Fatty acid profile of agro industry waste of pumpkin *Cucurbita maxima*

[1]Karina Marin Morocho*, [1]Elsalous Ahmed Elkotb Khairat, [2]Patricia Manzano, [2]Ivan Choez

[1]Facultad de Ciencias Agrarias, Universidad Agraria del Ecuador, Ecuador

[2]Facultad de Ciencias de la Vida, Escuela Superior Politécnica del Litoral Ecuador

Abstract

The use of pumpkin seeds has not been considered as important resource by the agroindustry, however the presence of fatty acids in *Curcubita maxima* seeds such as myristic, palmitic, linoleic, oleic and stearic have been identified by the traditional soxhlet method using solvents as ethanol, chloroform and hexane, except for the linoleic that was not identified in chloroform, the intensity of the area peaks identified by GC-MS did not show significant difference between ethanol and hexane that were higher with respect to chloroform, for this reason the use of ethanol to be a solvent called GRAS is very useful for the agro-industrial use of this seed.

Keywords: Fatty acid, Pumpkin, Seed oil, Agroindustry waste-pumpkin, Organic solvents.

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Introduction

The production of pumpkin seed oil and its consumption have been increasing during the last five years [1].

The edible oil is obtained from the seed denominated *Curcubita*. The seed is flat, oval, green and lies inside a white hull, which is used for culinary practices and comestibles [2,3].

For instance, in African countries is a snack popular with healing properties and USA is using in desserts [4].

Pumpkin seed oil has interest features since it presents fatty acids, such as oleic, linoleic and palmitic acid (Rezig et al.) [2].

The presence of fatty acids implied related with a nutritional sources and exhibits beneficial physiological effects in the prevention of disease such as coronary heart and cance.

Additionally, pumpkin seed oil is considered an edible oil and a nutraceutical for its natural rich content of proteins and, phytosterols polyunsaturated fatty acids, antioxidants and vitamins, such as carotenoids and tocopherol and trace elements such as zinc [5-8].

From of the point of agro industry the seed pumpkin has been registered that this seeds high content of carbohydrates for this reason have been reported it since has using in bakery process as alternative source [9-18].

The literature there are many reports on the composition and properties of pumpkin seeds which concern mainly at identity varieties and showing the composition of fatty acids according at Winkler et al., Younis et al., El-Adawy and Taha [19], Ryan et al. and Murkovic et al. [12].

In Ecuador, pumpkin seeds are generally considered to be agro-industrial wastes, and to our best knowledge, the information related to the composition of fatty acids in this seed is limited.

The production of edible oils is mainly produced from of palm, soybean and sunflower. These vegetable oils are processed and refined to produce high-quality oils suitable f such as frying or salad dressings.

Ecuador, the seventh largest exporter of palm oil in the world and the production of palm oil has been an important step in the reduction of poverty in the country [10,11].

The pumpkin seed oil dark is green and has a high content of free fatty acids for this reason, cannot be used for cook, however, the consume has been increasing in the southern parts Austria, Slovenia, and Hungary as salad dressing [12].

The organic solvents are used to extract oil; for example, hexane has been used for decades to extract oil from cottonseed.

However, environmental safety regulations and increased public health risk are necessitating the industry to consider alternatives to the organic solvents for use in oil extraction [13].

In this paper, the oil of the pumpkin is extracted *via* soxhlet method using three solvents: 1. Chloroform; 2. Hexane; 3. Ethanol.

The objective is to identified fatty acids and the physicochemical properties to contribute to the valorization of pumpkin seed oil as resource in agro-industry process, pharmaceutical and cosmetic.

Materials and Methods

Seed material

The raw materials used in this study were pumpkins collected from Daule city (Guayas, Ecuador). The seeds were washed with neutral detergent for complete removal of the fibrous strands surrounding them.

Subsequently, the samples were dried in stove (Memmert, SN75) at 50°C until constant weight, the dried material was

milled in a knife mill (Solab, Sl-50) and filtered using mesh #70 (212 μm).

Oil extraction

Pumpkin seed oil extraction was carried by "soxhlet" according of the Methods of Analysis Used for Chemical Control of Food of the US National Institutes of Health,1996 (Reports ISTISAN 96/34).

The sample was 20 g of milled seed were placed in cellulose cartridges (Whatman) the solvents used to extract were chloroform, hexane and ethanol, each extraction was carried out during 8 h, at a speed equivalent to one cycle every 20-30 min.

The volumes of solvents have been adapted to the volumes of soxhlet extractors used a range from 50 to 200 mL. three extractions by each solvent were continuously performed to test the repeatability of the method.

The extract was concentrate with a rotary evaporator ROVA-100 (Germany) at 40°C temperature, a pressure of 400 psi, until 5 ml of volume of extract. This extract was filtered at vacuum using a membrane with a pore size of 0.45 μm (General Electric Scientific, Pittsburgh, USA).

