters (FAME) following the method of (Hartman & Lago, 1973). About 0.5g of lipids were weighed and immediately re-suspended in 5 ml of chloroform and stored at -20°C. To determine its fatty acid composition, a 50 µl subsample of the lipid-in- chloroform was treated with 100 µL of 0.5 N sodium methoxide in methanol (prepared with a solution of dimethoxypropane and methanol (95:5, v/v). Esterification of fatty acids to fatty acid methyl esters (FAME) was completed after standing at room temperature for 15 minutes.

Gas Chromatography Analysis: About one µl of fatty acid methyl esters in petroleum ether was injected into the gas chromatograph (Hewlett Packard model 5890A), equipped with a Supelco fused silica capillary column No. 11484-02A, catalogue No. 2-4019 (30 m x 0.25mm ID x 0.2 µm film Mfg.) and a flame ionization detector (FID). The temperature was 100°C initially, then increased by 15°C per minute to 190°C and held at 190°C for 25 minutes. Injector and detector temperatures were at 200 and 220°C respectively. The fatty acid peaks in lipid samples were identified by comparison with the retention times of fatty acids in the standard mixture, and the amount calculated as a percentage of the total lipids and as grams of fatty acid per 100 grams of fruit (fresh weight).

Experimental Design and Statistical Data Analysis

Response Surface Methodology: Response surface methodology is a powerful method that enables determination and optimization of the best conditions to maximize the intended responses.Numerous research have utilized various multilevel designs such as central composite design (CCD) and Box–Behnken design (BBD) (Grosso et al., 2014) for the optimization of variables. The Box-Behnken design was selected in this study as it has a higher efficiency compared with CCD and is more efficient than a factorial design (Ferreira et al., 2007). The optimum conditions were found by analyzing the response surface plots aiming for the highest reachable response variable for each independent parameter. Optimization of different oil extraction conditions were carried out as follows. There were two extraction methods: 1) Ultrasound assisted extraction with three factors and three levels: Particle size (1.4mm, 2mm, 2.6mm), Solvent to sample ratio (5:1, 10:1, 15:1) and extraction time (30, 60, 90 min); 2) Conventional soxhlet extraction with three factors and three levels: Particle size (1.4mm, 2mm, 2.6mm), and sample to solvent ratio (15:1, 20:1, 25:1) and Extraction time (4, 6, 8 hour).

Data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test using SPSS (version 20).The values (the responses) were entered into the appropriate column in Design Expert after each experiment's results (oil yield) were obtained.

Result and Discussions:-

Yield of avocado oil extracted using soxhlet and ultrasound assisted extraction

The soxhlet extraction parameters that provided the highest yield was optimized as follows: particle size of 1.4 mm, Solvent to solid ratio of 20: 1 and extraction time of 8hr which yields 69.7 g oil/100g DW (Table 3.1). Also, the ultrasonic extraction conditions were optimized as follows: particle size of 2 mm, Solvent to solid ratio of 15: 1 and extraction time of 90 min which yields 67.1 g oil/100g DW (Table 3.1).

A comparably higher yield of avocado oil was obtained using the soxhlet extraction (69.7%) than ultrasonic extraction (67.1%), which is in agreement with a study performed by (de Oliveira et al., 2014), (Döker et al., 2010) and (Reddy et al., 2012). But, this extraction method used high organic solvent and was more time consumer (8hr compared with 90 min). In other words, ultrasonic extraction yields interesting amount by using less solvent and consuming less time compared to soxhlet extraction. Yields by ultrasound and soxhlet techniques lay between 38.8 to 67.1% and 42.1 to 69.7%, respectively. The comparison between oil extraction yield (69.7%) from avocado pulp by soxhlet and the oil content percentage in the initial matrix (70.75±0.35%), shows that almost all (about 98.5%) of the oil in the initial matrix was extracted.

Table 3.1:- Actual and predicted oil Yield value by soxhlet extraction.

