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Extraction and Evaluation of Some Bioactive Compounds from Tomato Pomace for Use in Food Support

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Abstract

This study aims to benefit from the tomato pomace produced from the manufacturing processes, by extracting and evaluating the bioactive compounds from it. Three methods were used to help extract these compounds which are Blending the Frozen (BF), Microwave Assisted (MA) and Ohmic Heating (OH). The results showed that the BF method was the best extraction method, and the amount of lycopene 487.4 µg/g, β - carotene 115.8 µg/g, total phenolics 1297.4 µg GAE/g, total flavonoids 462.5 µg QE/g and antioxidant activity 89.1%. It was found that the most abundant phenolic compounds in tomato pomace were gallic acid and ellagic acid, as it contains seven essential amino acids, and that glutamic acid was the dominant acid. Fatty acid estimation showed that linoleic cis and oleic cis were the dominant acids, as they were 585.50 and 164.67 µg/g total fatty acids, respectively. It also contained on unsaturated fatty acids 820.44 µg/g.

Keywords: Extraction • Evaluation • Bioactive compounds • Tomato pomace

Introduction

One of the agro food businesses with the most impact is the production of tomatoes (Lycopersicon esculentum), which are produced annually at a global rate of 170 million tonnes, of which 127.5 million are used for fresh consumption and 42.5 million for industrial processing [1]. The pomace skin, pulp, and tomato seeds are frequently present in larger concentrations in processed tomato products. They are great sources of Bioactive Compounds (BC), including vitamins, β-carotene, lycopene, and flavonoids, which can be utilised to make pharmaceuticals, food coloring, and other additives.

Clinical studies also support the beneficial effects of several compounds found in tomato pomace, such as lycopene, which fights reactive oxygen species and helps to prevent some non- communicable diseases in humans [2,3]. In addition to their anti- oxidant activity, anti-inflammatory, anti-diabetic, anti-obesity, anti- microbial, anti-proliferation, anti-allergic capabilities, and the prevention of chronic disease, polyphenols are well known for their beneficial effects on health. Based on the evidence of their action, polyphenols are of interest to the nutraceutical, cosmetic, and food industries. Their global market was estimated at $757 million in 2015 and is expected to grow at a Compound Annual Growth Rate (CAGR) of 8.26% from 2014 to 2022. The supply of polyphenols will be significantly increased by the functional food and functional beverage segments, and the dietary supplement segment is anticipated to continue growing steadily over the forecast period due to an increase in the number of elderly people in various countries.

Because there aren't enough extraction processes that are both environmentally acceptable, affordable, and effective at preserving the functionality of volatile bioactive compounds, the use of food waste for the recovery of valuable bioactive compounds is frequently constrained. These limits of traditional extraction techniques have been overcome by the advent of numerous non-thermal new extraction techniques over the past 20 years. New extraction methods have been developed to get around the limitations and drawbacks of traditional extraction methods, including pressured liquid extraction, microwave extraction, enzyme extraction, supercritical fluid extraction, and pulsed electric field extraction.

Aim of this study is to identify and evaluate a method for extracting bioactive compounds from tomato pomace under freezing conditions for use in food fortification, and to compare it with some other extraction methods to determine the possibility of increasing the amount extracted from these compounds and maintaining their activity.

Materials and Methods

Fresh samples of tomato pomace used in industry (seeds and skins) were gathered from Sun Top, Nile Street, Rajib, Jordan Sahab, and Amman, Jordan. One third of the quantity is stored by freezing at -17.5 °C until extraction, and the other two thirds were dried in a vacuum oven (at 50 °C for 5 hours) to measure Dry Matter (DM.) content. Thus, the dried samples were ground in a laboratory mill until obtaining a very fine powder. The samples were stored at 4 °C and in darkness until further analyses [4].

Extraction technique evaluation: Three methods were used to extract bioactive compounds from tomato pomace and compare among them to choose the best ones. The three methods are:

The first method: By BF tomato pulp with an electric mixer for five minutes.

The second method: According to Lasunon, et al., they used MA extraction, which was carried out by adding 5 g of tomato pomace powder to 100 mL of 95% ethanol and heating it at 300 W for 60 s using a (2450 MHz household microwave oven).

The third method: By using of OH technology OH extraction experiments were done in the presence of electric fields (MEF) was 55 °C for 15 min using 35% ethanol as a solvent according to technology.

