Comparison of different methods of extraction for pomegranate seeds

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Introduction

The pomegranate is often recognized as the earliest and sacred fruit that belongs to the Punicaceae family.

It is the source of many medicinal raw materials and functional foods, such as fresh and processed pomegranate juice and pomegranate cortex.

Pomegranate juice has antioxidant and anti-inflammatory effects due to the presence of anthocyanins, flavonoids and phenolic acids, which inhibit the activity of inflammation activators. Pomegranate cortex is a rich source of alkaloids and tannins, which have anti-parasitic effects.

The oil from pomegranate seed has also attracted considerable attention because of its potentially beneficial health effects: lipid fraction extracted from pomegranate seeds can improve immune function in vivo, reduce hepatic triacylglicerols accumulation and act as a chemopreventive agent against hormone-related human cancers.

The aim of the work

The aim of this work was to compare the properties of pomegranate oil seeds (from two different regions of Croatia) obtained by cold extraction, the Soxhlet extraction and Accelerated Solvent Extraction.

The conventional Soxhlet method and other shaking or stirring methods require long extraction periods, large sample sizes, and large amounts of toxic solvents that are expensive and can cause environmental problems. Accelerated (pressurized) solvent extraction (ASE) is the alternative method for the extraction of oil from seeds. This method which uses organic solvents at high temperatures and pressures above the boiling point for a short time can increase the solubility of the compound, solvent diffusion rate and mass transfer. Extraction with ASE is gaining more and more attention nowadays due to the lower amount of solvents and the lower process time required compared to other methods of extraction.

Materials and Methods

Pomegranate seeds were obtained from the Neretva river region of Croatia - south Dalmatia (pomegranate dark - PD) and Šibenik region of Croatia - north Dalmatia (pomegranate light - PL). The seeds have been ground to prepare the test sample.

Extraction of oil from seeds:

- Soxhlet extraction - was in accordance with reference method ISO 659.
- Cold extraction - was performed by hexane.
- Accelerated solvent extraction - the procedure was conducted on Dionex™ ASE™ 350 Accelerated Solvent Extractor (Thermo Fisher Scientific Inc., Sunnyvale, CA, USA) using n-hexane as the extraction solvent.

Methods

❖ Fatty acid composition

The determination of fatty acid composition was carried out by gas chromatographic analysis of fatty acid methyl esters (FAME). FAME were prepared according to the standard ISO method 5509 and injected into a gas chromatograph equipped with an FID detector according to ISO method 5508.

❖ Positional distribution of fatty acids in the sn-2 and sn-1,3 positions of TAG

Method of determination of positional distribution of fatty acids in the sn-2 and sn-1,3 positions of triacylglicerols was based on the ability of the pancreatic lipase to selectively hydrolyse ester bonds in the sn-1,3 positions.

❖ Determination of sterols

Sterols were determined with accordance with reference method ISO 12228 .

❖ PDSC measurements

The oxidative stability of oils was carried out by a differential scanning calorimeter (DSC Q20 TA) coupled with a high-pressure cell (PDSC).

Results

Table 1. Extraction yields (%, mean ± SD) and induction time (min, mean ± SD) of light and dark pomegranate seed oil (PL and PD) after using different methods of extraction (cold extraction-CE, Accelerated Solvent Extraction-ASE, Soxhlet extraction-SOX)

Type of sample	Extraction yield[1]	Induction time[1]
PD_CE	$16.95 \pm 0.59_{d}$	$4.55 \pm 0.15_{e}$
PD_ASE	$12.05 \pm 0.13_{b}$	$3.75 \pm 0.06_{d}$
PD_SOX	$16.31 \pm 0.67_{d}$	$0.71 \pm 0.03_{b}$
PL_CE	$13.34 \pm 0.30_{c}$	$3.81 \pm 0.25_{d}$
PL_ASE	$10.99 \pm 0.22_{a}$	$2.63 \pm 0.08_{c}$
PL_SOX	$14.02 \pm 0.17_{c}$	$0.32 \pm 0.11_{a}$

[1]The different lower case letters in the same column indicate significantly different values ($p < 0.05$).

