International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Unveiling the Purity: The Physico-Chemical and Fatty Acid Profiling of Unbranded Ghee for Quality Assurance

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Abstract

Analysing unbranded ghee is critical for authenticating its purity and quality using various physiochemical analyses and fatty acid profiling methods. In order to conduct the analysis, 30 unbranded ghee samples were collected from six districts: Chennai, Villupuram, Cuddalore, Thiruvannamalai, Vellore, and Madurai. The adulteration of the ghee can be analysed by the BR value, RM value, polenske value, saponification value, and iodine value. Cow's ghee samples collected from Vellore districts show a lower saponification value is 187.44 ±0.03, RM value (21.9 ±0.4 and 20.4 ±0.20), polenske value (3.61 ±0.02 and 3.58 ±0.30), and BR value (39.8 and 45.7), which doesn't comply with FSSAI 2011 regulations. Vanaspati, a substance containing trans-fat, contaminated 12 samples. Trans-fat extracted from linoleic acid in Madurai district found to have the highest level of 0.2954 ±0.02 by GC-FID. Vegetable oil (Soybean and sunflower oil) detected in six samples using RP-HPLC. This study could have an impact on providing insightful reports on the quality, safety, integrity, and authenticity of unbranded ghee, which does not fulfil FSSAI requirements.

Keywords: Adulteration, Ghee, Purity, Safety, Trans-fat, Vegetable oil.

Full-length article **Corresponding Author*, e-mail: *gurumoop@gmail.com* Doi # https://doi.org/10.62877/107-IJCBS-24-25-19-107

1. Introduction

Ghee, a form of clarified butter traditionally used in South Asian cuisine, prized for its rich flavor, high smoke point, and numerous health benefits [1]. Made by simmering butter to remove water content and milk solids, pure ghee is compose mainly of saturated fats and contains essential fatty acids, fat-soluble vitamins, and antioxidants [2]. Despite its nutritional value and culinary significance, the growing demand for ghee has led to the emergence of unbranded varieties in the market [3]. This unbranded ghee often adulterated with various substances, compromising their quality and safety. Adulteration of ghee with vegetable oils is a common malpractice aimed at reducing production costs. Vegetable oils, being cheaper, mixed with ghee to increase volume and profit margins [4]. Food Safety and Standards Authority of India (FSSAI) in 2019 found that approximately 30% of the sampled ghee products were adulterated. To a study by the Indian Council of Medical Research (ICMR), adulterated ghee samples showed cholesterol levels 20-30% higher than pure ghee. Plant sterols like stigmasterol and β-sitosterol, while beneficial in reducing cholesterol, indicate the presence of vegetable oils [5]. These compounds found in significant amounts in 40% of the adulterated samples tested in a 2021 survey by the National Dairy Research Institute (NDRI). However, this not only dilutes the authentic taste and aroma of ghee but also alters its nutritional profile and can potentially pose health risks.

Moreover, the presence of cholesterol, stigmasterol, and β-sitosterol in adulterated ghee further complicates its health implications. Cholesterol, a sterol found in animal fats, is naturally present in ghee but its levels can be significantly elevated due to adulteration. High cholesterol intake is associated with increased risk of cardiovascular diseases. On the other hand, stigmasterol and β-sitosterol are plant sterols typically found in vegetable oils [6]. While lower cholesterol levels in humans, their presence in ghee know plant sterols indicates adulteration and misrepresentation of the product's composition. Understanding the extent and impact of these adulterations is crucial for ensuring consumer safety and maintaining the integrity of this traditional food product. This research specified the unbranded ghee samples collected from six distant districts of Tamil Nadu. Unbranded ghee is made and sold without a brand name or label attached. It is essentially ghee-packed and supplied without any business branding or trademark. This is frequently available in local marketplaces or manufactured by smaller-scale businesses

that have not yet established brand identification. Ghee costs about three times more than edible vegetable oils/fats [5]. The aim of the study is to assess the safety and quality of unbranded ghee through evaluation of many criteria such as Physico-chemical characteristics, fatty acid content, and detection of vegetable oil adulterants in unbranded ghee samples, highlighting the need for stringent quality control and regulation in the production and marketing of ghee.

2. Materials and Methods

2.1 Material Procurement

This study evaluated the quality of unbranded ghee samples collected from six districts in Tamil Nadu, India: Chennai (CI), Villupuram (VM), Cuddalore (CE), Tiruvannamalai (TI), Vellore (VE) and Madurai (MI). All the unbranded ghee samples were numbered and named according to their region and they are stored in a glass container to maintain the purity and quality of the unbranded ghee over time in refrigerator at 4°C. The chemicals and reagents procured from Sigma Aldorich, Chennai.

2.2 Determination of physical and chemical analysis of unbranded ghee

Indian Standard (IS) and FSSAI Manual of method for analysis of oil fats [7] methods adopted analyse both the physical and chemical parameters of unbranded ghee.

2.3 Determination of fatty acid profiling using Gas Chromatography - Flame Ionization Detector

The fatty acid composition of cow ghee was determined using Gas Chromatography with Flame Ionization Detection (GC-FID) following a derivatization procedure with a TR-CN100 column [8]. Briefly, the method involved saponification with methanolic sodium hydroxide followed by methylation with boron trifluoride (BF3). After extraction and separation of the organic phase, samples were injected into the GC-FID equipped with a helium carrier gas. Specific temperature programs were employed for both overall fatty acid analysis and targeted analysis of ruminant trans-fatty acids (rTFA). Fatty acid methyl esters (FAME) identified based on retention times compared to chromatographic standards. Finally, the fatty acid profile established by calculating the percentage of each peak area from triplicate analyses for enhanced accuracy and reliability.