Standard Order	Run Order	Factor 1 A: Particle Size	Factor 2 B: Solvent: Sample ratio	Factor 3 C:Extraction time	Actual Value	Predicted Value	Residual
1	15	1.4	15	6	59.5	58.37	1.13
2	17	2.6	15	6	47.2	51.39	-4.19
3	13	1.4	25	6	67.9	68.12	-0.22
4	8	2.6	25	6	60.5	61.14	-0.64
5	5	1.4	20	4	45.6	49.78	-4.18
6	14	2.6	20	4	42.1	42.81	-0.71
7	6	1.4	20	8	69.7	70.36	-0.66
8	7	2.6	20	8	65	63.38	1.62
9	16	2	15	4	42.4	39.41	2.99
10	2	2	25	4	45.2	43.31	1.89
11	12	2	15	8	54.2	54.13	0.069
12	3	2	25	8	68.7	69.73	-1.03
13	11	2	20	6	65.8	64.69	1.11
14	10	2	20	6	65.3	64.69	0.61
15	4	2	20	6	65.4	64.69	0.71
16	1	2	20	6	64.7	64.69	0.01
17	9	2	20	6	66.2	64.69	1.51

Table 3.2:- Actual and predicted oil Yield value by ultrasound extraction.

Standard Order	RunOrder	Factor 1 A:particle size	Factor 2 B:solvent: sample ratio	Factor 3 C:extraction time	Actual Value	Predicted Value	Residual
1	4	1.4	5	60	56.76	56.22	0.54
2	8	2.6	5	60	45.17	48.39	-3.22
3	6	1.4	15	60	65.79	65.31	0.48
4	13	2.6	15	60	57.12	57.47	-0.35
5	1	1.4	10	30	44	46.88	-2.88
6	11	2.6	10	30	39.98	39.05	0.93
7	10	1.4	10	90	66.09	68.18	-0.69
8	9	2.6	10	90	60.42	60.34	0.081
9	17	2	5	30	38.8	35.66	3.14
10	14	2	15	30	43.56	44.75	-1.19
11	12	2	5	90	56.5	56.95	-0.46
12	2	2	15	90	67.1	66.04	1.06
13	5	2	10	60	60.55	59.61	0.94
14	16	2	10	60	59.24	59.61	-0.38
15	7	2	10	60	61.84	59.61	2.23
16	3	2	10	60	60.09	59.61	0.48

17	15	2	10	60	58.9	59.61	-0.71

Quality characterization of the extracted avocado oil

The highest percentage oil yield was achieved by soxhlet (69.7%) and lower yield was by ultrasonic extraction (67.1%). The oil yield differences of the extraction method could be attributed to the differences in their chemical properties and their fatty acid composition. The avocado oil obtained from soxhlet and ultrasonic extraction was analyzed for various parameters as shown in Table 4.8. The oil extracted by soxhlet extraction (SEO) comprises lower moisture and volatile matter, free fatty acid, acid value, alkalinity and Peroxide value than the oil extracted by Ultrasound assisted extraction (UEO). The obtained result was superior to the result reported by (Woolf et al., 2009),where Moisture <1%, FFA <0.5%, AV <1% except in the range for PV (<4).

Acid Value:

Acid value refers the free fatty acids found in fats and oils. High degree acid value can be related with degree of oxidation during preparation or storage. Since SEO (soxhlet extracted oil) has lower acid value (2.31±0.01 mgKOH/g) than UEO (ultrasound extracted) (2.65±0.00 mgKOH/g), it indicated that the quality of SEO was better than UEO. These results are found within the range of minimum (1) and maximum (7) values for the acid value properties of avocado oil reported by Eckey (1954). The analyzed oil also comprises higher than the result (1.23 ± 0.02) reported for the Fuerte cultivar by (Bora et al., 2001), and (1.46) reported for avocado grown in Mexico (Ortiz Moreno et al., 2003). The low acid value obtained for avocado oil in this study suggests that the oil is edible and less susceptible to rancidity.

Free fatty acid:

The percentage free fatty acid (FFA) value of oil is an important factor in determining oil quality because the lower the free fatty acid, the higher the quality of the oil particularly in terms of edibility. The percentage free fatty acid of UEO (1.33±0.00 g/100g), is slightly higher than SEO (1.16±0.86 g/100g).