The yield was 10 ml for each 454 of pumpkin oil. Finally, the sample was stored in a fridge at 4°C.

CG-MS by seed pumpkin

The analysis by CG-MS was carried using a gas chromatography (Agilent Technologies, Model 7890A), coupled with a mass spectrometer 5975 INSERT.

The chromatographic column was an Agilent J and W Scientific DB5-MS capillary (30 m length × 0.32 mm inner diameter × 0.25 μm film thickness).

The carrier gas was He (0.9 ml/min). The oven temperature program began with an initial temperature 60°C for 5 min and then the temperature was increase at a rate of 3°C/min to 300°C, and then it was maintained for 25 min.

The data were recorded by MSD ChemStation software and the identification of the constituents was achieved using mass spectral matches with Wiley7 NIST 05 mass spectra database.

Chemical composition of seed pumpkin and oil pumpkin

The content of moisture and water activity in seed pumpkin before and after it dried determined by AOAC methods (925.10). The density, index acidic, index peroxide, index saponification, index iodo was determined by AOAC methods in pumpkin oil (920.212, 940.28, 965.33, 920.16 and 920.159, respectively).

Results and Discussion

The results using ethanol to extraction are presented in Tables 1 and 2. It can be observed that the intensity of peaks are less intense than with hexane.

For example: linoleic, oleic and palmitic acid show the highest peaks. The ethanol is considered GRAS it has been reported has solubility for some fatty acids mainly in pumpkin seed due to H-bonding interaction.

According to report the ethanol has been used with supercritical carbon dioxide, demonstrated the identified of fatty acids such as linoleic, palmitic, oleic and stearic acid.

This results confirm that an ethanol is good solvent to identified fatty acids in seed pumpkin. This was supported through analysis by scanning electron microscope (SEM), where observed that the destruction of the cell walls using ethanol as solvent, in this way the lipid phase might be easy extracted [14].

The myristic contributed insignificantly to the fatty acid profile in pumpkin seed of Curcubita maxima.

Table 1. *GC-MS peak relative area of fatty acids identified in oil pumpkin with extracted with ethanol.*

Fatty acids	a RT	%Qual	CAS	Mw	Formula	Peak Area
Myristic	26,44	98	018603-17-3	228,37	$C_{14}H_{28}O_2$	1,45 ± 0,12
Palmitic	29,32	99	000628-97-7	256,4	$C_{16}H_{32}O_2$	19,03 ± 3,03
Linolenic	33,26	97	056259-07-5	278,43	$C_{18}H_{30}O_2$	6,07 ± 1,27
Linoleic	33,28	97	056259-07-5	280,44	$C_{18}H_{32}O_2$	30,24 ± 0,12
Oleic	33,38	99	021556-26-3	282,47	$C_{18}H_{34}O_2$	25,07 ± 2,47
Stearic	33,87	98	018748-91-9	284,48	$C_{18}H_{36}O_2$	18,13 ± 0,39
a Time Retention b values are the mean and standard deviation of three replicates From NIST available in http://webbook.nist.gov/chemistry/cas-ser.html for DB5 column						

The chloroform was used to identified fatty acid in pumpkin seed. The results are presented in Table 2. The palmitic acid is 25% more high than stearic acid. The peak of myristic was not significant.

The chloroform usually is not used in agro-industry, because in case of trace solvent have to removal to be considered an edible oil, our date is in accord with Glew and coworkers

which show the presence the same fatty acids with chloroform solvent [15].

Table 2. *GC-MS peak relative area of fatty acids identified in oil pumpkin with extracted with chloroform.*

Fatty acids	RT	%Qual	CAS	Mw	Formula	Peak Area
Myristic	24,52	98	000544-63-8	228,37	$C_{14}H_{28}O_2$	0,22 ± 0,01
Palmitic	28,96	99	000057-10-3	256,4	$C_{16}H_{32}O_2$	34,26 ± 0,90
Linoleic	32,16	99	000060-33-3	280,44	$C_{18}H_{32}O_2$	26,78 ± 4,63
Oleic	32,25	99	000506-17-2	282,47	$C_{18}H_{34}O_2$	29,94 ± 1,14
Stearic	32,97	96	000111-61-5	284,48	$C_{18}H_{36}O_2$	8,80 ± 0,37
[a] Time Retention [b] values are the mean and standard deviation of three replicates From NIST available in http://webbook.nist.gov/chemistry/cas-ser.html for DB5 column						

In the Table 3, the fatty acids were identified using hexane categorized as common solvent used in identified fatty acids in pumpkin seed oil, with respect at myristic acid show the peak higher than ethanol and chloroform, linoleic acid and palmitic acid show peak higher than ethanol and chloroform, it is worth mentioning that hexane is one of the most used solvents and the investigations carried out previously, however is not safe to food industry [16,17].

