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Evaluation and Analysis of the Ultrasound-Assisted Extracted Tomato Seed Oil

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ABSTRACT: Approximately 10-30% of tomatoes are wasted during processing. Tomato pomace consists of crushed skin and seeds of tomatoes. Seeds might be considered as a potential source of vegetable oil. The extracted oil is high in unsaturated fatty acids, namely linoleic acid. The extracted oil after purification or refining might be employed for consumption. The object of this study was to evaluate the extraction yield of tomato seed oil by using ultrasound at different ratios of seed to solvent, temperatures and times variations and compare this method to the percolation procedure. Series of values and chemical components namely unsaponifiable matter, iodine value, saponification value and fatty acid composition, sterol and tocopherol profiles of the extracted oil were determined.

Keywords: *Fatty Acid, Sterols, Tocopherols, Tomato Seed Oil, Ultrasound-Assisted Extraction.*

Introduction

Tomatoes (Lycopersicon esculentum) are among the main vegetables worldwide with the production value of 126 million tons in 2005 (FAOSTAT, 2007). Tomato is an excellent source of many nutrients and secondary metabolites that are important for human health namely minerals, vitamins C and E, β-carotene, lycopene, flavonoids, organic acids, phenolic compounds and chlorophyll (Giovanelli & Paradise, 2002).

When tomatoes are processed into products such as catchup, salsa, and souce among others, 10-30% is wasted as pomace (King & Zeidler, 2004). Pomace consists of crushed skin and seeds being rich in protein (20-23%, dry basis), fat (12-18% contained mostly in seeds) and crude fiber (12-30%). To maximize the use of this product and increase profitability, there is a need for investigation (Schieber *et al*., 2001). Tomato seeds contain about 20% oil (Rabak, 1917) with a fatty acid composition similar to that of low linolenic soya bean oil (Firestone, 1999). Therefore, it might serve as an edible oil (Vigo *et al*., 1977). The meal resulted from the oil extraction has been paid much attention for recovery of protein isolates because dried meal contains 40-50% protein with high nutritional value. Carotenoids especially lycopene are valuable compounds that are substantially present in the skin (Schieber *et al*., 2001).

Seeds are complicated networks with characteristics including size and moisture contents that significantly affects the extraction process. Most lipids (75-85%) are easily extracted using a solvent however, better yield might be obtained by application of other means. In order to utilize the functional compounds of seed oil hard

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cellulose texture of hull must be disintegrated to extract the oil (Mason *et al*., 1996).

There have been significant advances in extraction techniques for shortening time of extraction and reducing the use of solvents as well as the increasing extraction yield and improving the quality of extracted substances especially chemically active and heat-sensitive compounds. New extraction methods consisted of ultrasound-assisted extraction, microwave-assisted extraction, extraction using supercritical fluid and extraction using accelerated fluid (Wang & Weller, 2006).

In recent years ultrasound (20- 100 KHz) has been extensively used in food industries for extraction of different nutraceuticals, homogenization, emulsification, filteration, crystallization, heat transfer acceleration, etc. (Mason & Riera, 2005). Ultrasound has been recognized for potential application in the extraction of herbals and oils (carnosic acid, ginseng saponins, carvone, limonene, antraquinones, amaranth oil, gingerols, soybeans oil, almond oil, apricot oil), proteins (soy protein), and bioactive compounds from plant (polyphenols, anthocyanins, tartaric acid, aroma compounds, polysaccharides and functional compounds) or animal (chitin, lutein) materials (Vilkhu *et al*., 2008).

The use of ultrasound is inexpensive and easier as compared to other methods such as microwave-assisted extraction (Chen *et al*., 2008). Systems operating with ultrasound are of bath and probe types being applicable at both experimental and industrial scales (Wu *et al*., 2001).

