Research Article

doi:10.25081/jpc.2023.v51.i3.8339
Journal of Plantation Crops, 2023, 51(3): 128-132

Phytosterol Composition in the Oil of Bi-clonal Seed Stocks of Tea (*Camellia sinensis L.*) in North East India

Pradeep Kumar Patel*, Rupak Sarma[1], Boby Gogoi[1], Shobhit Kumar Singh[1], Namita Handique[1] and Azariah Babu[1]

[1]*Department of Plant Physiology and Breeding, Tocklai Tea Research Institute, Tea Research Association, Jorhat-785008, Assam, India*

(Manuscript Received: 01-09-23, Revised:01-12-23, Accepted:13-02-23)

Abstract

The phytosterols are naturally occurring, physiologically active substances found in foods of plant origin. In present study, the phytosterols profile of four bi-clonal tea seed stock oils viz., TS 462, TS 464, TS 491, and TS 506 released from Tocklai are presented. Phytosterols were evaluated using GC-FID. Among the phytosterols, 24-methylene cycloartan-3-ol was recorded as the most abundant sterol in tea seed oil, accounting for 31% of total phytosterols. Results showed that the majority of the sterol differences were significant (p 0.05). TS 506 has the highest 24- methylene cycloartan-3-ol (476.03.7 mg kg^{-1}) followed by TS 491 (475.97 mg kg^{-1}). β-sitosterol, cycloartenol, canophyllol, lanosterol and campesterol were also present with their contents of 371.03 ± 0.26, 291.38 ± 0.12, 120.44 ± 0.13, 89.12 ± 0.19 and 69.05 ± 0.24 mg kg^{-1} respectively. The outcomes of the present study provided a foundational understanding of the phytosterols profiles of the four bi-clonal tea seed oils and highlighted their similarities and differences.

Keywords : *Bi-clonal, β-sitosterol, Phytosterols, Tea Seed, Oils*

Introduction

More than 250 different sterols and related compounds collectively referred to as phytosterols or plant sterols, have been documented in a variety of plants of land and marine origin (Salehi *et al*., 2021). Vegetable oils, nuts, seeds, and cereals are all prevalent sources of phytosterols, which are naturally found in plant cell membranes which consist of stigmasterol, campesterol, and -sitosterol (Witkowska *et al*., 2021). Phytosterols have a wide range of biological effects, including antioxidant (Van Rensburg *et al*., 2000), antidiabetic (Zaklos-Szyda, 2015), antiatherosclerotic (Moghadasian, 2000), antibacterial, and antifungal effects, anti-inflammatory (Lopez-Garcia *et al*., 2019a), antieryptotic, antihemolytic effects (Alvarez-Sala *et al*., 2018) and anticancer properties Lopez-Garcia *et al.,* 2019b). Research has demonstrated that phytosterols can lower blood total cholesterol by preventing the absorption of low-density lipoprotein cholesterol (Plat *et al*., 2019).

Bi-clonal tea seeds are widely used as planting material for the commercial production of tea (Sarmah *et al*., 2018). At the moment, there is a lot of interest in product diversification and value addition. However, there is currently no systemic information available on the phytosterols profile of the bi-clonal tea (*Camellia sinensis* L.) seed oils that the Tocklai Tea Research Association (TRA) has released. This is the first comprehensive analysis of the phytosterols from the bi-clonal seed stock oil of North-East India origin. The purpose of this study

**Corresponding author: pradeepk.bhu@gmail.com*

was to identify the types of phytosterols and their concentration in the oils derived from bi-clonal seed stocks that were released by the TRA in North-East India. This research is the first to provide insights into expanding the uses of tea seed.

Materials and methods

Experimental

Four bi-clonal seed stocks of *Camellia sinensis* L. seeds were collected from Tocklai Tea Research Institute experimental site (Figure 1), New Botanical Area (26.75 ◦N latitude and 94.22 ◦E longitude) TRA, Jorhat district, South Bank, Assam. The seed stocks released by TRA to the tea industry are presented in Table 1 and Figure 1.

Fig. 1. Tea Bi-clonal seed stocks

Table 1. TRA released bi-clonal hybrid tea seed stocks.

Stock no.	Year of release	Parents / generative clones
TS 462	1980	(TV1 x 124/48/8)
TS 464	1984	(TV1 x 19/29/2)
TS 491	1989	(TV1 x S.3A/1)
TS 506	1994	(TV1 x 19/22/4)

Tea seed oil extraction

The oil was extracted from freshly harvested dried cotyledons of matured tea seed of four different bi-clonal tea seed stocks that were kept for commercial multiplication following the method described by Lee and Yen (2006). Briefly, tea seeds were first roasted at 120 °C for 20 minutes to obtain tea seed oil, and then they were pressed using a twin screw extruder. Tea seed oil (100 g) was extracted with 200 mL of each of the following: acetone, methanol, ethanol, ethyl acetic acid, and acetonitrile for an hour at room temperature in a shaking incubator. The extracts were filtered, and the leftover material was used for extraction three more times following the same protocol. The combined filtrates from each solvent were evaporated in a rotary evaporator under vacuum at a temperature of 40 °C, and then the mixture was weighed to determine the extraction yield before being kept at -20 °C until use.