Results

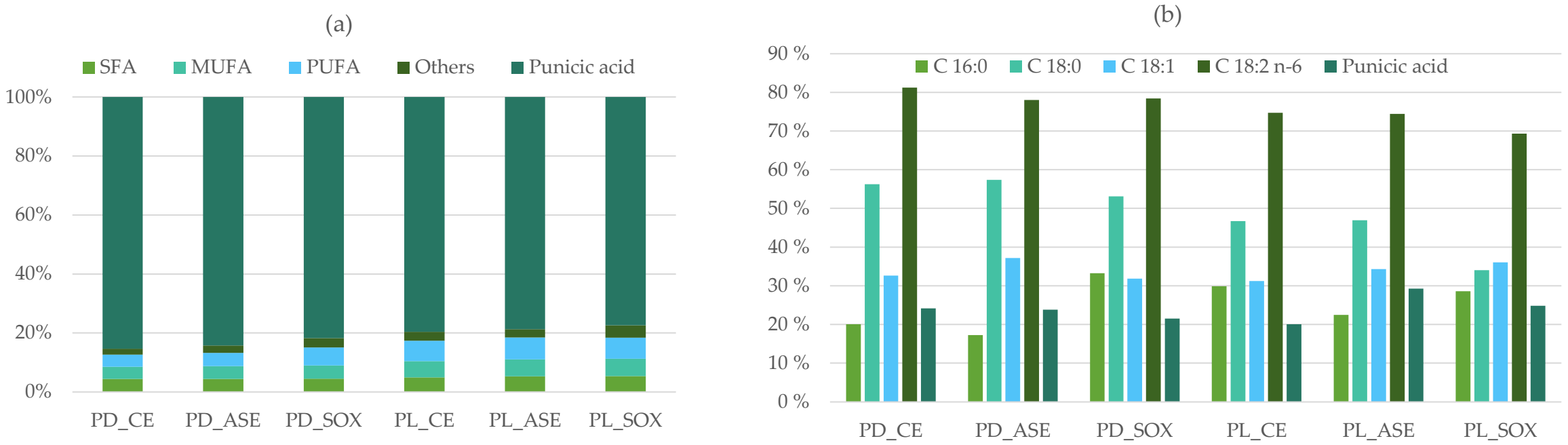


Figure 1. Fatty acid composition (a) and distribution in TAG (B) for dark and light pomegranate seed oil (PD and PL)) after using different methods of extraction (cold extraction-CE, Accelerated Solvent Extraction-ASE, Soxhlet extraction-SOX). PUFA – polyunsaturated fatty acids, MUFA – monounsaturated fatty acids, SFA – saturated fatty acids.