The enhancement of extraction efficiency of organic compounds by ultrasound is attributed to the phenomenon of cavitation produced in the solvent by the passage of an ultrasonic wave. Cavitation bubbles are produced and compressed during the application of ultrasound. The increase in the pressure and temperature caused by the compression leads to the collapse of the bubble. With the collapse of bubble, a resultant ‘‘shock wave’’ passes through the solvent enhancing the mixing (Paniwnyk *et al*., 2001). Ultrasound also exerts a mechanical effect, allowing greater penetration of solvent into the sample matrix, increasing the contact surface area between solid and liquid phase. This is coupled with the enhanced mass transfer and significant disruption of cells, via cavitation bubble collapse, increases the release of intracellular product into the bulk medium. The use of higher temperatures in UAE can increase the efficiency of the extraction process due to the increase in the number of cavitation bubbles formed (Palma & Barroso, 2002; Paniwnyk *et al*., 2001; Wu *et al*., 2001).

Ultrasound-assisted extraction is an inexpensive, simple and effective method of extraction. The main advantages are increased extraction yield and faster kinetics. Ultrasound might also be operated at lower temperature of operation helping to extract the heat-sensitive compounds (Wang & Weller, 2006).

Li *et al*. (2004) investigated the ultrasound-assisted extraction of oil from soybeans (Li & Pordesimo, 2004). Stanisavlyevic *et al*. (2007) studied the ultrasound-assisted extraction of oil from tobacco seed (Stanisavljevic & Lazic, 2007).

Cravatto *et al*. (2008) examined the extraction of vegetable oils using ultrasound and microwave (Cravotto *et al*., 2008). Esclapez *et al*. (2011) reviewed the ultrasound-assisted extraction of natural products (Esclapez *et al*., 2011). Li *et al*. (2012) evaluated ultrasound-assisted extraction and profile characteristics of seed oil from *Isatis indigotica* Fort (Li *et al*., 2012). Goula (2013) investigated the ultrasound-assisted extraction of pomegranate seed oil and its kinetics (Goula, 2013). In order to extract valuable oils such as tomato seed oil with high nutritional value, favorable conditions are provided therefore maximum extraction yield will be

achieved within the shortest time to avoid damaging the resulted oil.

The aim of this study was to determine the extraction yield of tomato seed oil using ultrasound-assisted extraction at different ratios of n-hexane, temperature and times and to compare the results with percolation method. The percentage of unsaponifiable matter, iodine value, saponification value, fatty acid, sterol and tocopherol profiles of the extracted oil were also determined.

Materials and Methods

All the chemicals were provided by Merck, Romile, Sigma and Sapleco chemical companies. Tomato seeds were obtained from the local suppliers in shahrood, Iran.

The oil was extracted by applying percolation and ultrasounic methods. For the percolation extraction, 5g of the sample was mixed with 25 and 50 ml of n-hexane separately in a 100 ml funnel and the oil was extracted at 25˚C for 20, 40, and 60 min. The samples were then filtered on a wattman No.1 filter paper for the separation of meal. The solvent was removed using a rotary evaporator and the extracted oil was quantified. For ultrasonic method, 5 g of the sample was mixed with 25 and 50 ml of n-hexane separately in 100 ml funnels. The samples were then heated at 25, 40 and 60˚C in an ultrasonic bath (techno-Gas, Italy) with the frequency of 28-34 KHz for 20, 40 and 60 min, respectively. The samples were then filtered through wattman No.1 filter paper for separation of meal and the solvent was removed using a rotary evaporator and then the extracted oil was quantified.

The quality of ultrasound-assisted extracted oil was determined by the fatty acid composition, iodine value, the amount of unsaponifiable matter and the composition of sterols and tocopherols fractions.

The extraction was carried out by soxhlet method according to ISO 659 (ISO 659:2009). The quantitative and qualitative identification of the fatty acids of the oil were carried out by methylation of the fatty acids according to ISO 5509: 2000 followed by the identification of methyl esters by a GC equipped with flame ionisation detector according to ISO 5508 (ISO 5508:1990).

The iodine value was calculated using the equation presented by AOSC Cd 1c – 85 directly from the fatty acid composition (Firestone, 1999a). The saponification value was determined according to ISO 3657 (ISO 3657: 2002).

The percentage of unsaponifiable matter was determined according to ISO 3596: 2000 by the saponification of the oil with alcoholic potassium hydroxide followed by the extraction of the unsaponifiable matter with ether. The unsaponifiable matter fractions were separated and identified by thin layer chromatography (TLC) according to AOAC 970.51 (Firestone, 1999a).