The abundance of peaks areas: palmitic, linoleic, oleic, linoleic and stearic using this solvent, might be it supported genetic characterization of the pumpkin [14].

Table 3. *GC-MS peak relative area of fatty acids identified in oil pumpkin with extracted with hexane.*

Fatty acids	RT	%Qual	CAS	Mw	Formula	Peak Area
Myristic	26,44	98	018603-17-3	228,37	$C_{14}H_{28}O_2$	1,45 ± 0,13
Palmitic	29,31	99	000628-97-7	256,4	$C_{16}H_{32}O_2$	23,28 ± 0,19
Linolenic	32,37	99	000544-35-4	278,43	$C_{18}H_{30}O_2$	25,39 ± 0,04
Linoleic	32,5	99	000111-62-6	280,44	$C_{18}H_{32}O_2$	26,23 ± 0,18
Oleic	33,26	99	056259-07-5	282,47	$C_{18}H_{34}O_2$	8,42 ± 0,54
Stearic	33,87	99	018748-91-9	284,48	$C_{18}H_{36}O_2$	15,20 ± 0,39
[a] Time Retention [b] values are the mean and standard deviation of three replicates From NIST available in http://webbook.nist.gov/chemistry/cas-ser.html for DB5 column						

The fatty acid identified as commons in three solvents are shown in the Figure 1.

The ethanol with hexane show peak comparable, because the six acids are found with two solvents, a exception of myristic acid that is insignificant using ethanol and linoleic acid that is not identified in chloroform acid.

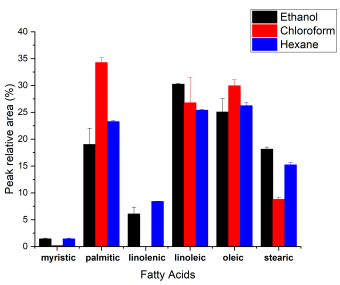

Figure 1. *Fatty acids identified in three solvents using.*

Finally,the chemical properties of pumpkin oil seed were analyzed with ethanol and are presented below in Table 4. The results are in accordance with the regulations for edible oils, the density is comparable with common edible oils obtained from seeds such as: sunflower, cotton [10].

The acidity index of pumpkin seed oil was low, the peroxide index was higher than Curcubita pepo [17], this value is a good indicator of greater oxidative stability. The indicated iodine number was high, indicating a high degree of unsaturation and low peroxide.

The saponification number has been reported that pumpkin seed oil was within the 175-250 range that is normally found in other seed oils such as raspberry, safflower, sunflower and corn seed [18].

These values demonstrate that pumpkin seed oil possesses the desirable qualities of an edible oil [17] and in urinary diseases [19].

Table 4. *The chemical properties of seed pumpkin oil extracted with ethanol.*

Parameters	Result[a]	Reference Method	Edible Oils
Density	0,9051 ± 0,23 g/ml	AOAC 20th 920,212	0,895-0,910
Acid Index	2,49 ± 0,15 mgKOH/g	AOAC 20th 940,28	2.96 b ± 0,16
Peroxid Index	1,32 ± 0,02 meqO_2/kg	AOAC 20th 965,33	Max.10,00
Saponification index	138,63 ± 0,46 mg/g	AOAC 20th 920,16	
Iodine Index	67,25 ± 0,47 cg/g	AOAC 20th 920,159	60-72
aDate are the average of three replicates, bAcidex Index was compared [19].			

The presence of palmitic and myristic acid in oil pumpkin might be considered because a certain population is sensitive to its consumption, generally the conventional edible oil such as soy and palm oils that are rich in fatty acids and additionally are subjected to high temperatures [14,15].

Particularly, pumpkin seeds oil are recommended for consumption without undergoing thermal process due to, approximately the caloric intake represented by fats constituted 35% and this must be in energy balance and the amount that will be finally absorbed [16]. This is supported by previous studies where the health of people is not affected slows the as trans fatty acid if the caloric is balanced [17].

In this context, myristic acid reported a relative area of 1.45 ± 0.12 and palmitic acid of 19.03 ± 3.03 with the use of ethanol as a solvent.

Conclusion

These findings suggest that oil of pumpkin Curcubita maxima might be useful edible oil due to the presence of fatty acids such as myristic, palmitic, linoleic, linoleic, oleic and stearic. These fatty acids are source nutrional until now the seeds of the pumpkin have been considered an agro-industrial waste.

The importance of its nutritional benefits is an opportunity for industrialization, using as solvent ethanol considered a safe food grade solvent.

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*Correspondence to

Karina Marin Morocho

Facultad de Ciencias Agrarias

Universidad Agraria del Ecuador

Ecuador

E-mail: kmarin@uagraria.edu.ec