2.4 Identification of vegetable oil adulteration using RP-HPLC

Vegetable oil adulteration of unbranded cow ghee was determined using Reversed Phase High Performance Thin Layer Chromatography (RP-HPLC). A one-gram fat sample underwent USM extraction using a KOH/methanol saponification at 90°C for 50 min with intermittent shaking. After water and hexane addition, vortexing, and centrifugation (3000 rpm, 5 min), the hexane layer containing USM was collected and dried. The dried USM then re-dissolved in a chloroform/methanol solvent mixture and filtered through a 0.22 µm filter prior to RP-HPLC analysis. Sterol standards (cholesterol, stigmasterol, β-sitosterol) at 1 mg/mL were analyzed by RP-HPLC with UV detection at 205 nm [9]. The HPLC system employed a 20-µL sample injection onto a C18 column (4.6 × 250 mm ID, 5-µm particle size) maintained at 30°C. A constant mobile phase flow rate of 1.5 mL/min used for 30 min, with sterol detection at 205 nm using a UV detector.

2.5 Statistical Analysis

The data was collected and analysed using statistical software IBM SPSS statistics 27. Each experiment was repeated thrice, and the results are presented as average values with a standard deviation.

3. Result and Discussion

3.1 Physico-chemical analysis of unbranded ghee

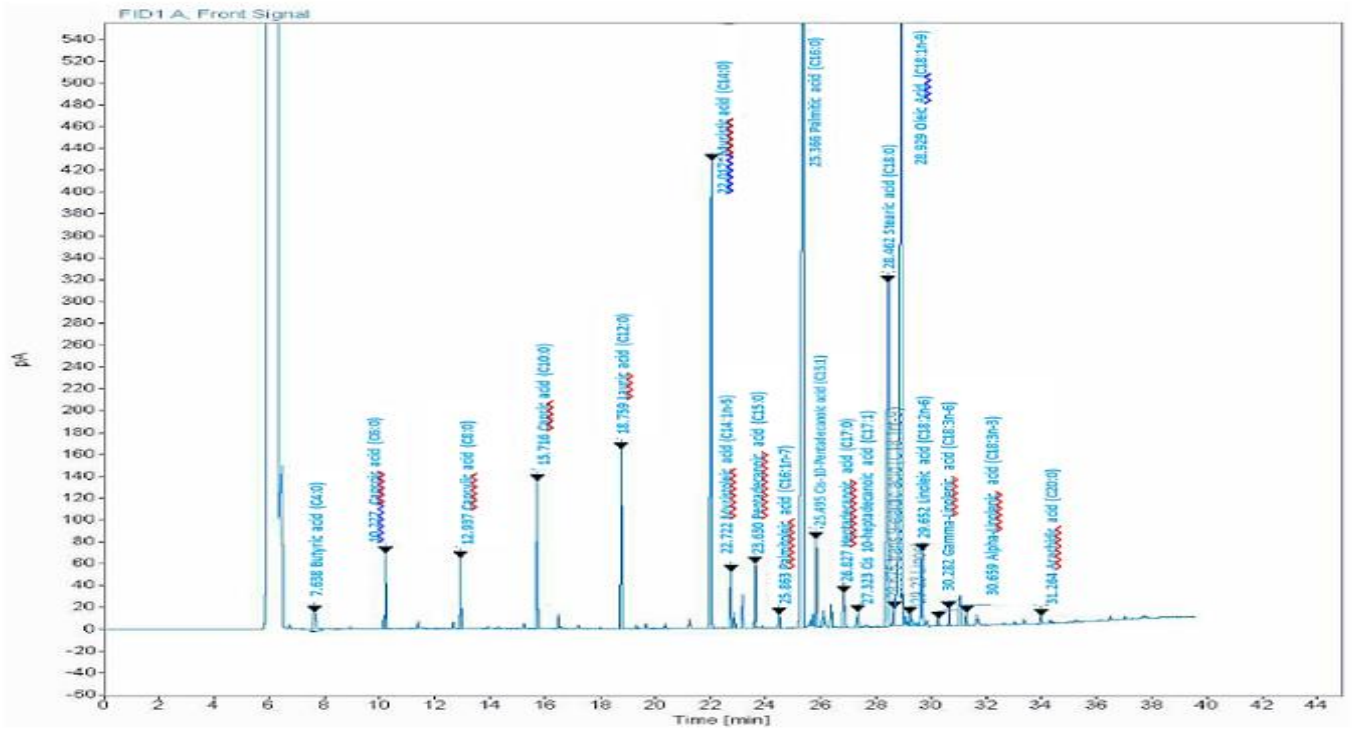
This study evaluated the physicochemical properties of 30 unbranded ghee samples to assess their quality and potential adulteration. Moisture content ranged from 0.1 ± 0.12% to 0.4 ± 0.15%. Higher moisture levels were observed in VM2, CE5, TI4, VE1, and MI4 regions when compared to the other districts, if the moisture level exceed 0.5% it may potentially impacting shelf life and microbial stability [10-11]. RM values ranged from 20.36 ± 0.25 to 35.62 ± 0.36. VE2, VE4, CI4, and TI2 regions exhibited significantly lower RM values compared to the standard minimum of 24, indicating adulteration with foreign fats [12-13]. Conversely, VE3 and CI4 regions displayed higher PV (3.61 ± 0.02 and 3.59 ± 0.02) exceeding the FSSAI limit (0.5 – 2.0) [14]. The saponification value varied between 187.44 ± 0.02 and 234.62 ± 0.02. Samples from VE3, CI4, and VM5 regions had significantly lower values, suggesting adulteration with plant oils with lower saponification values [15-14].