The identification and quantification of the sterols were carried out according to ISO 12228:1999 using a gas-chromatograph equipped with flame ionisation detector.

Tocopherols and tocotrienol were determined according to ISO 9936:2006 by the application of HPLC apparatus.

The effects of extraction method, processing duration and processing temperature on extraction yield were investigated using factorial test with completely randomized blocks in triplicate order. The means were compared using Duncans multiple range test at confidence level of $p<0.01$. SPSS 16 software was used to analyze data. The diagrams were drawn by using the Excel 2007 software.

Results and Discussion

- The effect of ultrasound-assisted extraction condition on the yield

As shown in tables 1 and 2, ultrasound-assisted extraction procedure yields different quantities of oil depending on extraction temperature, duration of extraction and the

ratio of seed to solvent. The results of variance analysis of the effect of ultrasound-assisted extraction on extraction yield showed that all three studied factors have significant ($p<0.01$) effect on the extraction yield.

- *The effect of extraction temperature*

The effect of extraction temperature was investigated when the other parameters including extraction time and the ratio of solvent to seed remained constant. Table 1 and 2 show that at 60˚C that is the boiling point of n-hexane the highest extraction yield was obtained. Increasing the extraction temperature significantly affected the extraction yield. This is consistent with the findings of Stanisavljevic & Lazic (2007) who reported that increasing the extraction temperature resulted in increased extraction yield of oil from tobacco. Increasing diffusion coefficient and solubility of oil in extracting solvent at higher temperature results in accelerated oil extraction.

- *The effect of the ratio of solvent to seed*

The effect of the ratio of the solvent to seed was examined when the other parameters including extraction time and temperature extraction remained constant. Tables 1 and 2 show that at 10:1 ratio of solvent to seed the highest extraction yield was obtained. This result is in consistence with the findings of Stanisavljevic & Lazic (2007) who reported that increasing the ratio of solvent to seed resulted in increased extraction yield.

The reason is that oil extraction yield increases as the ratio of seed to solvent decreases from 1:5 to 1:10 g/ml due to a higher driving force in more diluted solution.

Table 1. The effects of temperature and duration of ultrasound-assisted extraction on the ratio of seed to solvent 1: 5

Yield (%)			Time (min)
Temperature (˚C)			
60	**40**	**25**	
9.02 e	7.3 g	4.14 i	**20**
11.88 b	8.5 f	6.61 h	**40**
13.58 a	11 c	9.62 d	**60**

∗Dissimilar letters represent significant difference at $p<0.01$.

Table 2. The effects of temperature and duration of ultrasound-assisted extraction on the ratio of seed to solvent 1:10

Yield (%)			Time (min)
Temperature (˚C)			
60	**40**	**25**	
12.1 d	10.1 f	7.9 j	**20**
15.6 b	13.3 c	11 e	**40**
17.9 a	15.5 b	13.6 c	**60**

∗Dissimilar letters represent significant difference at $p<0.01$.

- *The effect of extraction time*

The effect of extraction time was investigated when the other parameters including extraction temperature and the ratio of solvent to seed remained constant.

Tables 1 and 2 show that the highest extraction yield was obtained when oil was extracted for 60 min. This result is in agreement with the findings of Li & Pordesimo (2004) who studied the oil extraction from other oilseeds such as soya bean and of Stanisavljvic & Lazic (2007) who reported that extraction yield of tobacco increased as the duration of sonication increased. The longer sonication duration is the longer cavitation time thus it causes the higher extraction yield.

- *The comparison of oil extraction using ultrasound and percolation methods*

As shown in tables 3 and 4, oil extraction yield is significantly higher using ultrasound as compared to percolation method. Oil extraction yield at 25˚ C for 20, 40 and 60 min at a 5:1 ratio of solvent to seed using percolation is lower than extraction yield using ultrasound under the same conditions.