According to Lasunon P, et al. [5], a cylindrical glass reactor with an inner diameter of 2.7 cm, a total length of 30 cm, and two stainless steel electrodes 316 positioned at each edge and isolated by PTFE covers was utilized to perform OH assisted extraction from tomato samples at a frequency of 25 kHz. This mixture prevents electrochemical reactions and corrosion. The system was controlled by a function generator (Agilent 33,220 A, Bayan Lepas, Malaysia; 1 Hz–25 KHz and 1-10 V) coupled to an amplifier (Peavey CS3000, Meridian, MS, USA; 0.3 V–170 V). For further investigation, the extracted crude was kept in storage at -18 °C.

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Determining of lycopene and β –carotene

Nagata and Yamashita method was used to determine the amounts of lycopene and -carotene. In a nutshell, 16 ml of acetone/ hexane (4:6) were violently agitated with 1 g of tomato pomace powder for 15 minutes. Using a Varian Cary 50 UV-Vis spectrophotometer, the light absorption values (A) of the hexane layer at 453, 505, 663, and 645 nm wavelengths were measured after phase separation [6].

The milligrammes of lycopene and β-carotene per 100 mL of solvent were determined using the following equations:

Lycopene= -0.0458 × A663+0.204 × A645+0.372 × A505-0.0806+A453

β-carotene= 0.216 × A663-1.220 × A645+0:304 × A505-0:452 × A453

Where,

A663, A645, A505 and A453 are the absorbance at 663, 645, 505 and 453 nm, respectively. The results were expressed in mg per kg.

Determination of total phenolic content

Based on Singleton and Rossi's, approach, the Folin- phenol Ciocalteu's reagent method was used to quantify the total phenolic content utilizing gallic acid as a typical phenolic substance. For extraction, 0.3 g of dried tomato pomace was combined with 5 mL of methanol, and the mixture was then ultrasonically processed for 50 minutes at room temperature. The extracts were then centrifuged for 5 minutes at 4200 rpm to separate the supernatants, which were then collected, filtered through 0.45 m polyamide membranes, and kept at 4 ºC until they were needed for the test. 500 mL of the 0.2N Folin-Ciocalteu reagent and 100 mL of the filtered extracts were combined. 1.5 mL of a 20% sodium carbonate solution was added after 5 minutes. To achieve a final volume of 10 mL, the reaction mixture was diluted with distilled water. After 30 minutes of incubation at 40 ºC with intermittent shaking, the solution developed a blue color, and its absorbance was measured at 765 nm on a Varian Cary 50 UV-Vis spectrophotometer. Results were given in mg/kg Gallic Acid Equivalents (GAE).

Determination of total flavonoid content

The aluminum nitrate method, as described by Mohammadzadeh, et al., was used to spectrophotometrically quantify the flavonoid content of dried tomato pomace. Briefly, 0.4 milliliters of tomato pomace methanolic extract, 0.1 milliliters of 10% aluminum nitrate

($AlCl_3$), 0.1 milliliters of 1 M aqueous potassium acetate, and 4.3 milliliters of methanol were combined. Using an evolution 600 UV-Vis spectrophotometer, the mixture's absorbance at 415 nm was measured after 40 minutes of reaction time at room temperature (Thermo Scientific, USA). Using a standard curve containing quercetin, the total flavonoid content was calculated. The results were represented in milligrammes of Quercetin Equivalents (QE) per kilogram.

DPPH radical scavenging activity

Hydrophobic and hydrophilic fractions' antioxidant activities were assessed. By adjusting the polarity of the extracted ethyl acetate, Erkan, et al., examined the hydrophobic fraction's DPPH radical scavenging activity, while Sengkhamparn and Phonkerd determined the hydrophilic fraction. The following equation was used to express the data as a percentage of radical scavenging activity (%):

Radical scavenging activity (%)= (Acontrol-Asample) × 100/A control

Where A control was the absorbance of ethanol and ethyl acetate instead of crude extract for the hydrophobic fraction and hydrophilic fraction, respectively, and A sample was the absorbance of the combination.

