Fatty acid and sterol profile of nutmeg (*Myristica fragrans*) and star anise (*Illicium verum*) extracted by three different methods

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INTRODUCTION

- Nutmeg (*Myristica fragrans*) and star anise (*Illicium verum*) are world popular spices originally from South East part of Asia
- The Nutmeg tree is indigenous to Banda islands in the Moluccas in east Indonesia
- Star anise is an aromatic evergreen tree with red flowers and star fruits. It belongs to the family Schisandraceae and grows mainly in southern China and Vietnam.
- Apart as food condiment they have been used extensively in traditional medicine and lately as a subject of research in the field of pharmacology and medical sciences.
- The main problems in oil extraction of nutmeg is hardness of the nut while for star anise seed is relatively low oil yield (around 10 %)

Aims of the research and used methods

The aims of this present paper were

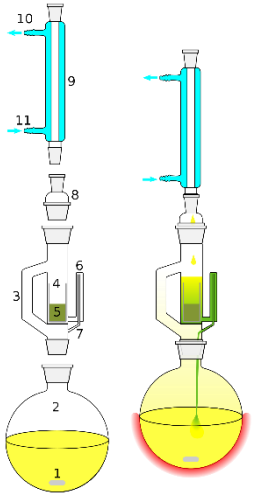
(1) to give more detail information about fatty acid and sterol composition of nutmeg and star anise seed oils

(2) to try to give alternative methods for oil extraction taking in account hardness of nutmeg and low oil yield in star anise and

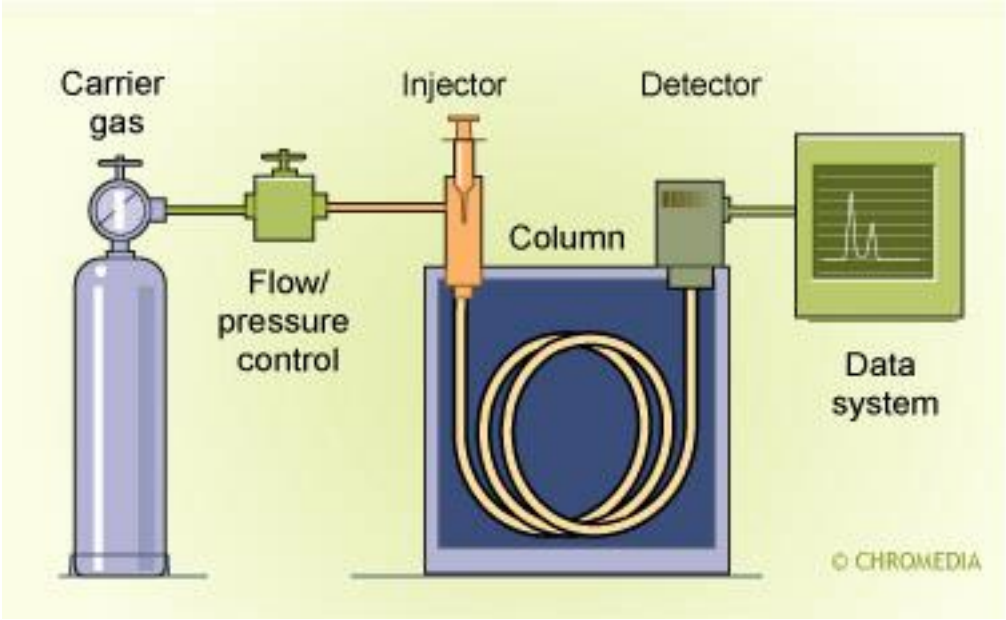
(3) to give some basis for further research of this seeds using ASE.

Oil extraction:

- Soxhlet extraction - SE
- Agitation Assisted Extraction - AAE
- Accelerated solvent extraction – ASE at 25 (ASE25) and 100 °C (ASE100)



Fatty Acid and Sterol Composition-GC/MS

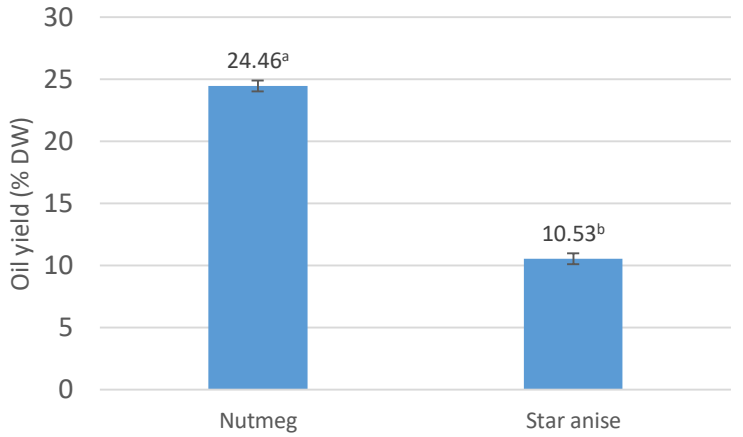


Accelerated Solvent Extraction at elevated pressure (ASE)