Gas chromatographic analysis

Sterol determination in seed oil was determined by the method of Association of Official Analytical Chemists (AOAC) 994.10 (1996). Quantification of phytosterols was done using the gas chromatographic (GC) system with capillary column (30 m x 0.250 mm x 0.15 mm, Agilent Technologies Inc., 5301 Stevens Creek Blvd Santa Clara, CA95051 United States). Analyte mixture of one microliter was injected into GC system with a split/splitless injector and flame ionization detector (FID, Agilent Technologies Inc. 5301 Stevens Creek Blvd Santa Clara, CA95051 United States). The split ratio was 10:1, and the inlet temperature was 250 ^{0}C. Helium was the carrier gas, flowing at a constant rate of 2.5 mL min^{-1}. Initially, the oven was set to start at 250^{0}C for 5 minutes, and then increased by 5^{0}C min^{-1} up to 260 ^{0}C for 8 minutes. The detector was adjusted to 300 ^{0}C with 200 mL min^{-1} of airflow, 80 mL min^{-1} of hydrogen flow, and 40 mL min^{-1} of helium makeup flow. The entire GC analysis took 15 minutes. SPE Fractionation of Sterols was carried out by Azadmard-Damirchi and Dutta (2006).

Phytosterol estimation

A standard calibration curve was created using the internal standard peak area to analyte peak area ratio at each concentration level. Each analyte was given its own calibration curve for calculation

purposes. High-level test solutions were diluted to fall within the permitted range. Measured as mg Phytosterols/100 g test portion, the amount of each phytosterols component in test portions was determined.

Methanol, ethanol, and other chemicals used were of HPLC grade and were purchased from Sisco Research Laboratories Pvt. Ltd. in Mumbai, India. All the standards for phytosterols profiling (standard mixture) were procured from Sigma-Aldrich (USA).

Statistical Analysis

Values were calculated in triplicate for each analysis, and the mean ± SEm was used to express all results. With the use of the Statistical Package of WASP 2.0 (ICAR), a one-way analysis of variance and Duncan's test were used to determine whether a difference was statistically significant at a p-value of 0.05.

Results and Discussion

In the current investigation, thirteen phytosterols in four bi-clonal tea seed stock oils were quantified (Table 1). According to Patel *et al.* (2020), the oil content of bi-clonal seed stocks ranged from 20.84 to 21.90%. Four bi-clonal tea seed stock oils varied in their phytosterols level and composition (Table 2). Thirteen compounds were identified in all of the examined bi-clonal seed stock oils, including b-amyrin, lupeol, campesterol, spinasterol, β-sitosterol, canophyllol, cycloartenol, stigmast-7-en-3-ol, betulin, lanosterol 24-methylene cycloartan-3-ol, lupanol and obtusifoliol. Three of the main ones were cycloartenol, -sitosterol, and 24-methylene cycloartan-3-ol. Further it was observed that the seed stock TS 506 oils had the highest concentrations (476.03 mg kg^{-1}) of 24- methylene cycloartan-3-ol, while the seed stock TS 462 oils presented the lowest concentration (474.84 mg kg^{-1}). 24-methylene cycloartan-3-ol was the dominant phytosterols in tea seed oils, representing more than 30% of all available phytosterols. Total sterol content ranged from 1544.98 mg kg^{-1} to 1559.12 mg kg^{-1} in tea bi-clonal seed stocks. Comparatively, total sterol content ranges from 520 to 1780 mg kg^{-1} for cereals (Zhang *et al*., 2017), In terms of fresh weight, phytosterols from vegetables varied from 50 to 370 mg kg-1, fruits have 60 to 750 mg kg-1, and berries exhibit 370 mg kg-1 (Bai *et al*., 2021). The total sterol content of vegetable oils, particularly soybean oil, varied from 2.29 to 4.49 g kg-1, while olive oil ranged from 1.19 to 1.88 g kg-1 (Yang *et al*., 2019) (Demirag and Konuskan, 2021). High moisture content is the primary cause of the low sterol content in fruits, berries, and vegetables; as a result, these foods are not usually regarded as being as good a source of sterols as cereals. Almonds, cashew nuts, and peanuts show 1.43, 1.58, and 2.20 g kg^{-1}, respectively, according to the USDA Nutrient Database (USDA, 1999). Hence, nuts are considered as a good source of sterols. The oil from TS 462 of the *Camellia sinensis* bi-clonal seed stock oil had the highest lanosterol contents (90.44 mg kg-1), and these compounds are effective in preventing cardiovascular disease (Lanzani *et al*., 2016) and delaying senescence (Lim *et al*., 2011). In addition, lanosterol is another significant non-polar active compound. However, it was found that several samples had unique sterol compositions. Both *Camellia sinensis* and Camellia *oleifera* seed oils had been found to have b-amyrin, β-sitosterol, and lanosterol (Li *et al*., 2022). All these compounds reported as above in the bi-clonal tea seed stock oil for the first time.