Determination of amino acids

The procedure described by Varzaru which entails acid hydrolysis for the release of amino acids from the protein molecules, was followed by performic acid oxidation for the sulfur amino acids, when preparing the dried tomato pomace samples for the amino acids determination. Thermo-Electron Corporation, Waltham, Massachusetts) HPLC finningan surveyor plus system, outfitted with a DAD and a Hypersil BDS C18 column (250 4.6 mm, particle size 5 m), was used to carry out the chromatographic separation. 50 mM phosphate buffer (pH 7.5) was used as eluent A at a flow rate of 1.7 mL/min, while water, acetonitrile, and methanol (20/20/60) was used as eluent B. The injection had a 20 L volume. The following conditions for a linear gradient elution were used to separate amino acids: Steps of 2 minutes at 0% solvent B, 23 minutes at 57% solvent B, 1 minute at 100% solvent B, 3 minutes at 100% solvent B, 1 minute at 0% solvent B, and 5 minutes at 0% solvent B. To keep track of the derivatized amino acids, the DAD was set to 338 nm. The standard amino acid combination was produced as a stock solution in hydrochloric acid (0.1 M), containing 500 g ml-1 of each amino acid. Utilizing calibration curves fitted by linear regression analysis, quantification was based on the external standard approach. Software called Chrom Quest was used to gather and process the data.

Determination of fatty acids

Fatty Acid Methyl Ester (FAME)/gas chromatography was used to determine the amount of fatty acids, in accordance with ISO/TS 17,764-2 (2008). By trans esterifying fatty acids from the whole lipid extracts in methanol containing 3% concentrated sulfuric acid at 80 ºC for 4 hours; fatty acids were changed to their methyl esters. Methyl esters of fatty acids were examined in a Perkin Elmer-Clarus 500 chromatograph fitted with a BPX70 capillary column (60 m 0.25 mm i.d., 0.25 m film thickness) and a Flame Ionization Detector (FID). The column temperature ranged from 180 ºC to 220 ºC, with a 5 ºC min 1 program. The splitting ratio was 1:100 and the carrier gas was hydrogen (35 cms-1 linear velocity at 180 ºC). Temperatures for the injector and detector were 250 ºC and 260 ºC, respectively. FAME was identified by comparing retention times to established benchmarks. Results were given in terms of grams of fatty acids per kilogram of total fatty acids.

Determination of phenolic compounds

Nour used a Finningan Surveyor plus HPLC system (Thermo Electron Corporation, San Jose, CA) with a vacuum degasser, surveyor plus LCPMPP pump, surveyor plus ASP thermoautosampler, and a PDA5P diod array detector to determine specific phenolic compounds (DAD). A reversed-phase Hypersil Gold C18 column (5 m, 250 4.6 mm) running at 20 ºC was used for the separation. Eluent A, a 1% aqueous acetic acid solution, and methanol made up the mobile phase (eluent B). The gradient programme looked like this: 90% A (27 minutes), 90% A to 60% A (28 minutes), 60% A (5 minutes), 60% A to 56% A (2 minutes), 56%A (8 minutes), 56% A to 90% A (1 minute), and 90% A (4 min). The injection had a 5 L volume. At a flow rate of 1 mL min-1, simultaneous monitoring was done at 254, 278 and 300 nm. Before injection, the methanolic extracts produced as previously reported were filtered using a nylon syringe filter (0.45 m). According to peak area measurements that were recorded in the calibration curves of the respective standards, each compound was quantified. In mg per kilogram, phenolic component concentration was specified [7].

Statistical analysis

For each sample, measurements were made in triplicate, and the results were presented as the mean value minus the standard deviation. Utilizing the statistical analysis programme Statgraphic Centurion XVI (StatPoint Technologies, Warrenton, VA, USA).

Results and Discussion

Tomato pomace content from lycopene, β -carotene, total phenolics, total flavonoids and antioxidant activity.

Table 1 shows the effect of the three extraction methods used in this study on the yield of lycopene, β-carotene, total phenols, and total flavonoids, and antioxidants activity. By comparing the results obtained from the three methods, it was found that the best results obtained from lycopene, β-carotene, total phenols, total flavonoids and antioxidant activity was from PF method where it was 487.4 µ/g, 115.8 µ/g, 1297.4 µg GAE/g, 462.5 µg QE/g and 89.1%, while

the lowest methods were (OH) 472.3 µ/g, 111.2 µ/g, 1236.4 µg GAE/g, 446.4 µg QE/g and 84.7%, respectively. These extraction methods are used in order to soften the cell walls and thus facilitate the release of their contents from the bioactive compounds. The amount of these compounds varies according to the efficiency of each method, as we find that the results of the Ohmic Heating method (OH) and the method of using Microwave (MA) are somewhat similar, while the results of the extraction method by mixing frozen (BF) were the best among those methods and this is due to the expansion of cell fluids during freezing and thus pressure on the cell walls, causing them to explode and easy to exit the contents during the mixing process.