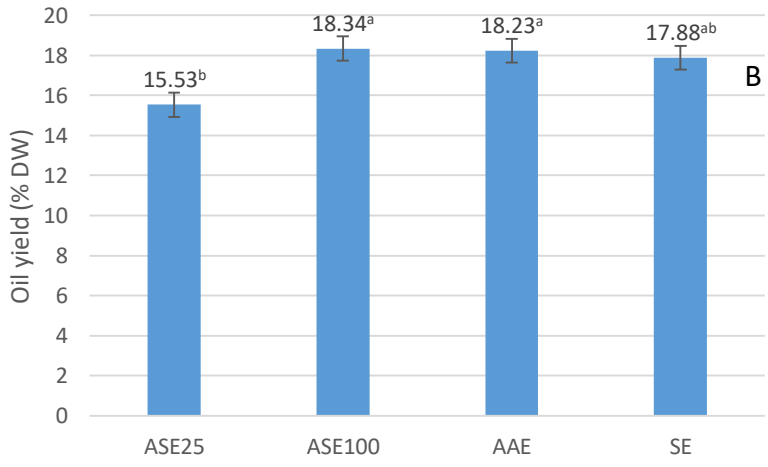
- ASE is a newer and more advanced extraction technique that has recently been increasingly used due to its many advantages.
- It is an automated method that represents an alternative to Soxhlet extraction with shorter extraction time and less solvent usage. This extraction method involves the use of elevated pressure which keeps the solvent in a liquid state during extraction at higher temperatures and thus prevents it from evaporating.
- The extraction is performed on a specially designed ASE device which contributes to the automation and ease of use of this extraction method.

Oil yield (%)

- Average yield of oil in **nutmeg** is 24,46% with highest value obtained by AAE at 26,90% and lowest with ASE25 at 22,10%.
- Average oil yield for **star anise** was 10,53% with lowest values at 9,55% with AAE and highest at 11,95 with ASE100.



The highest yield in oil extraction was obtained with ASE100 and lowest with ASE25 (Fig.1). It is evident that the influence of elevated pressure alone (ASE25) does not result in satisfactory utilization and that the combination of elevated temperatures and pressures is key to more efficient ASE. Following all the above, for future research on the extraction of oil from spice seeds with ASE, it is important to further optimize the extraction conditions using temperatures >100 °C, but also other solvents to increase efficiency or recovery.



Oil yields obtained from nutmeg and star anise (A) by different extraction methods (B) (p≤0,05). Results are shown as mean ± standard error; the values within the samples marked with different letters differ statistically at p≤0,05.

Fatty acids (%)

Table 1. Influence of seed type and extraction method on composition and content (%) of fatty acids in nutmeg and star anise seed oils

(%) fatty acids	C12:0ǂʃ	C14:0ǂʃ	C14:1ǂʃ	C16:0ǂʃ	C16:1ǂʃ	C17:0ǂʃ	C18:0ǂʃ	C18:1ǂʃ	C18:2ǂʃ	C18:3ǂʃ	C20:0ǂʃ
	Nutmeg										
AAE	nd	77,20^{a}±0,09	nd	7,5^{c}±0,05	0,11^{cde}±0,01	0,89^{a}±0,01	0,81^{f}±0,05	12,14^{c}±0,09	1,06^{f}±0,02	0,14^{ab}±0,02	0,15^{b}±0,01
SE	nd	75,31^{b}±0,15	nd	8,13^{b}±0,07	0,13^{a}±0,00	0,86^{a}±0,03	1,14^{d}±0,01	13,00^{b}±0,05	1,12^{ef}±0,05	0,13^{abc}±0,01	0,18^{ab}±0,02
ASE 25	nd	74,89^{b}±0,32	nd	7,89^{b}±0,24	0,12^{bcd}±0,00	0,73^{b}±0,00	0,97^{e}±0,02	13,74^{a}±0,49	1,31^{e}±0,09	0,19^{a}±0,01	0,17^{ab}±0,01
ASE 100	nd	75,36^{b}±0,02	nd	8,06^{b}±0,06	0,13^{bc}±0,00	0,87^{a}±0,03	1,10^{d}±0,01	13,11^{ab}±0,08	1,08^{f}±0,01	0,13^{abc}±0,00	0,17^{ab}±0,01
	Star anise										
AAE	60,86^{c}±0,05	0,06^{c}±0,00	0,91^{c}±0,00	8,89^{a}±0,02	0,10^{cde}±0,01	nd	1,69^{a}±0,00	13,00^{b}±0,02	14,30^{a}±0,02	nd	0,19^{a}±0,00
SE	59,97^{d}±0,12	0,06^{c}±0,00	1,23^{b}±0,01	8,74^{a}±0,02	1,33^{bc}±0,01	nd	1,64^{ab}±0,00	12,77^{bc}±0,05	14,06^{b}±0,05	nd	0,20^{a}±0,00
ASE 25	63,90^{a}±0,13	0,05^{c}±0,00	0,86^{d}±0,01	8,14^{b}±0,03	0,09^{c}±0,01	0,04^{c}±0,00	1,59^{bc}±0,01	12,41^{bc}±0,08	12,63^{c}±0,07	0,10^{c}±0,04	0,18^{ab}±0,00
ASE 100	64,48^{b}±0,14	0,05^{c}±0,00	1,26^{a}±0,00	7,96^{b}±0,04	0,15^{c}±0,01	0,04^{c}±0,00	1,55^{c}±0,01	12,09^{c}±0,03	12,35^{d}±0,05	0,07^{b}±0,00	nd