Results

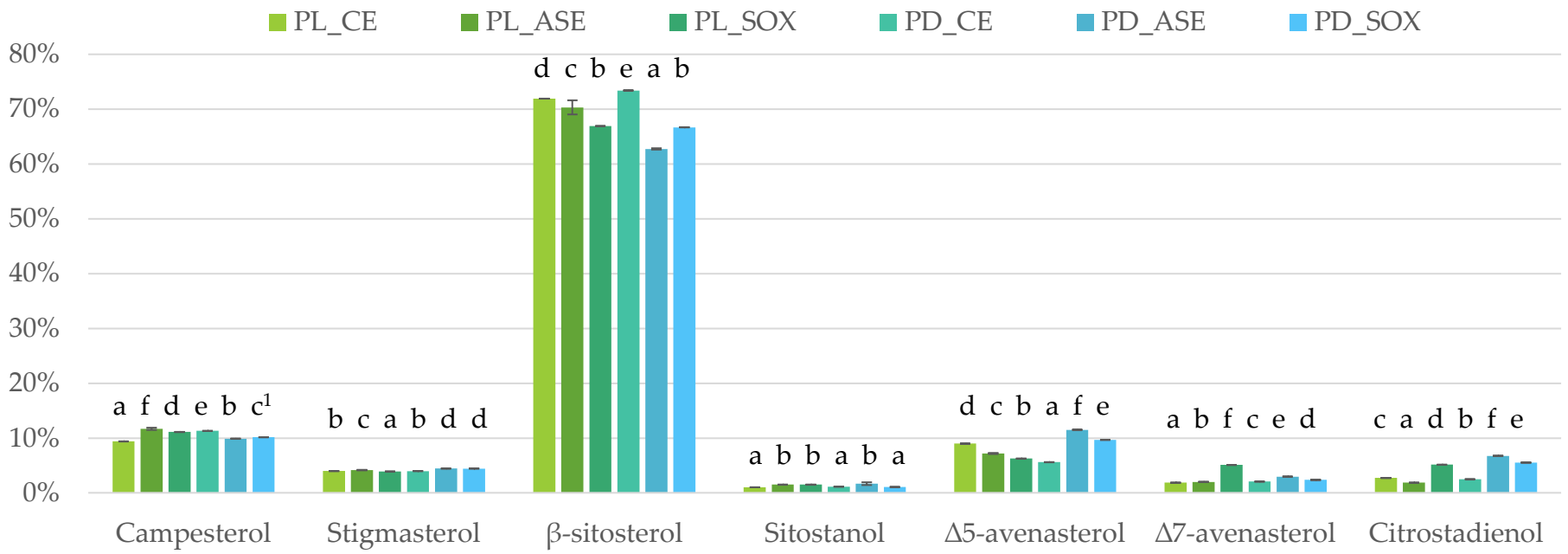


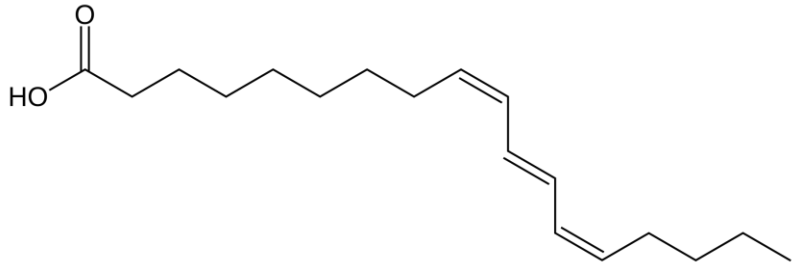
Figure 2. Individual phytosterols (%, mean ± SD) in light and dark pomegranate seed oil (PL and PD) after using different methods of extraction (cold extraction-CE, Accelerated Solvent Extraction-ASE, Soxhlet extraction-SOX).
[1]Different letters indicate that the samples are significantly different at $p<0.05$ for each type of phytosterols.

Conclusions

- The oil content in pomegranate seed oil (PSO), according to the literature data, is ranged from 12 to 20% . The obtained results were consistent with the literature data. The extraction method, among other, affects the content of the extracted oil.
- The results confirm that the fatty acid present in the largest amount in the pomegranate seed oil is geometric and positional isomers of unsaturated octadecatrienoic acid - punicic acid (cis-9, trans-11, cis-13 C18:3).
- In the external positions of triacylglycerols there is mainly punicic acid, while in the internal positions there are oleic and linoleic acids.
- The results of other researchers suggest that stigmasterol, Δ5-avenasterol, campesterol, and β-sitosterol, in order of increasing abundance, were the most common sterols in PSO. The results obtained in this study are consistent with the literature data.
- Due to the high content of unsaturated fatty acid, PSO exhibits desirable nutritional and medical properties, although it would be vulnerable to oxidation. The results obtained with PDSC confirm the low oxidative stability of PSO. Microencapsulation techniques are the methods that scientists believe can be used to increase the oxidative stability of the oil.

Conclusions

- The type of method used to extract the PSO has an effect on yield, sterol content and fatty acid composition. The ASE method produces an oil containing a lot of unsaturated fatty acids, but the amount of extracted oil is lower compared to other methods.



Thank you for your attention!