Comparing these results with what you get Khedr A and Shabana M [1] where they extracted the bioactive compounds from tomato pomace after drying them in an air-drying oven, and therefore their results were completely different from what we reached.

Tomato pomace content of phenolic compounds

The phenolic compounds in tomato pomace extract were estimated from the three extraction methods used in this research as shown in Table 2. As shown in the table, the best results obtained were from the BF extraction method, while the other two methods were close in results. The results also showed that Gallic acid and ellagic acid were the most abundant phenolic compounds that were

190.2 and 142.6 µg/g, respectively. On the contrary, it was found that the least present compounds were sinapic acid, catechin hydrate and caffeic acid, which were 0.1, 0.2 and 0.4 µg/g, respectively. Absence of some compounds such as epicatechin, ferulic acid, Quercetin and trans-cinnamic acid was also observed. The results showed that the amount of rutin and myricetin was low as it was 32.1 and 73.9 µg/g, respectively. It is known that these two compounds have a high capacity as antioxidants and also have the ability to search for free radicals in the environment around them, and therefore their presence among the phenolic compounds in tomato pulp is a very good indicator, as he explained.

The phenolic compounds shown by the results of this study agree to some extent with those shown by Violeta, et al., but they are more quantitative than obtained.

Tomato pomace content from amino acids

The amino acid content of tomato pomace was estimated as shown in Table 3.

It was observed that the PF extraction method was the best method for obtaining the largest amount of amino acids compared to the other two methods of MA and OH extraction whose results were somewhat similar.

It was also found that there are 7 essential amino acids are phenylalanine, tyrosine, threonine, lysoleucine, valine, methionine and lysine in the following quantities 5.8, 5.7, 5.3, 7.2, 4.9, 3.1 and 10.5 (µg/g) respectively. It was also noted that the most abundant acid was glutamic acid, a non-essential amino acid, which was 73.2 µg/g and the least present among the acids found in the tomato pulp was serine and cysteine, which were 2.2 and 2.5 µg/g respectively. Elbadrawy and Sello found also glutamic acid as the predominant amino acid in the tomato peel protein fraction.

The amino acid profile of dried tomato residues will rely on the peel/seed ratio in the pomace because prior research showed that the peel by-product was typically lower in essential amino acids than the seed by-products [8].

Tomato pomace content from fatty acids

The findings in Table 4 demonstrated that the PF method was the most efficient extraction method. The findings indicated that palmitic acid was the predominant saturated acid 135.21, 204.43 and 202.57 µg/g, whereas linoleic acid represented the major fatty acid 585.50,584.11 and 579.53 µg/g of the total fatty acids by BF, MA and OH extraction, respectively. The dominance of unsaturated fatty acids over saturated fatty acids may be seen in the dried

Table 1. Tomato pomace content from lycopene, β-carotene, total phenolics, total flavonoids and antioxidant activity on dry weight.

Component	Extraction Method		
	BF	MA	OH
Lycopene (µ/g)	487.4 ±0.26a	477.5 ± 0.30b	472.3 ± 0.31c
β-carotene (µg/g)	115.8 ± 0.14a	112.4 ± 0.15b	111.2 ± 0.17b
Total phenolics (µg GAE/g)	1297.4 ± 51.3a	1242.7 ± 49.2b	1236.4 ± 54.5c
Total flavonoids (µg QE/g)	462.5 ± 17.1a	446.7 ± 14.6b	446.4 ± 16.2b
Antioxidant activity (%)	89.1 ± 0.02a	86.5 ± 0.03b	84.7 ± 0.03c
Different a substantial difference between samples is indicated by letters in the same row (<0.05).			

Table 2. Tomato pomace content of phenolic compounds on dry weight (Âµg/g).

