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Original Research Paper

Fatty acid profiling of bottle gourd [*Lagenaria siceraria* (Mol.) Standl.] seed oil using gas chromatography-mass spectrometry

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ABSTRACT

The present investigation was carried out to study the oil quality and estimate fatty acid (omega-3 and omega-6) composition in bottle gourd using gas chromatography-mass spectrometry. Results indicated that the extracted bottle gourd seed oil was estimated to have an oil content (25.2%), polyunsaturated fatty acids *viz*., omega-3 (6.63%), omega-6 (69.74%) and others (0.17%), which play a major role in, cardiovascular health and has anti-inflammatory, anti-allergic and anti-cancer benefits. It also consisted of saturated fatty acids *viz*., palmitic acid (9.78%), stearic acid (4.45%) and others (0.97%) along with monounsaturated fatty acids (MUFA) (5.99%) without oleic acid (omega-9). Due to increased demand for specialty edible oil in both domestic and industrial use, it becomes necessary to exploit the potential of bottle gourd seeds as a novel source of edible oil. Being a rich source of omega-6 and omega-3 fatty acids, which lacks omega-9 known for oxidative stability required for storage and cooking, it can be commercialized as cooking oil by blending it with edible oil rich in oleic acid or can also be supplied as an omega-6-rich capsule as a food supplement in future.

Keywords: Bottle gourd, oleic acid, omega-3, omega-6, polyunsaturated fatty acids and seed oil

INTRODUCTION

Bottle gourd [*Lagenaria siceraria* (Mol.) Standl.] belongs to the family Cucurbitaceae, with chromosome number 2n = 22. It is also known as *Calabash*, *Doodhi*, and *Lauki* in different parts of India (Deore, 2009). Bottle gourd is one of the most common cucurbits grown in India and is said to be one of the first species domesticated by humans. It grows throughout the *kharif* season. India, Sri Lanka, South Africa, Indonesia and Malaysia are the major bottle gourd-producing countries in the world. India produced 3,742.71 mt of bottle gourd over an area of 1.58 mha. In India, it is mainly grown in Bihar, Uttar Pradesh, Haryana, Madhya Pradesh, Chattisgarh, Orissa, Punjab, Assam, Andhra Pradesh and Karnataka. Bihar is the largest producer of bottle gourd with a production of 655.55 tonnes during 2021-22 (Anonymous 2022).

Bottle gourd is a rich source of minerals, vitamins and essential fatty acids and is an abundant source of medicinal compounds since ancient times. Tender fruits are used as cooked vegetables and also to make desserts. Beyond the fruit, the seeds are also gaining attention for their nutritional benefits and have been recognized as a good source of edible oil due to their favourable oil characteristics. The sources of oils are mostly of plant/vegetable origin i.e. seed oils. Vegetable oils are essential components of diets and are used in many manufactured foods such as ice cream, cookies, and margarine apart from their usage as cooking oil. Despite the vast range of sources for vegetable oils, world consumption is dominated by soybean, palm, rapeseeds and sunflower oils (Stevenson et al., 2007). Due to the increasing demand for oil, both as domestic and industrial functional ingredients, it is necessary to exploit the potential of some lesser-known underutilized sources like cucurbit oil seeds. Bottle gourd seeds have high levels of oil, similar to sunflower seeds (Axtell, 1992). According to reports, bottle gourd seed oil is high in sterolic compounds (Axtell, 1992), with many health benefits (Milind, 2011), which can be due to the high content of polyunsaturated fatty acids (PUFAs) including linoleic and linolenic acids, which lower the risk of cardiovascular and atherosclerosis diseases by their resistance to oxidation (Abramovic, 2007). El-dengawy et al. (2001), showed that bottle gourd seeds contain nearly 39.22% fatty acid. Which are primarily linoleic acid (63.32%), oleic acid and palmitic acid (21.36%). The bottle gourd seed oil has medicinal use that helps in reducing prostate cancer and also for treating BPH, acne, hyper-seborrhea, alopecia and hirsutism. It is also considered anthelmintic and is applied externally for headaches (Piccirilli et al., 2007). Bottle gourd seed oils of the oleaginous gourds are a good source of polyunsaturated fatty acids (PUFAs) - linoleic acid, an essential fatty acid for the human diet and could also be used by the food industry for formulating functional foods enriched with PUFAs (Essien, 2015). The active ingredients in bottle gourd seed oil haven't been extensively investigated. In this regard, the present study aimed to determine the fatty acid profile, sterol composition, spectroscopic and physicochemical characterization as well as the GC-MS analysis of seed from *Lagenaria siceraria*.

MATERIALS AND METHOD

Bottle gourd fruits were collected during *kharif*, 2022 from Division of Vegetable Crops, ICAR-Indian Institute of Horticultural Research, Bangalore. After the collection of bottle gourd fruits, the seeds were separated manually, washed and dried in the shade.

Determination of oil content

The oil content from the matured seeds was extracted in the Soxhlet apparatus according to the AOCS method (AOCS, 1993). The dried seeds were then thoroughly ground and mixed with ether to extract the total oil and the oil content was calculated from the weight of oil and seeds using the formula (Rai et al., 2018).