Table 2. Phytosterols (mg kg^{-1}) of bi-clonal tea seed stock oils

No	Phytosterols	TS 462	TS 464	TS 491	TS 506
1.	b- Amyrin	30.17 ± 0.39^{b}	30.46 ± 0.15^{j}	31.55 ± 0.25^{b}	31.14 ± 0.23^{j}
2.	Lupeol	9.45 ± 0.82^{i}	8.40 ± 0.04^{k}	8.69 ± 0.10^{j}	8.59 ± 0.18^{k}
3.	Campestrol	67.17 ± 1.14^{f}	68.28 ± 0.10^{f}	68.97 ± 0.37^{f}	69.05 ± 0.24^{f}
4.	Spinasterol	9.74 ± 0.97^{i}	11.64 ± 0.11^{j}	12.00 ± 0.19^{i}	11.92 ± 0.20^{j}
5.	β-sitosterol	368.41 ± 1.53^{b}	370.57 ± 0.46^{b}	370.69 ± 0.21^{b}	371.03 ± 0.26^{b}
6.	Canophyllol	120.00 ± 0.38^{d}	120.04 ± 0.36^{d}	120.77 ± 0.25^{d}	120.44 ± 0.13^{d}
7.	Cycloartenol	286.08 ± 4.29^{c}	289.51 ± 1.17^{c}	291.04 ± 0.58^{c}	291.38 ± 0.12^{c}
8.	Stimas-7-en-3ol	31.68 ± 0.45^{h}	31.78 ± 0.03^{h}	31.99 ± 0.27^{h}	31.94 ± 0.30^{h}
9.	Betulin	45.28 ± 0.32^{g}	46.09 ± 0.10^{g}	46.08 ± 0.33^{g}	46.10 ± 0.27^{g}
10.	Lanostero	$l90.44\pm0.76^{e}$	89.40 ± 0.05^{e}	88.99 ± 0.39^{e}	89.12 ± 0.19^{e}
11.	24- Methylene cycloartan-3-ol	474.84 ± 0.82^{a}	475.30 ± 0.36^{a}	475.97 ± 0.53^{a}	476.03 ± 0.42^{a}
12.	Lupanol	8.01 ± 0.11^{i}	8.07 ± 0.16^{k}	8.28 ± 0.33^{j}	8.14 ± 0.11^{k}
13.	Obtusifoliol	3.68 ± 1.18^{j}	3.67 ± 0.06^{l}	4.09 ± 0.20^{k}	3.93 ± 0.36^{l}
	Total sterols	1544.98	1553.23	1559.12	1558.82

Values are Mean ± Standard error of the mean (SEm). Different parameters were analyzed using ANOVA to detect a significant difference between means using Web Agri Stat package-WASP 2.0. Means were compared using Duncan's Multiple Range Test (DMRT) at P <0.05. Mean the same alphabets in columns are not significant.

Conclusion

The tea seed oil contains phytosterols in varying levels and compositions, similar to those found in vegetables, cereals, fruits, berries, nuts, and vegetable oils. Total Phytosterols in the bi-clonal tea seed oil tested were in the following order: TS491 > TS506 > TS464 > TS 462. Bi-clonal tea seed stocks oil has a higher total sterol content than vegetables, cereals, fruits, and berries, but it is lower than that of nuts and vegetable oils. Tea seed oil contains significant amounts of β-sitosterol and lanosterol, which can delay aging and prevent cardiovascular disease. These properties imply that tea seed oil could potentially be used as a nutraceutical and a raw material for cosmetics in the near future. This is the first report to study the phytosterols profile of tea seed oil obtained from bi-clonal tea seed stocks of North East India.

Acknowledgments

The authors acknowledge the funding support from SERB-DST, Government of India, New Delhi, the Director TRA for their critical comments on the article, and Edward Food Research & Analysis Centre Ltd (EFRAC), Kolkata, for their analysis support.

Funding

Science and Engineering Research Board (SERB), Department of Science and Technology (DST), Government of India, New Delhi (Sanction no. ECR/2016/000002)

Data Availability Statement: Not applicable.

Disclosure statement

No potential conflict of interest was reported by the authors.