Iodine values ranged from 26.44 ± 0.03 to 40.94 ± 0.02. VM1, TI2, VE2, VE3, VE4, and MI2 regions displayed values exceeding the FSSAI standard (25 – 38), potentially indicating higher unsaturation and decreased stability [16-14]. Measured at 40°C, the butyro-refractometer reading ranged from 40.0 ± 0.1 to 47.2 ± 0.2. VE4 and TI2 regions significantly exceeded the FSSAI standard (40.0 – 44.0), possibly due to adulteration or presence of contaminants [17-12]. Acid values ranged from 0.13 ± 0.04 to 1.57 ± 0.04, falling within the FSSAI limit (maximum 2%) for all samples [18-7].Twelve samples tested positive for the presence of vanaspati (hydrogenated vegetable fat) using the Baudouin test, indicating adulteration. All samples exhibited a peroxide value of zero, suggesting no initial oxidation had occurred. The analysis revealed significant variations in the physicochemical properties of the unbranded ghee samples. Several samples exhibited evidence of adulteration with foreign fats or vegetable oils, raising concerns about their quality and adherence to food safety regulations.

3.2 Determination of fatty acid profiling using GC-FID

Studies have shown that the specific fatty acids present in milk fat significantly influence the physical and chemical properties of ghee [19]. Examining the fatty acid profile offers valuable information regarding the quality and flavor profile of the resulting ghee [20]. A ghee's quality determined by its fatty acid makeup, which also affects its flavor, texture, and the potential health benefits it may provide. Ghee primarily consists of saturated fatty acids (SFAs) such as C4:0 (Butyric), C6:0 (caproic), C8:0 (caprylic), C10:0 (capric), C12:0 (lauric), C14:0 (myristic), and C16:0 (palmitic), monounsaturated fatty acids (MUFAs) like C16:1 and C18:1 (oleic), and polyunsaturated fatty acids (PUFAs) including C18:2 (linoleic) and C18:3 (linolenic) (Mone im Sulie man et al. 2013).

(a)



(b)

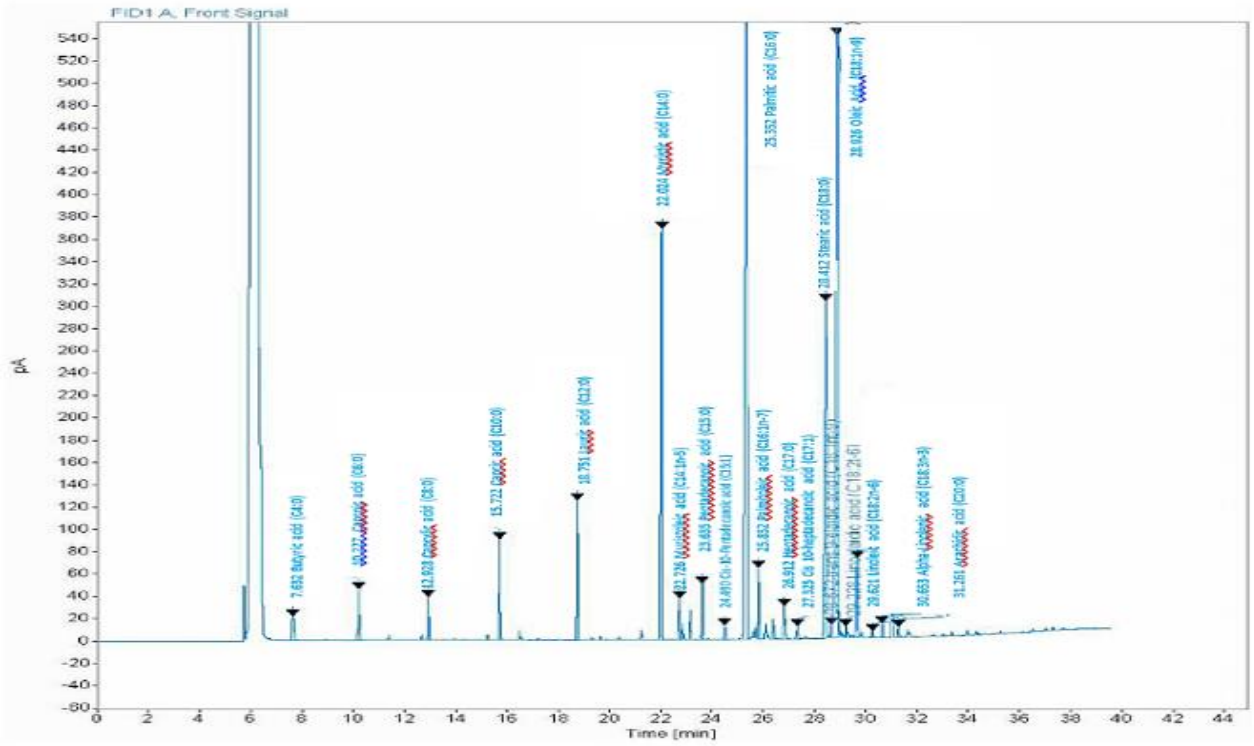


Figure 1. Fatty Acid Composition (FAC) of unbranded ghee sample from a. Vellore and b. Madurai districts.