Peroxide Value:

The peroxide value of the UEO (2.74±0.36meq/kg) was higher than that of the SEO (0.23±0.00 meq/kg), indicating that UEO is unstable against oxidation than SEO. Nearly similar peroxide value of the pulp oil were found by other researchers, but a lower value than UEO and higher value than SEO (1.4), was reported by (Bora et al., 2001). Much higher values of peroxide values, varying from 3.7 to 12.74, were reported by (Ortiz Moreno et al., 2003). These differences may be due to fruit production in different geographical places, climate, and soil composition. An oil with peroxide value above 10 meq kg^{-1} is classified at high oxidation state (Besbes et al., 2004). Furthermore, due to its high content of unsaturated fatty acids, avocado oil ought to exhibit a high rate of oxidation. Peroxides are formed when unsaturated fatty acids react with oxygen. Avocado oil peroxide values have been reported to be in the range of 5.1-12.3 meqkg-1 (Quiñones-Islas et al., 2013).

Iodine Value:

The Iodine Value (IV) of oils and fats indicates their degree of unsaturation (Sun, 2005). Higher iodine value is attributed to high unsaturation. The slightly higher iodine value (78.6) of SEO gives the indication that SEO contains more unsaturated fatty acid than UEO (75.3). These results are lower than the range of 82-95 reported by (Ortiz Moreno et al., 2003), but consistent with some reports stating that the number of IV for avocado oil was in the range of 65-95 (AOCS, 1998). The iodine value obtained also agrees with the data (77.6) reported by (Bora et al., 2001) for avocado oil.

Fatty acid (FA) Profile of avocado oil

Consumers are concerned about quality of oil, particularly the levels of oleic and linoleic acids, which have been proved to be healthy sources of oil for the human body. Avocados are primarily high in oleic, palmitic, palmitoleic and linoleic acids (Para et al., 2004). Thus, in the current study, oil analyses were conducted to determine the content of the aforementioned fatty acids. The fatty acid composition of oils obtained using various extraction methods is shown in Table 4.8. The analyzed oil consisted of four different FAs; two unsaturated FAs and two saturated FAs. MUFAs, oleic (C18:1) and SFA, palmitic (C16:0) acids, are predominant constituents of avocado oils. Linoleic acid (C18:2) was only detected in oil extracted by soxhlet and for saturated fatty acids, butyric acid was only detected in oils extracted by ultrasound extraction. The lower percentages of FAs produced by USAE could be due to short extraction time and low solvent to sample ratio. USAE is relatively rapid; increasing the extraction time could increase the yield as well as fatty acid composition.

In this study, only four fatty acids in the pulp oil of Hass cultivar were identified. The obtained principal fatty acid concentration was 46.46-59.59%, 23.66-24.66% and 7.62% for oleic, palmitic and linoleic acid respectively, which is nearly similar (in the range) with that of reported by (Woolf et al., 2009), which was: Palmitic acid (16:0) 10%-25%, Oleic acid (18:1) 60%-80%, Linoleic acid (18:2) 7%-20%. But, (MARTINEZ NIETO et al., 1988) reported a range of 60-65, 15-19 and 11-12%, respectively for the same fatty acids in the oil from Bacon, Fuerte, and Hass cultivars.

The lipid content and fatty acid composition in avocados varies greatly with the cultivar, region of cultivation, geographical locations within the same country, due to variation in climate, soil composition, and other factors including ripening stage, solvent type, extraction time and storage temperature (Landahl et al., 2009) and (Para et al., 2004). Therefore, the selection of an avocado cultivar for oil extraction might be based on both lipid content and on fatty acid composition (which is related to the intended use of the oil) (Santana et al., 2015).

Table 3.3:- Quality characteristics of oil extracted by UAE and Soxhlet Extraction.

Parameters	USAE	SOXHLET	Unit
Moisture and volatile matter	8.47±0.09	2.27±0.01	%(g/100g)
Free Fatty Acid	1.34±0.01	1.17±0.01	%(g/100g)
Acid Value	2.65±0.00	2.32±0.01	mgKOH/g
Alkalinity (Soap Content)	0.11±0.00	0.004±0.00	%(g/100g)
Peroxide Value	2.75±0.01	0.23±0.01	meq/kg
Iodine value	75.3±0.14	78.6±0.14	(g/100 g)
palmitic acid (16:0)	24.01	23.66	%
oleic acid (18:1)	46.46	59.59	%
Linoleic acid (18:2)	ND	7.62	%
Butyric acid (4:0)	1.38	ND	%