Author Contributions

PKP: Experimental design, original draft preparation. RS and NH: Data curation and analysis. BG and SKS: Bi-clonal tea Seed collection from experimental site and maintenance of seed bari. AB: Review MS and editing. All authors contributed significantly, directly, and intellectually to the work and gave their consent for it to be published.

References

Álvarez-Sala, A. *et al*. 2018. Physiological concentrations of phytosterols enhance the apoptotic effects of 5-fluorouracil in colon cancer cells. *J. Funct. Foods* **49**, 52–60.

AOAC,1996. *International Official Method 994.10: Cholesterol in Foods-Direct Saponification-Gas Chromatographic Methods*, Association of Official Analytical Chemists, Arlington, VA, USA.

Azadmard-Damirchi, S. and Dutta, P.C. 2006. Novel solid-phase extraction method to separate 4-desmethyl-, 4-monomethyl-, and 4,4′-dimethylsterols in vegetable oils. *J. Chrom. A*. **1108**, 183–187.

Bai, G., Ma, C. and Chen, X. 2021. Phytosterols in edible oil: Distribution, analysis and variation during processing. *Grain Oil Sci. Tech.* **4**, 33–44.

Demirag, O. and Konuskan, D.B. 2021. Quality Properties, Fatty Acid and Sterol Compositions of East Mediterranean Region Olive Oils. *J. Oleo Sci*. **70** (1):51–58. doi:10.5650/jos.ess20179

Han, J.H., Yang, Y.X. and Feng, M.Y. 2008. Contents of Phytosterols in Vegetables and Fruits Commonly consumed in China. *Biomed. Envir. Sci*. **21**, 449–453.

Lanzani, *C. et al*. 2016. Lanosterol Synthase Gene Polymorphisms and Changes in Endogenous Ouabain in the Response to Low Sodium Intake. *Hypertension*. **67**, 342–348.

Lee, C.P. and Yen, G.C. 2006. Antioxidant Activity and Bioactive Compounds of Tea Seed (Camellia oleiferaAbel.) Oil. *J. Agri. Food Chem*. **54**, 779–784.

Li, G. *et al*. 2022. Extraction of Oils and Phytochemicals from Camellia oleifera Seeds: Trends, Challenges, and Innovations. *Processes* **10**, 1489.

Lim, L. et al. 2011. Lanosterol induces mitochondrial uncoupling and protects dopaminergic neurons from cell death in a model for Parkinson's disease. *Cell Death and Differentiation*. **19**, 416–427.

López-García, G. et al. 2019a. Anti-Inflammatory and Cytoprotective Effect of Plant Sterol and Galactooligosaccharides-Enriched Beverages in Caco-2 Cells. *J. Agri. Food Chem*. **68**, 1862–1870.

Lopez Garcia, G. *et al*. 2019b. Effect of a Milk-Based Fruit Beverage Enriched with Plant Sterols and/or Galactooligosaccharides in a Murine Chronic

Colitis Model. *Foods*. **8**, 114.
Moghadasian, M.H. 2000. Pharmacological properties of plant sterols in vivo and in vitro observations. *Life Sciences*. **67**, 605–615.
Patel, P.K. *et al.* 2020. Tea seed oil: Physicochemical profiling. *J. Plant. Crops*, **48**(3)247–251. doi: 10.25081/jpc.2020.v48.i3.6633.
Plat, J. *et al.* 2019. Plant-based sterols and Stanols in health & disease: "Consequences of human development in a plant-based environment?" *Progress Lipid Res*.**74**, 87–102.
Salehi, B. *et al*. 2021. Phytosterols: From Preclinical Evidence to Potential Clinical Applications. *Front. Pharm*. 11.
Sarmah, K. *et al*. 2018. Biochemical characterization of tea (*Camellia spp*.) seed oil cake. *B. Envir. Pharm. Life Sci*. **7**, 98–102.
USDA, 1999. Nutrient Database, Release 12, US Department of Agriculture, Washington, DC.
Van, Rensburg, S.J. *et al*. 2000. A comparative study of the effects of cholesterol, beta-sitosterol, beta-sitosterol glucoside, dehydroepiandrosterone sulphate and melatonin on in vitro lipid peroxidation. *Metabolic Brain Disease* **15**, 257–265.
Witkowska, A.M. *et al.* 2021. Assessment of Plant Sterols in the Diet of Adult Polish Population with the Use of a Newly Developed Database. *Nutrients* **13**, 2722.
Yang, R. *et al*. 2019. Phytosterol Contents of Edible Oils and Their Contributions to Estimated Phytosterol Intake in the Chinese Diet. *Foods* **8**, 334.
Zakłos-Szyda, M. 2015. Phytosterols in type 2 diabetes and obesity – molecular mechanisms of action. Research Signpost.
Zhang, H. *et al*. 2017. Rapid recovery of high content phytosterols from corn silk. *Chem. Central J*. **11.**