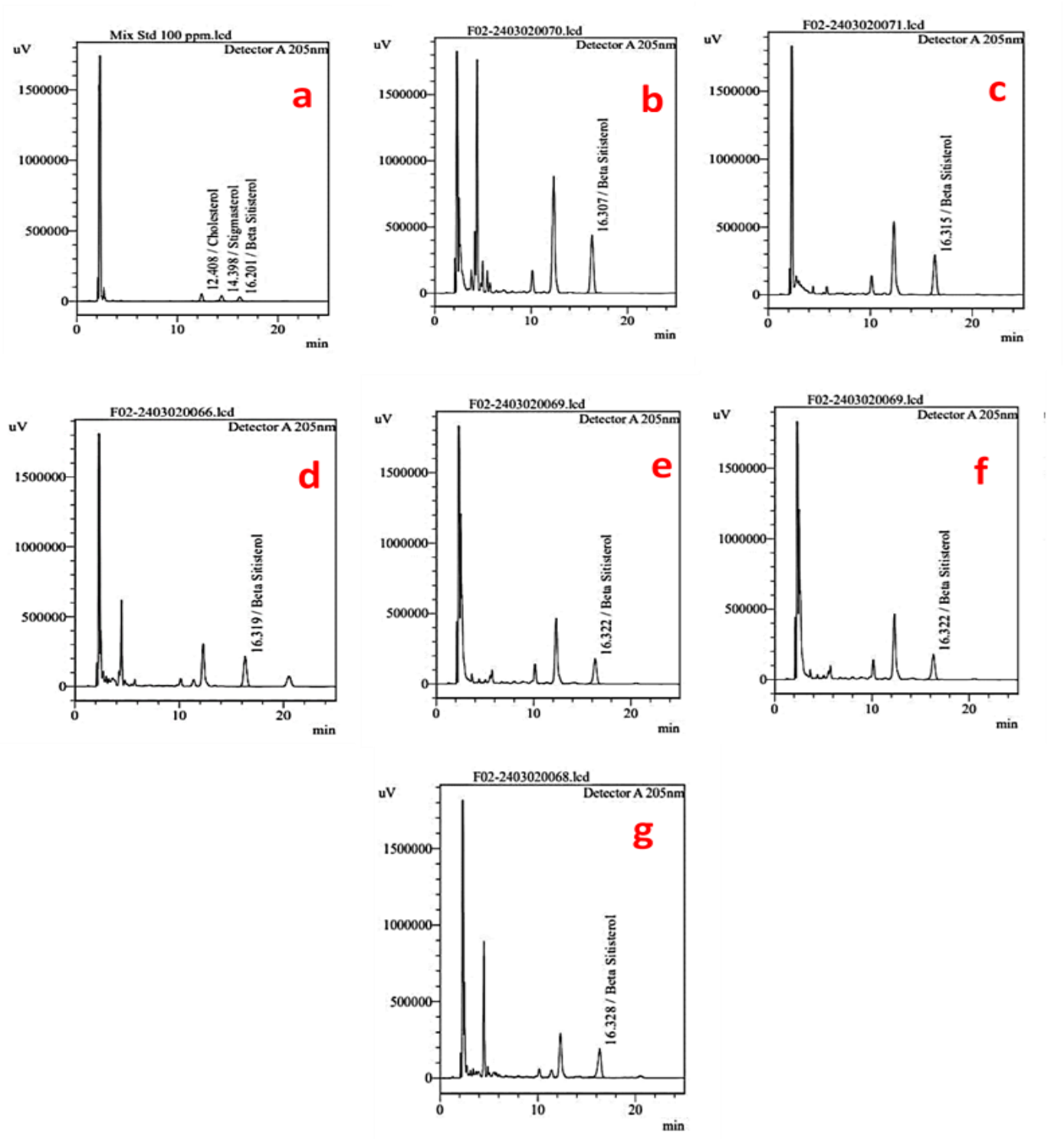


Figure 2. RP-HPLC chromatogram results a. Reference standards for cholesterol, stigmasterol and β-sitosterol, b. CI4 (Chennai) district, c. VM1 (Villupuram) district, d. VM5 (Villupuram) district, e. CE1 (Cuddalore) district, f. VE2 (Vellore) district and g. VE3 (Vellore) district.