Extraction of seed oil

Bottle gourd seeds (50 g) were weighed and transferred to a 30 mm × 200 mm cellulose thimble. It was placed in the extraction chamber of a 250 mL Soxhlet apparatus fitted with a condenser, which was placed on a 500-mL distillation flask containing 250 mL of solvents n-hexane. Bottle gourd seeds oil was then extracted under reflux with n-hexane for 1, 2, 4 and 6 h (10– 12 cycles/h). After that, hexane was then removed by using a heated rotary evaporator (Stuart, England), under vacuum conditions. All extraction processes were performed in replications. The yield of oil extracts was expressed as a percentage of the weight of extracts obtained from extraction relative to the weight of date seeds used for extraction. The yield of oil extraction is calculated using the below-mentioned formula

$$\text{Yield of oil extraction} = \frac{\text{weight of oil extracted}}{\text{weight of bottle gourd seeds used}} \text{ X } 100$$

Profiling of fatty acids

Lipid fractionation by column chromatography: Homogenize the known weight of the sample in 10 mL of 3:1 chloroform/methanol solvent. The homogenate is filtered through filter paper and repeat the extraction 3 times with the same solvent. Once the extraction is complete take the entire filtrate material and separate the water phase and solvent phase with a separating funnel. Add sodium sulphate to the solvent phase once separated and make up the volume to 15 mL. Dry the whole sample in a round bottom flask.

Esterification: To the dry round bottom flask add 1 mL of BF3/methanol (commercially available) with a pipette through the condenser and boil for 10 min at 45°C -50°C. Again, add 3 mL of heptane through the condenser and boiling further continues for 10 min. Firmly add 2 mL of heptane and keep it for cooling. Once the condenser comes to room temperature, remove the conical flask from the condenser and add 2 mL of saturated NaCl solution to the conical flask, shake it nicely and pour the entire content into the test tubes. Again, transfer the upper heptane layer to another test tube containing sodium sulphate, then filter the heptane layer through nylon filter paper with the help of a syringe and inject it to the GC-MS.

Gas chromatography-mass spectrometry (GC-MS): GC-MS analysis was performed on Varian-3800 gas chromatograph coupled with Varian 4000 GC-MS-MS ion trap mass selective detector. Fatty acids are separated on VF-SMS fused silica capillary column (Varian, USA) (30 mx 0.25 mm id with 0.25 um film thickness) by applying the same temperature program as described above for GC-FID analysis. The carrier gas is helium at a flow rate of 1 mL/min; injector temperature, 260°C: ion source temperature, 220°C; trap temperature, 200°C and transfer line temperature, 260°C. Mass detector conditions are El-mode at 70 eV with full scan range 50-450 amu. Fatty acids are identified by comparing the relative retention times of Fatty acid methyl esters (FAME) peaks with those of reference standards (Sigma-Aldrich, USA) and also by comparing the spectra with those available in Wiley and NIST-2007 spectral libraries (Liu, 2016). The total quantity of FAME is estimated as the sum of all GC-FID peak areas in the chromatogram and individual compounds are quantified by comparing the known individual FAME procured as standard.

RESULTS AND DISCUSSION

Total seed oil content

The total seed oil content in bottle gourd seeds was found to be 25.2% which was closely similar (28%) in the study conducted by Warra et al*,* (2016). Other studies showed that the total oil content in the bottle gourd seeds is almost in the similar range of popularly used edible oils (Table 1). This leads to fatty acid profiling of bottle gourd.

Table 1 : Seed oil content across the popular edible oil crops

Crop	Seed oil content (%)	Reference
Bottle gourd	32.24	Akuma Oji et al. (2020)
Coconut	65	Appaiah et al. (2014)
Mustard	27	Gyanendra Kumar Rai et al. (2018)
Palm	12	Hamza Ourradi et al. (2021)
Safflower	27	Gunstone & Harwood, 2007 and Pelin Günç Ergonul & Zeynep Aksoylu Ozbek et al. (2020)
Sunflower	32	Murat Reis Akkaya (2018)
Soya bean	22	Gunstone & Harwood (2007)
Olive	20	Garcia-Inza et al. (2016)
Groundnut	50	Gunstone & Harwood (2007)