Oil extraction yield at 25˚C for 20, 40 and 60 min at a 10:1 ratio of solvent to seed using percolation is lower than extraction yield by ultrasound under the same conditions. This might be explained by cavitation phenomenon. As stated earlier, ultrasound is defined as the application of waves with high frequency and their interaction with substances (Luque-Garc_ıa & Luque de Castro, 2003). Ultrasound emission and interaction change the physicochemical properties of the exposed matter (Mason & Lorimer, 1988). For raw plant tissue ultrasound disrupts the plant cell wall resulting in increased releasing extractable compounds and enhanced mass transfer of solvent from continuous phase to plant cells (Vinatoru, 2001).

It should be noted that oil extraction yield by applying soxhlet method is higher as compared to the ultrasound because of using high amount of solvent, longer duration and solvent recycling. The disadvantages of soxhlet method include long extraction time and requires considerable quantity of solvent that is quite costly, while ultrasound-assisted extraction requires short extraction time with low amount of solvent (Ormen˜o *et al*., 2011).

Table 3. The effect of extraction method on oil extraction at 25˚C within different durations at a 5:1 ratio of solvent to seed

Yield (%)		Time (min)
Ultrasound	**Percolation**	
4.14 e	2.96 f	**20**
6.61 b	4.44 d	**40**
9.62 a	6.52 c	**60**

∗Dissimilar letters represent significant difference at p<0.01.

Table 4. The effect of extraction method on oil extraction at 25˚C within different durations at a 10:1 ratio of solvent to seed

Yield (%)		Time (min)
Ultrasound	**Percolation**	
7.9 d	5.14 e	**20**
11b	8.78 c	**40**
13.6 a	10.86 b	**60**

∗Dissimilar letters represent significant difference at p<0.01.

- *Analysis of chemical composition of ultrasound-assisted extracted tomato seed oil*

Due to the fact that fatty acid composition of tomato seed oil is similar to low linoleic soya bean oil, therefore soya bean oil is taken as a reference in this project (Firestone, 1999b).

Table 5. The results of the tests carried out on tomato seed oil

Factor	Content
Moisture content (%) (seed)	3.90
Oil content using Soxlet method (%)	23.72
Saopnification value (mg KOH/g of oil)	193
Unsaponifiale matter (%)	1.01
Iodine value $(gI_2/100g)$	115
Total sterols (mg/kg)	2911
Total tocopherols (mg/kg)	1009

As shown in table 5, the oil content of tomato seed was 23.72% that is similar to soyabean that is 18-20% (Malek, 2000). The study conducted by Lazos *et al*. (1998) on tomato seed oil indicated that the oil content was 21.8%.

Saponification value that represents the average molecular weight of the fatty acids in the triglycerides molecule shows a value of 193 (mg KOH/g of oil) for tomato seed oil that is similar to the value of soyabean oil (Ghavami *et al*., 2008). Lazos *et al*. (1998) in their study on tomato seed oil reported the saponification value of 184. Demirbas (2010) reported the saponification values of the oils using supercritical fluid and soxhlet methods; 183.6 mg KOH/g of oil and 190.2 mg KOH/g of oil, respectively.

The percentage of the unsaponificable matter of tomato seed oil (1.01%) is similar to that of soya bean oil (1.5%) (Ghavami *et al*., 2008). Lazos *et al*. (1998) in their study on tomato seed oil reported that the unsaponificable matter of this oil was 1.4%.

All the oils and fats contain some chemical compounds in their unsaponifiable matters that consisted of sterols, 4-methysterol, triterpene alcohols, tocopherols, dimeric compounds and hydrocarbons.

The iodine value of tomato seed oil (115 $gI_2/100g$) is lower than that of soyabean oil (124-139 $gI_2/100g$), this might be due to higher linolenic acid content in soyabean oil (Ghavami et al., 2008). Lazos *et al*. (1998) reported that iodine value of tomato seed oil was 105($gI_2/100g$). The difference between our result and the value obtained by lazos *et al*., (1998) might be due to different factors namely variety. Demirbas (2010) determined the iodine values of the oils extracted using supercritical fluid and soxhlet using acetone as the solvent and obtained values of 109.7 ($gI_2/100g$) and 126.8 ($gI_2/100g$) respectively.