Table 1: Physico-chemical parameter of unbranded ghee samples

Places	Moisture Content %	Reichert Meissel Value	Polenske Value	Iodine value (g/100g)	Saponification value (mg KOH/g)	BR readings at 40 ˚C	Acid Value (mg KOH/g)
FSSR clause no 2.1.8, 2011 regulation	**Maximum of 0.5%**	**Not Less Than 24**	**Between 0.5 – 2**	**Between 25-38**	**Between 205-235**	**Should be between 40.0 to 44.0**	**Maximum of 2.0%**
CI1	0.22±0.09^{m}	25.41±0.30^{gh}	1.77±0.02^{cd}	34.64±0.03^{h}	206.25±0.04^{k}	43.66±0.18^{de}	0.28±0.02l ^{lm}
CI2	0.22±0.10^{m}	27.60±0.32^{e}	1.56±0.03^{f}	28.63±0.05^{s}	234.45±0.03^{a}	43.45±0.24^{e}	0.48±0.05^{ghij}
CI3	0.29±0.17^{g}	23.70±0.18^{i}	3.53±0.02^{b}	38.35±0.04^{f}	195.94±0.03^{l}	39.44±0.17^{l}	0.42±0.04i^{jk}
CI4	0.23±0.08^{l}	21.59±0.31^{mn}	3.56±0.0 2^{ab}	38.73±0.03^{d}	189.65±0.02^{qr}	45.50±0.33^{b}	0.35±0.04^{kl}
CI5	0.30±0.15^{f}	26.46±0.29^{f}	1.74±0.02^{d}	32.35±0.03^{l}	232.84±0.04^{b}	41.59±0.28^{hi}	0.25±0.03^{lm}
VM1	0.32±0.17^{d}	22.39±0.25^{klm}	3.55±0.02^{ab}	39.25±0.04^{c}	193.64±0.03^{no}	46.41±0.35^{a}	0.49±0.04^{ghi}
VM2	0.32±0.10^{d}	27.73±0.24^{de}	1.07±0.01^{jk}	29.64±0.03^{q}	234.64±0.02^{a}	43.82±0.12^{de}	0.62±0.06^{f}
VM3	0.35±0.09^{b}	27.68±0.23^{e}	1.06±0.03^{k}	28.45±0.04^{t}	209.52±0.34^{j}	41.17±0.24^{ijk}	0.82±0.06^{e}
VM4	0.34±0.12^{c}	26.32±0.22^{fg}	1.44±0.02^{g}	29.54±0.04^{q}	205.75±0.02^{k}	41.42±0.22^{ij}	1.55±0.04^{a}
VM5	0.31±0.17^{e}	22.44±0.35^{klm}	3.55±0.03^{ab}	38.67±0.01^{d}	190.44±0.03^{q}	46.40±0.30^{a}	0.60±0.04^{fg}
CE1	0.24±0.13^{k}	23.55±0.39^{ij}	3.54±0.02^{b}	38.44±0.04^{ef}	193.34±1.70^{o}	38.80±0.12^{lm}	1.45±0.02^{ab}
CE2	0.24±0.07^{k}	25.11±0.19^{h}	1.45±0.03^{g}	30.94±0.03^{n}	231.33±0.03^{c}	43.51±0.3^{1e}	0.13±0.04^{n}
CE3	0.28±0.14^{h}	28.68±0.23^{d}	1.76±0.03^{d}	30.63±0.02^{o}	230.05±0.04^{d}	40.49±0.15^{k}	0.36±0.03^{jkl}
CE4	0.28±0.12^{h}	30.69±0.24^{c}	1.25±0.03^{i}	34.44±0.03^{i}	224.53±0.02^{f}	41.37±0.17^{ij}	0.12±0.04^{n}
CE5	0.34±0.09^{c}	25.60±0.20^{fgh}	1.35±0.03^{h}	32.49±0.08^{k}	232.82±0.02^{b}	40.67±0.30^{jk}	0.57±0.04^{fgh}
TI1	0.25±0.07^{j}	21.20±0.41^{nq}	1.04±0.03^{k}	31.62±0.01^{m}	220.65±0.04^{g}	42.35±0.19^{gh}	1.34±0.03^{cb}
TI2	0.32±0.14^{d}	21.94±0.43^{lmn}	3.56±0.03^{ab}	39.34±0.02^{bc}	191.76±0.03^{p}	46.44±0.25^{a}	0.25±0.03^{lm}
TI3	0.30±0.16^{f}	25.85±0.40^{fgh}	0.53±0.02^{m}	34.94±0.03^{g}	228.63±0.02^{e}	43.28±0.13^{ef}	1.32±0.03^{c}
TI4	0.35±0.13^{b}	25.49±0.13^{gh}	1.85±0.02^{c}	34.14±0.02^{j}	221.56±0.03^{g}	40.66±0.34^{jk}	1.24±0.02^{c}
TI5	0.24±0.09^{k}	22.93±0.19^{ijk}	1.47±0.01^{g}	30.72±0.02^{o}	232.83±0.02^{b}	46.54±0.33^{a}	0.94±0.03^{d}
VE1	0.35±0.10^{b}	23.45±0.35^{ij}	3.54±0.02^{b}	38.53±0.03^{e}	195.33±0.02^{lm}	45.49±0.18^{b}	0.53±0.01^{fghi}
VE2	0.28±0.16^{h}	20.47±0.26^{q}	3.56±0.01^{ab}	39.26±0.03^{bc}	189.33±0.02^{r}	39.39±0.32^{l}	1.26±0.02^{c}
VE3	0.32±0.12^{d}	22.69±0.21^{jkl}	3.63±0.02^{a}	40.93±0.02^{a}	187.44±0.03^{s}	45.43±0.44^{b}	0.84±0.04^{de}
VE4	0.27±0.14^{i}	21.57±0.39^{mn}	3.54±0.02^{b}	39.36±0.01^{b}	195.46±0.02^{lm}	47.26±0.21^{a}	0.88±0.03^{de}
VE5	0.31±0.18^{e}	25.49±0.33^{gh}	1.15±0.02^{j}	29.12±0.01^{r}	218.75±0.02^{h}	42.51±0.33^{fg}	1.23±0.03^{c}
MI1	0.22±0.11^{m}	35.62±0.36^{a}	1.83±0.02^{cd}	29.76±0.02^{p}	230.24±0.02^{d}	38.29±0.20^{m}	1.28±0.02^{c}
MI2	0.23±0.06^{l}	23.45±0.25^{ij}	3.53±0.02^{b}	39.25±0.04^{c}	195.54±0.02^{lm}	45.27±0.12^{bc}	0.14±0.03^{mn}
MI3	0.30±0.12f	20.36±0.18^{q}	3.55±0.03^{ab}	38.54±0.03^{e}	194.65±0.03^{m}	44.42±0.34^{cd}	0.54±0.03^{fgh}
MI4	0.37±0.10^{a}	31.42±0.40^{c}	1.66±0.02^{e}	27.04±0.01^{u}	234.53±0.02^{a}	41.56±0.31^{hj}	0.34±0.03^{kl}
MI5	0.28±0.10^{h}	33.42±0.21^{b}	0.74±0.04^{l}	26.44±0.03^{v}	211.74±0.03^{i}	42.50±0.33^{fg}	0.46±0.01^{hijk}