Phenolic Compounds	Extraction Method		
	BF	MA	OH
Coumaric acid	20,2 ± 0.3b	21.5 ± 0.3a	18.9 ± 0.4c
Caffeic acid	0.4 ± 0.01a	nd	0.1 ± 0.01a
Chlorogenic acid	84.4 ± 0.3a	83.1 ± 0.2b	83.4 ± 0.3b
Catechin hydrate	0.2 ± 0.01a	0.09 ± 0.01a	nd
Epicatechin	nd	nd	nd
Ellagic acid	142.6 ± 0.4a	136.5 ± 0.3c	140.5 ± 0.3b
Ferulic acid	nd	nd	nd
Gallic acid	190.2 ± 0.5a	174.5 ± 0.6b	173.8 ± 0.3b
Myricetin	73.9 ± 0.4a	68.6 ± 0.4b	68.4 ± 0.2b
Quercetin	nd	nd	nd
Rutin	32.1 ± 0.4a	30.6 ± 0.4b	28.6 ± 0.6c
Syringic acid	3.3 ± 0.4a	2.9 ± 0.2b	2.5 ± 0.2c
Sinapic acid	0.1 ± 0.01a	0.2 ± 0.01a	nd
Salicylic acid	81.2 ± 0.34a	77.5 ± 0.3b	75.3 ± 0.4c
trans-Cinnamic acid	nd	nd	nd
Vanillic acid	35.2 ± 0.2a	34.9 ± 0.1a	32.3 ± 0.4b
Different a substantial difference between samples is indicated by letters in the same row (P<0.05).			

Table 3. Tomato pomace content from amino acids on dry weight (µg/g).

Amino Acid	Extraction Method		
	BF	MA	OH
Phenylalanine	5.8 ± 0.4b	6.1 ± 0.3a	5.2 ± 0.3c
Serine	2.2 ± 0.3a	1.7 ± 0.4c	2.0 ± 0.5b
Tyrosine	5.7 ± 0.4b	6.1 ± 0.3a	5.1 ± 04c
Leucine	11.3 ± 0.4a	10.7 ± 0.2b	9.8 ± 0.3c
Cystine	2.5 ± 0.5b	2.3 ± 0.4b	3.1 ± 0.5a
Arginine	13.9 ± 0.3a	12.8 ± 0.5c	13.3 ± 0.4b
Aspartic acid	16.1 ± 0.3a	14.7 ± 0.2c	15.5 ± 0.3b
Alanine	6.9 ± 0.4a	6.3 ± 0.2b	7.2 ± 0.4a
Threonine	5.3 ± 0.5b	6.1 ± 0.3a	5.6 ± 0.3b
Isoleucine	7.2 ± 0.4a	6.3 ± 0.5b	6.5 ± 0.3b
Glutamic acid	73.2 ± 0.5a	70.9 ± 0.4c	71.3 ± 0.4b
Valine	4.9 ± 0.2a	4.5 ± 0.3b	4.1 ± 0.4c
Glycine	6.6 ± 0.3a	5.7 ± 0.3c	6.1 ± 0.5b
Methionine	3.1 ± 0.2c	4.0 ± 0.5a	3,7 ± 0.4b
Lysine	10.5 ± 0.3a	10.1 ± 0.4b	9.9 ± 0.5c
Total amino acids	175.2 ± 0.2a	168.3 ± 0.4b	168.4 ± 0.3b

Different a substantial difference between samples is indicated by letters in the same row (P<0.05).

Table 4. Tomato pomace content from fatty acids profile on dry weight (µg/g total fatty acids).