Results are expressed as the mean of duplicate ± Standard deviation. ND: Not Detected

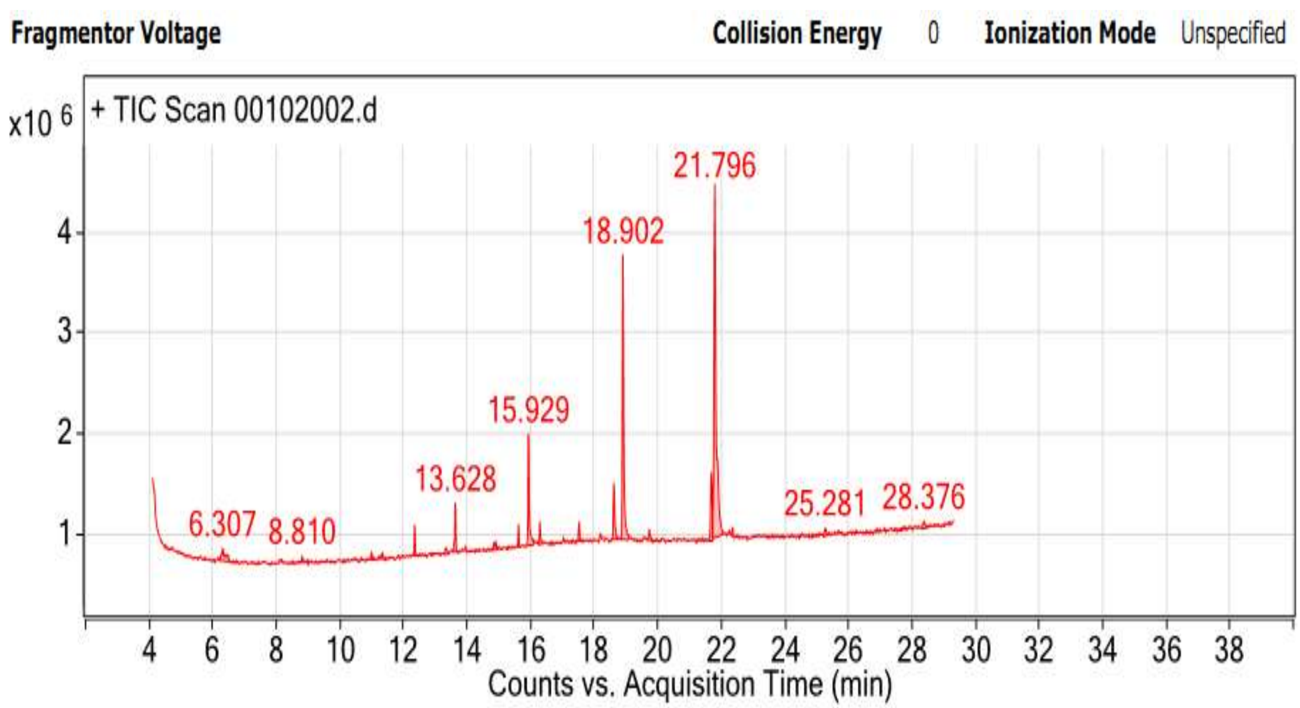

Figure 3.1:- GC-MS result for ultrasound extracted oil.