Total sterols content of tomato seed oil (2911 mg/kg) is lower than that of soyabean oil (1800-4500 mg/ kg) (Ghavami *et al*., 2008). Lazos *et al*., (1998) determined the total sterol content of tomato seed oil as 4550 mg/kg. The difference in the figure might be due to the type of the samples and extraction methods. Eller *et al*. (2010) reported that the total sterol content of tomato seed oil extracted using supercritical fluid, hexane and ethanol were 2100 mg/kg, 2200 mg/kg and 2400 mg/ kg respectively.

The tocopherols concentration in tomato seed oil (1009 mg/kg) is in agreement with the work carried out by Eller *et al*. (2010).

Figure 1 shows the fatty acid composition of tomato seed oil extracted by the application of ultrasound. The results indicate that the extracted oil contains high quality of linoleic acid with the value of 47.6%. Other unsaturated fatty acids such as oleic acid (27.76%), α-linolenic acid (1.81%) and others (1.21%) were present. High concentration of unsaturated fatty acids (~ 78.38%) makes this oil a valuable oil.

Lazos *et al*. (1998) determined the unsauration degree of tomato seed oil (78%). Demirbas (2010) determined the unsaturation degree of oil extracted by using supercritical fluid and soxhlet and found the

unsaturation in the order of 75.8% and 84% respectively.

Figure 2 indicates the sterol composition of ultrasound- assisted extracted tomato seed oil. The results indicate that β-sitosterol is the predominant sterol (1440.69mg/kg), followed by cholesterol (499.32 mg/kg) and stigmasterol (364.33 mg/kg).

Sterol profile of tomato seed oil is different from that of soya bean oil. Sterol composition of tomato seed oil consist of β-sitosterol (49.49%), cholesterol (17.15%), stigmasterol (12.51%), campesterol (11.24%), brassicasterol (2.9%), Δ-5-avenasterol (2.84%) and clerosterol (0.45%) while sterols composition of soya bean oil includes β-sitosterol (47-60%), campesterol (15.8-24.2%), stigmasterol (14.9-19.1%), Δ-5-avenasterol (1.5-3.7%), Δ -7- stigmasterol (1.4-5.2%), Δ-7-avenasterol (1.0-4.6%), cholesterol (0.2-1.4%) and other sterols (1.8%) (Ghavami *et al*., 2008).

The high content of cholesterol, relative to other vegetable oils that usually only contain trace amounts of this sterol (Gunstone *et al*., 1986), is the characteristic of phytosterols from the seeds from the Solanaceae family (Itoh *et al*., 1977a ; 1977b).