All values are mean ± SD of triplicates. Values with alphabetic characters (a-v) in the same column are significantly different ($p < 0.05$).

Table 2. Fatty acid profile of unbranded ghee samples

Fatty Acid	CT3 (%)	CT4 (%)	VM1 (%)	VM5 (%)	CE1 (%)	TI2 (%)	VE1 (%)	VE2 (%)	VE3 (%)	VE4 (%)	MI2 (%)	M13 (%)
(C4:0) Butyric acid	1.16±0.02^{c}	1.05±0.02^{c}	1.14±0.04^{c}	1.15±0.02^{c}	1.15±0.03^{c}	1.15±0.03^{c}	1.77±0.33^{b}	1.83±0.02^{b}	2.49±0.23^{a}	1.74±0.20^{b}	1.78±0.06^{b}	2.49±0.52^{a}
(C6:0) Caproic acid	1.44±0.44^{cd}	0.70±0.35^{f}	0.75±0.01^{ef}	0.72±0.02^{ef}	1.09±0.03^{de}	0.89±0.03^{ef}	0.05±0.01^{g}	2.18±0.01^{a}	0.87±0.04^{ef}	1.70±0.07^{bc}	1.90±0.02^{ab}	0.65±0.05^{f}
(C8:0) Caprylic acid	0.90±0.08^{cd}	0.65±0.02^{e}	0.85±0.04^{d}	0.65±0.03^{e}	1.05±0.03^{bc}	0.89±0.08^{d}	0.88±0.06^{d}	1.26±0.02^{a}	1.18±0.01^{ab}	1.27±0.05^{a}	1.23±0.03^{a}	1.13±0.05^{ab}
(C10:0) Capric acid	2.09±0.06^{b}	1.39±0.02^{cd}	1.63±0.04^{c}	1.36±0.02^{d}	2.40±0.04^{a}	2.07±0.02^{b}	2.06±0.01^{b}	0.88±0.02^{e}	1.04±0.03^{e}	1.64±0.28^{c}	2.08±0.01^{b}	2.08±0.01^{b}
(C12:0) Lauric acid	2.90±0.01^{d}	3.07±0.01^{c}	3.93±0.07^{a}	2.47±0.01^{e}	3.27±0.05^{b}	2.90±0.03^{d}	3.93±0.03^{a}	2.47±0.03^{e}	3.28±0.01^{b}	1.99±0.02^{f}	3.87±0.03^{a}	3.90±0.01^{a}
(C14:0) Myristic acid	10.95±0.01^{d}	8.84±0.02^{j}	8.48±0.03^{k}	9.84±0.02^{i}	11.06±0.02^{c}	10.97±0.01^{d}	10.16±0.04^{g}	10.85±0.03^{e}	9.95±0.03^{h}	11.16±0.02^{b}	10.26±0.02^{f}	11.26±0.02^{a}
(C15:0) Pentadecanoic acid	1.25±0.03^{bc}	0.95±0.02^{bcd}	0.85±0.04^{cd}	0.49±0.54^{d}	1.24±0.04^{bc}	1.36±0.04^{ab}	0.84±0.02^{cd}	0.65±0.14^{d}	1.84±0.02^{bc}	1.36±0.03^{b}	1.24±0.03^{bc}	1.17±0.02^{bc}
(C16:0) Palmitic acid	34.05±0.04^{de}	35.26±0.04^{b}	34.55±0.03^{bcde}	35.20±1.18^{bc}	34.25±0.04^{bcde}	34.37±0.04^{bcde}	34.14±0.04^{cde}	34.21±0.01^{bcde}	35.03±0.03^{bcd}	33.84±0.56^{e}	33.66±0.04^{e}	36.94±0.03^{a}
(C17:0) Heptadecanoic acid	0.94±0.04^{ab}	0.75±0.04^{efg}	0.64±0.02^{h}	0.87±0.03^{abcd}	0.84±0.03^{cde}	0.96±0.03^{a}	0.65±0.03^{gh}	0.69±0.01^{fgh}	0.77±0.03^{def}	0.85±0.03^{bcd}	0.88±0.02^{abc}	0.65±0.03^{gh}
(C18:0) Stearic acid	12.65±0.04^{b}	12.44±0.02^{bc}	11.13±0.01^{f}	13.28±0.54^{a}	11.74±0.03^{de}	12.36±0.03^{b}c	12.63±0.02^{bc}	12.17±0.03^{cd}	13.65±0.03^{a}	9.74±0.03^{g}	11.17±0.02^{f}	11.34±0.03^{ef}
(C20:0) Arachidic acid	0.24±0.03^{d}	0.34±0.02^{ab}	0.34±0.02^{ab}	0.36±0.03^{a}	0.25±0.02^{bcd}	0.33±0.01^{abcd}	0.25±0.02^{bcd}	0.25±0.04^{bcd}	0.24±0.02^{cd}	0.24±0.04^{d}	0.36±0.03^{a}	0.33±0.01^{abc}
(SFA) Saturated Fatty Acid	68.57	65.44	64.29	66.39	68.34	68.25	67.36	67.44	70.34	65.53	68.43	71.94
(C14:1n-5) Myristoleic acid	0.94±0.02^{bc}	0.55±0.04^{fg}	0.66±0.04^{ef}	0.55±0.03^{g}	1.06±0.02^{a}	0.94±0.03^{bc}	0.96±0.04^{ab}	0.85±0.03^{cd}	0.56±0.04^{fg}	0.76±0.04^{de}	0.93±0.01^{bc}	1.06±0.03^{a}
(C15:1) Cis-10Pentadecanoic acid	0.35±0.04^{ab}	0.26±0.02^{cdf}	0.25±0.04^{ef}	0.30±0.01^{bcde}	0.29±0.01^{bcde}	0.32±0.02^{abcd}	0.31±0.01^{bcde}	0.29±0.01^{bcde}	0.34±0.03^{abc}	0.26±0.03^{def}	0.39±0.01^{a}	0.20±0.02^{f}
(C16:1n-7) Palmitoleic acid	1.63±0.03^{bc}	1.32±0.01^{f}	1.17±0.03^{g}	1.67±0.02^{b}	1.64±0.03^{bc}	1.45±0.03^{e}	1.45±0.03^{e}	1.56±0.03^{cd}	0.93±0.05^{h}	1.59±0.01^{bcd}	1.53±0.02^{de}	1.80±0.01^{a}