ǂ statistically significant influence of seed type at p≤0,05; ʃ statistically significant influence of extraction method at p≤0,05; nd-not detected; the values within the column marked with different letters differ statistically at p≤0,05.

- Dominant fatty acid in all **nutmeg oil samples was myristic C14:0** with average of 75,69%
- Dominant fatty acid in **star anise oil was lauric C12:0** with average of 62,30%.

Sterols (mg/100g)

Table 2. Influence of seed type and extraction method on composition and content (mg/100g) of sterols in nutmeg and star anise seed oils

(mg/100g)	Campesterol ǂ∫	Campestanol ǂ∫	Stigmasterol ǂ∫	β-sitosterol ǂ∫	Sitostanol ǂ∫	d5-avenasterol ǂ∫	d5,24-stigmastadienol ǂ∫	d7-stigmasterol ǂ∫	Citrostadienol ǂ∫	d7-avenasterol ǂ∫	Total sterols ǂ∫
	Nutmeg										
AAE	72,90^{c}±0,1	nd	13,0^{c}±0,1	198,8^{c}±0,1	nd	26,1^{c}±0,1	nd	nd	nd	nd	310,7^{e}±0,3
SE	168,0^{b}±2,6	nd	15,9^{b}±0,3	231,2^{b}±2,7	nd	32,8^{b}±0,3	nd	nd	8,6^{a}±0,1	nd	456,5^{b}±5,7
ASE 25	71,8^{c}±0,5	nd	16,0^{b}±0,1	213,5^{bc}±1,0	nd	36,9^{a}±0,5	nd	nd	nd	nd	338,2^{d}±2,0
ASE 100	213,3^{a}±2,8	nd	16,3^{b}±0,1	215,6^{bc}±1,3	0,3^{a}±0,0	35,8^{a}±0,0	nd	nd	10,0^{b}±0,0	nd	491,2^{a}±3,5
	Star anise										
AAE	54,4^{d}±0,4	4,0^{b}±0,2	17,0^{ab}±0,5	263,2^{a}±3,5	nd	2,1^{d}±0,4	6,2^{a}±0,2	9,3^{a}±0,8	nd	3,6^{a}±0,1	359,9^{cd}±4,7
SE	36,0^{e}±1,2	nd	18,4^{a}±0,1	287,7^{a}±3,9	nd	0,7^{e}±0,0	5,9^{a}±0,6	1,2^{b}±0,3	nd	0,8^{b}±0,0	350,7^{cd}±6,0
ASE 25	39,7^{e}±0,1	8,0^{a}±0,0	10,7^{c}±0,0	191,4^{c}±0,8	nd	nd	4,5^{b}±0,0	nd	nd	nd	254,3^{f}±0,9
ASE 100	58,3^{d}±1,4	4,2^{b}±0,6	16,0^{d}±1,5	285,4^{a}±16,7	nd	0,3^{e}±0,5	5,4^{ab}±0,1	nd	nd	nd	369,6^{c}±16,3

ǂ statistically significant influence of seed type at p≤0,05; ∫ statistically significant influence of extraction method at p≤0,05; nd-not detected; the values within the column marked with different letters differ statistically at p≤0,05.

- Dominant sterol in all samples was **β-sitosterol** (average nutmeg 214,8 and star anise 256,9 mg/100g)
- Other significant sterols in **nutmeg oil** were **campesterol and Δ5-avenasterol** while in **star anise oil** were **campesterol and stigmasterol.**

Conclusion

With most of the research and industry focused on essential oils from nutmeg and star anise there is a possibility of utilizing the by-products as a source of myristic and lauric fatty acids. The application of ASE in extraction shows promising results with reduction of time and solvent usage. Apart from higher oil yield it enhances sterol extraction of around 50%. Its influence on polyunsaturated fatty acids in star anise oil demands further optimization of the process.