It has been reported that an ideal edible oil should comprise of low saturated fats (<6%), high oleic acid (>50%), moderate amounts of linoleic (<40%) and low linolenic acid (<14%) (Potts et al., 1999). The results of the fatty acid composition of the bottle gourd (*Lagenaria siceraria)* seed oils revealed that the predominance fatty acid group in bottle gourd seed oils is polyunsaturated fatty acid (76.54%) followed by saturated fatty acid and mono-unsaturated fatty acid (Table 2). The percentage of unsaturated fatty acid in the seed oils consisted of monounsaturated fatty acid (MUFA) about 5.99% where oleic acid was found nil and polyunsaturated fatty acid (PUFA) was 76.54% consisting mainly of linoleic acid (ω"6) (69.74%), α-linolenic acid (ω"3) (6.63%) and others (0.17%). The saturated fatty acid (15.20%) in the seed oils includes the most commonly found saturated fatty acids viz., palmitic acid (9.78%), stearic acid (4.45%) and others (0.97%). These results are in agreement with Chinyere et al., (2009), Ogunbusola & Magu et al. (2017), who found the predominant fatty acid group in *Lagenaria siceraria* seed oils to be unsaturated fatty acid. The percentage of unsaturated fatty acid in the seed oils of *Lagenaria siceraria* variety 1 and variety 2 ranged from 78.34 to 81.31 % respectively, consisting mainly of linoleic (60.15 to 60.40%), linolenic (0.12 to 0.10), oleic (13.36 to 20.62%) and others 0.11%. In variety 1 and variety 2, saturated fatty acid in the seed oils consists of palmitic acid (12.11 to 10.84%), stearic acid (6.71 to 8.49%), total saturated fatty acid ranging from 18.64 to 21.56%, respectively.

Table 2 : Fatty acid composition of bottle gourd (*Lagenaria siceraria*) seed oil

Fatty acids	Per cent
Saturated fatty acid	15.20
Palmitic acid	9.78
Stearic acid	4.45
Others	0.97
Mono-unsaturated fatty acid	5.99
Oleic acid (ω–9)	-
Others	5.99
Polyunsaturated fatty acid	76.54
Linoleic acid (ω–6)	69.74
α-Linolenic acid (ω–3)	6.63
Others	0.17

Dorni et al. (2018) in coconut, groundnut and mustard oil reported oleic acid content to be 7.34, 53 and 10.16, linoleic acid content 1.90, 26.96 and 15.58, respectively. Mass spectral analysis showed the existence of saturated fatty acids such as the total saturated fatty acid (TSFA) in bottle gourd was 15.20% which was closely related to soybean (15.90%), sunflower (11.39%) and groundnut (19.27%). The total monounsaturated fatty acid (TMUFA) in bottle gourd was found to be 5.99%. The total polyunsaturated fatty acid (TPUFA) content in bottle gourd was found to be 76.54% (Aremu et al., 2006), which was similar to safflower (76.78%) but comparatively found lower in coconut (1.90%), groundnut (26.96%), mustard oil (27.28%), palm oil

(11.54%), soybean (59.33%) and sunflower (62.69%) as reported (Dorni et al. 2018).

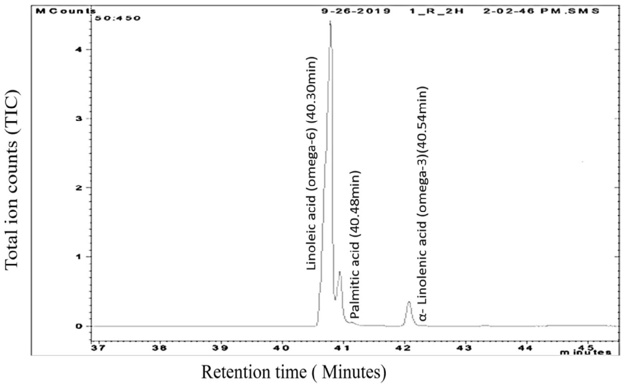

Fig 1 : Fatty acid profile – GCMS chromatogram

The chromatographic profile of these fatty acids showed that bottle gourd seed oil is rich in PUFA with higher proportions of linoleic acid (ω"6) and α-linoleneic acid (ω"3) (Fig 1.). Chromatographic profiles reveals that higher peak for linoleic acid (ω"6) at 40 minutes 30 seconds, another second peak for palmitic acid (saturated fatty acids) appears at 40 minutes and 48 seconds and last significant peak for α-linoleneic acid (ω"3) comes out at 40 minutes 54 seconds.

The present results supported by Rahaman et al. (2022) and Cui et al. (2017) described all interested peaks that represented fatty acids between C16 to C18. The enlarged section of the chromatogram between 37 and 45 min contained the peaks of the major UFAs in the oil samples, including C16:0, C18:2 and C18:3 in 10 different oils.

Warra et al. (2016) revealed that following fatty acids; palmitic acid, stearic acid, linoleic acid, behenic acid which were potential in cosmetic, perfumery and pharmaceutical industries. Omega-6 fatty acid polyunsaturated fatty acids also referred to as ω-6 fatty acids or n-6 fatty acids, they are a family of pro-inflammatory and anti-inflammatory. The results showed the potential of the hexane extract of the seed oil in cosmetic, perfumery and pharmaceutical industries as well.

Components of bottle gourd seed oil which has scope in pharmaceutical industries as it is rich in omega 3 and omega 6 which has abundant health benefits such as healthy heart, stronger bones, improved vision, better cognition, anticancerous and improved blood circulation.

CONCLUSION

This study revealed that *Lagenaria siceraria* seeds possess oils that are exceptionally rich in essential fatty acids and offer numerous health benefits. These oils have the potential to be utilized in both domestic and industrial applications. Furthermore, the GC-MS analysis indicated that seeds of *Lagenaria siceraria* produce a substantial amount of oil that can be processed for commercial use.

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