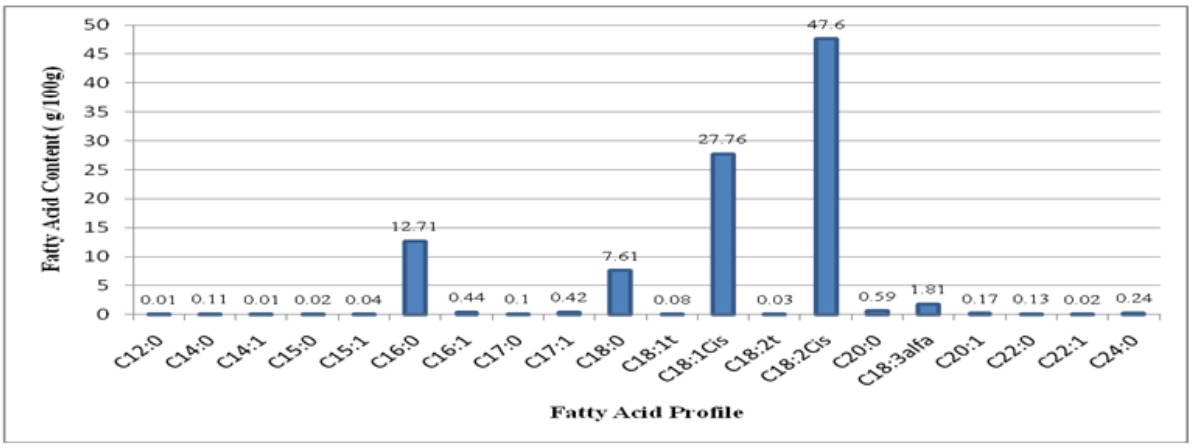

Fig. 1. Fatty acid composition of ultrasound-assisted extracted tomato seed oil

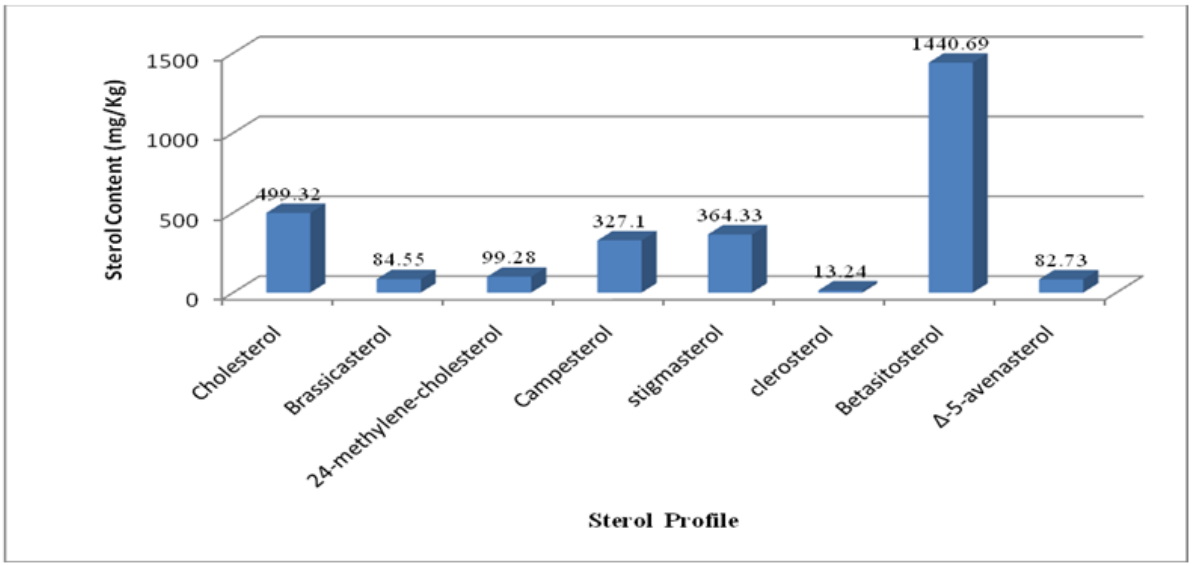

Fig. 2. Sterol profile of ultrasound-assisted extracted tomato seed oil

Lazos *et al*., (1998) showed that total sterols of tomato seed oil included β-sitosterol (52%), cholesterol (15%), stigmasterol (14.4%), campesterol (15.8-24.2%), Δ-5-avenasterol (6.7%), brassicasterol (1.5%), Δ-7-stigmasterol (0.4%), Δ-7- campesterol (0.3%), Δ-7-avenasterol (0.1%) and traces of clerosterol.

Tomato seed oil only included Gamma – tocopherol (1009 mg/kg). Lazos *et al*., (1998) determined the total tocopherols content as 1260 mg/kg. The difference is likely due to the type of sample and the method applied.

Eller *et al*. (2010) determined the total tocopherols of tomato seed oil using accelerated solvent extraction, ethanol; 940 mg/kg and hexane; 1080 mg/kg and supercritical fluid; 1110 mg/kg and found that Gamma – tocopherol was the dominant tocopherol. α – and Δ – tocopherols were absent or present in trace concentrations.

Conclusion

In this study the effects of ultrasound-assisted extraction parameters including temperature, time and the ratio of solvent to seed on the oil yield were investigated and oil extractions by the application of ultrasound and percolation methods were compared. The chemical composition of the ultrasound-assisted extracted oil was also analysed. The results showed that ultrasound application is an effective and fast technique to extract the oil from tomato seeds and the optimum conditions of the extraction were at 60˚C, for 60min with 10:1 solvent to seed ratio.

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