Fatty Acid	Extraction Method		
	BF	MA	OH
Myristic C 14:0	3.20 ± 0.42c	3.61 ± 0.35a	3.55 ± 0.52b
Pentadecanoic C 15:0	2.15 ± 0.45a	1.14 ± 0.26c	1.46 ± 0.71b
Pentadecenoic C 15:1	1.43 ± 0.42a	1.26 ± 0.62b	1.24 ± 0.54b
Palmitic C 16:0	135.21 ± 0.25a	134.24 ± 0.61b	133.74 ± 0.35c
Palmitoleic C 16:1	8.92 ± 0.35a	9.14 ± 0.32a	8.92 ± 0.34a
Heptadecanoic C 17:0	1.82 ± 0.26a	1.43 ± 0.32b	1.22 ± 0.34c
Heptadecenoic C 17:1	5.96 ± 0.44c	6.23 ± 0.35b	6.43 ± 0.27a
Stearic C 18:0	63.20 ± 0.52a	61.35 ± 0.42b	60.27 ± 0.65c
Oleic cis C 18:1	164.67 ± 0.41a	163.24 ± 0.51b	162.48 ± 0.35c
Linoleic cis C 18:2n-6	585.50 ± 0.63a	584.11 ± 0.72b	579.53 ± 0.29c
Linolenic γ C 18:3n-6	0.95 ± 0.36a	0.67 ± 0.25b	0.59 ± 0.37c
Linolenic α C 18:3n-3	30.26 ± 0.52a	29.45 ± 0.51b	28.27 ± 0.57c
Octadecatetraenoic C18:4n-3	4.90 ± 0.42a	4.54 ± 0.52b	4.47 ± 0.41c
Eicosadienoic C20(2n-6)	1.93 ± 0.52a	01.75 ± 0.42b	1.45 ± 0.52c
Eicosatrienoic C20(3n-6)	0.74 ± 0.42a	0.55 ± 0.41b	0.64 ± 0.44c
Docosadienoic C22(2n-6)	4.71 ± 0.26a	4.28 ± 0.41b	4.35 ± 0.65c
Docosatrienoic C22(3n-6)	5.10 ± 0.47b	5.39 ± 0.55a	4.85 ± 0.66c
Docosatrienoic C22(3n-3)	1.91 ± 0.64a	1.46 ± 0.45c	1.54 ± 0.52b
Eicosapentaenoic C20(5n-3)	3.46 ± 0.33a	3.09 ± 0.28b	2,87 ± 0.52c
Lignoceric C 24:0	2,96 ± 0.42a	2.66 ± 0.54b	2.33 ± 0,36c
Other fatty acids	2.36 ± 0.52a	1.94 ± 0.29b	2.17 ± 0.44c
Fatty Acids Profile			
Saturated fatty acids (SFA)	208.54 ± 0.36a	204.43 ± 0.66c	202.57 ± 0.45b
Monounsaturated fatty acids (MUFA)	180.98 ± 0.32a	179.87 ± 0.31b	179.07 ± 0.44c
Polyunsaturated fatty acids (PUFA), of which:	639.46 ± 0.51a	635.20 ± 0.63b	628.65 ± 0.51c
▪ n-3	40.53 ± 0.24a	38.54 ± 0.62c	37.15 ± 0.46b
▪ n-6	598.93 ± 0.44a	596.66 ± 0.37b	591.41 ± 0,26a
n-6/n-3	14.78 ± 0.27b	15.48 ± 0.42a	15.92 ± 0.44a

Different a substantial difference between samples is indicated by letters in the same row (P<0.05).

tomato pomace, where unsaturated fatty acids account for 820.44, 815,07 and 807.72 µg/g of the total fatty acids and saturated fatty acids for 208.54, 204.43 and 202.57 µg/g by BF, MA and OH extraction, respectively. The results of this study are slightly higher than the previous results obtained when using the OH method of extraction [9].

Elevated n-6/n-3 PUFA in human nutrition is recognized as a risk factor for cancer and coronary heart disease [10]. This ratio for tomato pomace was 14.78:1; 15.48:1 and 15.92:1 by BF, MA and OH extraction respectively, which is greater than the 10:1 ratio in the normal American diet but lower than the 15:1 ratio Simopoulos reported for the typical Western diet.

Conclusion

Three methods were used to extract bioactive compounds from tomato pomace, and it was found that the best of them was freezing and then mixing with a blender. It also found a good amount of lycopene, beta-carotene, total phenols, total flavonoids and an antioxidant activity of 89.1%. We estimated the phenolic compounds, and it was found that gallic acid and ellagic acid were the predominant compounds, and both myricetin and rutin were found to be highly free radical-seeking compounds. It was also found that tomato bagasse contains seven essential amino acids, and that the acidic acid was glutamic acid. Showed that linoleic cis and oleic cis were the dominant acids, as they were 585.50 and 164.67 µg/g total fatty acids, respectively. It also contained on unsaturated fatty acids 820.44 µg/g.

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Conflict of Interest

The author declared that he has no conflict of interest.

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