(C17:1) Cis 10 heptadecanoic acid	0.31±0.01^{abcd}	0.22±0.01^{e}	0.23±0.05^{de}	0.27±0.02^{bcde}	0.33±0.01^{abc}	0.34±0.03^{ab}	0.23±0.04^{de}	0.21±0.01^{e}	0.20±0.01^{e}	0.20±0.01^{e}	0.38±0.02^{a}	0.24±0.03^{cde}
(C18:1n-9) Oleic acid	25.22±0.02^{g}	26.92±0.02^{e}	28.76±0.02^{a}	27.05±0.04^{d}	25.56±0.02^{f}	25.06±0.02^{h}	26.93±0.05^{e}	27.30±0.01^{c}	24.91±0.02^{i}	27.82±0.01^{b}	24.15±0.04^{j}	21.97±0.02^{k}
(C18:2n-6) Linoleic acid	2.10±0.01^{bc}	3.97±0.02^{a}	3.05±0.03^{ab}	2.15±1.60^{bc}	1.58±0.01^{c}	2.01±0.02^{bc}	1.65±0.02^{c}	1.43±0.02^{c}	2.05±0.02^{bc}	2.15±0.03^{bc}	3.06±0.03^{ab}	1.05±0.03^{c}
(C18:3n-3) Alpha-Linolenic acid	0.37±0.01cd	0.36±0.04cd	0.24±0.03e	0.36±0.03d	0.36±0.02d	0.46±0.04bc	0.24±0.02^{e}	0.25±0.03^{e}	0.42±0.01^{cd}	0.92±0.01^{a}	0.54±0.02^{b}	0.84±0.03^{a}
(UFA) Unsaturated Fatty Acid	30.92	33.60	34.36	32.35	30.82	30.58	31.77	31.89	29.41	33.70	30.98	27.16
(C18:1nt-9) Trans 9-elaidic acid	0.43±0.01^{e}	0.43±0.02^{e}	0.43±0.01^{e}	0.45±0.02^{c}	0.43±0.02^{e}	0.48±0.01^{a}	0.45±0.01^{c}	0.43±0.03^{e}	0.44±0.01^{d}	0.44±0.01^{d}	0.43±0.01^{e}	0.47±0.02^{b}
(C18:2t-6) Linoelaidic acid	0.24±0.03^{e}	0.24±0.01^{e}	0.21±0.01^{g}	0.26±0.01^{c}	0.22±0.02^{f}	0.24±0.04^{e}	0.25±0.04^{d}	0.25±0.03^{d}	0.27±0.01^{b}	0.27±0.01^{b}	0.24±0.02^{e}	0.28±0.01^{a}
(TFA) Trans-fatty Acid	**0.67**	**0.67**	**0.64**	**0.71**	**0.65**	**0.72**	**0.70**	**0.68**	**0.71**	**0.71**	**0.67**	**0.75**

All values expressed in percentage (g/100g) and mean ± SD of triplicates. Values with alphabetic characters (a-k) in the same row are significantly different ($p < 0.05$).