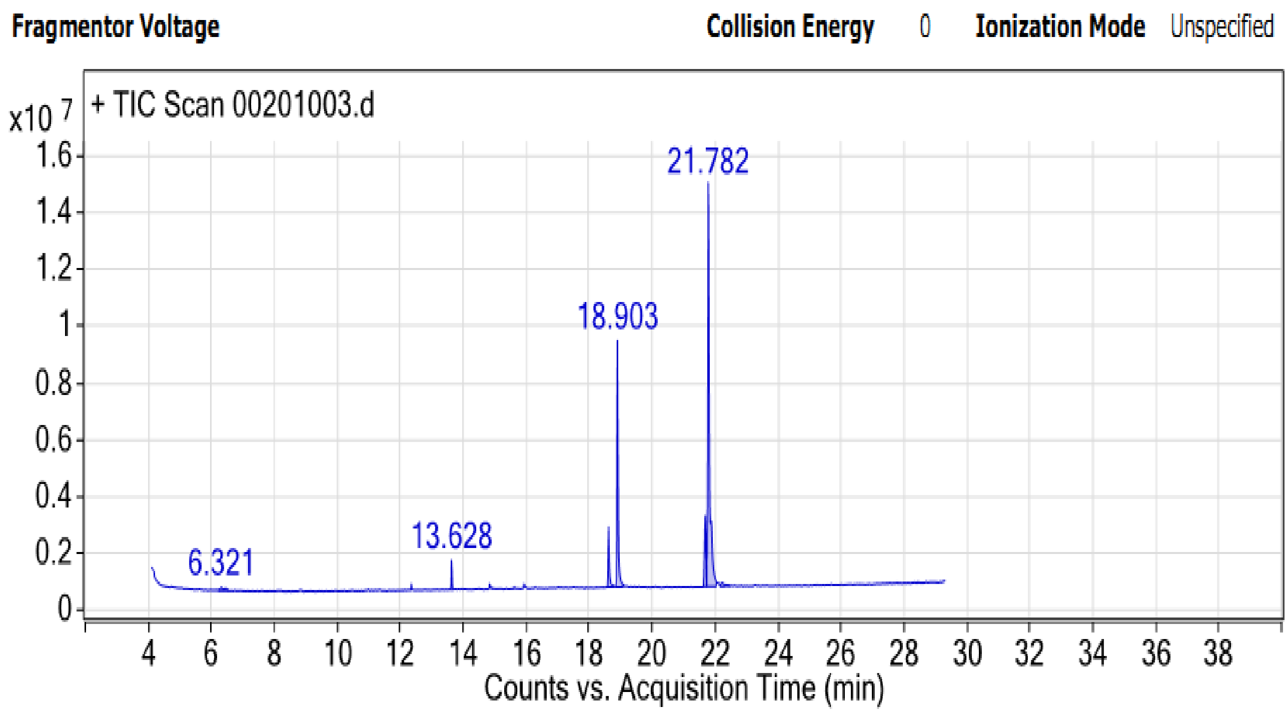

Figure 3.2:- GC-MS result for soxhlet extracted oil.

Generally, Soxhlet extracted avocado oil had the lowest acid value, peroxide values, FFA, MVM than ultrasonic extracted oil.Brazilian legislation for oils and fats (A Guide to Brazil ' s Oil and Oil Derivatives Compliance Requirements, n.d. BRASIL, 2005) does not propose specific standards for avocado oil. However, acid and peroxide values are standardized for pressed and crude oils, where the maximum allowed acid value is 4.0mgKOH/g and the peroxide value cannot exceed 15mEq O2/kg. In contrast, Mexico does have specifications for avocado oil (NMX-F-052-SCFI-2008); the maximum allowed acid and peroxide values are 1.5% oleic acid and 10mEqO2/kg, respectively. In the present work, oils from both processes presented acid and peroxide values below the limits set by Brazilian legislation.

Conclusion:-

In the present study, ultrasonic and soxhlet extraction of avocado oil was optimized. The yield of oil increased as extraction time and solvent to solid ratio increased, but vice versa for particle size. The comparison of the two techniques shows that the soxhlet extraction provided the greater yield. This fact may be explained because extractions were conducted above the boiling point of the solvents, takes longer extraction time and consumes more solvents. It may also occur because this technique uses practically pure solvent in each refluxing which makes the mass transfer easier. Eventhough the method presented, UAE, gives lower quality and quantity, but it has the advantage of easy operation, shorter extraction time and reduced solvent consumption and can be used for sequential extraction of fatty acids. Gas chromatography analysis showed that the fatty acid composition of the avocado oil obtained by ultrasonic extraction was nearly similar to that of oil obtained by Soxhlet extraction. The results also show that, relatively high contents of oleic and linoleic acids present in the avocado pulp oil reveal that this cultivar was rich sources of ω-6 fatty acids and it is needed to reduce LDL and maintain HDL, which is responsible in reducing cancers and cardiovascular diseases. So, Ultrasonic extraction is a useful and environmentally friendly extraction method that could be applied to the production of other plant oils and active substances.

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