Milk fats are particularly rich in short and medium-chain fatty acids (C4-C10) [21]. Table 2 detail the fatty acid profiles of unbranded ghee samples collected from six districts. Out of 30 samples, 12 samples did not meet up with the physico-chemical parameters according to the FSSAI regulation (2011) as they subjected to FAC analysis. The MI3 region sample exhibited the highest concentration of palmitic acid (C16:0), a dominant SFA, at 36.94 ± 0.03 g/100g. This was followed by oleic acid (C18:1n-9), a MUFA, found at 28.76 ± 0.02 g/100g in the VM1 region (Table 3). These findings align with previous research who identified palmitic and oleic acids as the primary fatty acids in Indian ghee [22]. The MI3 region also displayed the highest proportion of SFAs (71.91% g/100g) compared to other locations. The content of short and medium-chain fatty acids (C4:0 to C12:0) ranged from 1.05 ± 0.02 to 3.93 ± 0.07 g/100g, while long-chain fatty acids (C14:0 to C18:0) varied between 8.48 ± 0.03 and 13.65 ± 0.03 g/100g. On average, ghee samples contained 35% Unsaturated Fatty Acid (UFAs), with MUFAs constituting a higher proportion compared to PUFAs. Studies suggest that MUFAs and PUFAs may play a role in reducing the risk of coronary heart disease and inflammatory conditions [23]. PUFAs are essential for proper growth, cell function, communication, and immune response [24]. Oleic acid (C18:1c9), a MUFA, was particularly abundant in the ghee samples, ranging from 21.97 ± 0.02 to 28.76 ± 0.02 g/100g.

Linoleic acid (C18:2n-6) was the most prevalent PUFA, detected between 1.05 ± 0.03 and 3.97 ± 0.02 g/100g. While linoleic acid, a long-chain unsaturated fatty acid, exists in dairy fat and animal storage fat, its presence is considerably more prominent in vegetable oils [25]. The study also identified small quantities of trans-fatty acids, specifically Trans-9-elaidic acid (C18:1nt-9) and Linoleic acid (C18:2t-6), ranging from 0.43 ± 0.01 to 0.48 ± 0.01 g/100g and 0.21 ± 0.01 to 0.28 ± 0.01 g/100g, respectively. Linoleic acid (C18:2) serves as a reliable marker for detecting milk fat adulteration with vegetable oil [25]. Their study revealed a detection limit as low as 5% adulteration. While analyzing 30 unbranded ghee samples, researchers found that 18 contained trans-fats below the 0.1% detection limit. The remaining 12 samples exceeded this limit. Despite ghee's reputation as a source of healthy fats, excessive trans-fats can negate these benefits. Trans-fats are a well-established health concern linked to increased risk of cardiovascular diseases, highlighting the importance of maintaining low levels in ghee.

3.3 Identification of vegetable oil adulteration using RP-HPLC

Vegetable oils contain a unique sterol profile compared to milk fat, with β-sitosterol being a prominent phytosterol alongside cholesterol [26]. The varied composition of vegetable oils makes general adulteration detection challenging. This study-evaluated β-sitosterol as a marker for detecting soybean and sunflower oil adulteration in ghee. B-sitosterol might be a more effective marker than stigmasterol due to its higher abundance in vegetable oils, particularly those with a higher proportion of unsaturated sterols [15]. To identify cholesterol and phytosterols, 20 mL of un-saponifiable matter (USM) from control milk fat (ghee) and test samples analyzed. Twelve unbranded ghee samples examined. Six samples (CI4, VM1, VM5, CE1, VE2, and VE3) displayed a β-sitosterol peak (Figures 2), indicating adulteration with 1% soybean oil and 2% sunflower oil. It is important to note that the limit of detection (LOD) can vary depending on the type of adulterating oil. Peak retention times compared to reference standards to identify specific sterols.

The presence of a β-sitosterol peak in adulterated samples confirmed vegetable oil adulteration [9]. As shown in Figure 2, reference standards for cholesterol, stigmasterol, and β-sitosterol exhibited retention times of 12.408, 14.398, and 16.201 min, respectively. Analysis of vegetable oils revealed a prominent β-sitosterol peak alongside a smaller cholesterol peak. Soybean and sunflower oils have distinct fatty acid profiles, including differing linoleic acid (C18:2t-6) content. Both oils are widely consumed; soybean oil is rich in polyunsaturated fats, particularly the essential omega-6 fatty acid linoleic acid. Partial hydrogenation, used to stabilize soybean oil, can convert linoleic acid to linolelaidic acid. This study demonstrates the effectiveness of β-sitosterol as a marker for detecting low concentrations of vegetable oil adulteration in ghee. Additionally, the peak height of β-sitosterol increased with the concentration of adulterant oil.

4. Conclusions

This study evaluated the physico-chemical properties, fatty acid composition, and adulteration potential of unbranded ghee samples from six districts in Tamil Nadu, India. Analyses focusing on Reichert Meissel (RM), Polenske (PV), saponification, iodine value, and butyro-refractometer readings revealed deviations from FSSAI standards (2011) in several samples. These deviations suggest potential adulteration or improper processing/storage practices. Higher saponification and iodine values may indicate adulteration with plant oils, impacting flavor, aroma, and shelf life. Fatty acid analysis provided insights into nutritional value and quality. The presence of short-chain fatty acids supports product genuineness, while trans-fatty acids, particularly linolelaidic acid, suggest contamination, reducing nutritional quality and posing health risks. Linolelaidic acid has linked to increased cardiovascular risk by elevating Low Density Lipoprotein (LDL) and lowering High Cholesterol Lipoprotein (HDL), highlighting the importance of ghee authenticity and quality control. Soybean and sunflower oils, being unsaturated, can increase trans-fat content when used as adulterants in saturated fat like ghee. While ghee offers a source of healthy fats, adulteration can negate these benefits and introduce health risks. These findings emphasize the critical role of stringent quality control measures and regulatory enforcement in ensuring the authenticity, safety, and nutritional value of ghee products. This research can provide valuable theoretical foundations for improving ghee quality across various regions, ultimately addressing both public health needs and the economic interests of producers and consumers.

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