Detection of tallow adulteration in cow ghee by derivative spectrophotometry

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Abstract

Context: Ghee is a widely consumed dairy product in India and that prepared from cow milk is mentioned in ayurvedic texts as an ingredient of many formulations/additive as well. Detection of cow ghee adulteration with vegetable oils/fats and animal body fats is a key concern. Indicated values for commonly used parameters to differentiate pure and adulterated ghee materials are many a times overlapping. Among reported techniques, ultraviolet fluorescence and paper chromatography technique are not that much sensitive while other methods require sophisticated instrumental facilities (such as gas chromatography, mass spectrometry) and costly analytical processes. Aims: The present paper deals with a promising spectroscopic method to determine the tallow adulteration in cow ghee. Materials and Methods: Ghee and tallow (taken in chloroform) as such and mixed in different proportions were scanned by spectrophotometer and their second order spectra were analyzed. Results: The value of the ratio of the absorbance of peaks at about 238 nm and 297 nm steadily decreases with the increasing proportion of tallow. This decrease shows consistent linearity suggesting its applicability for quantitative estimation of tallow in cow ghee. Conclusion: The developed derivative spectroscopic method is a rapid, sensitive, cost-effective method for detection of tallow adulteration in cow ghee.

Key words: Cow ghee, second order spectra, tallow adulteration, ultraviolet spectrophotometry

INTRODUCTION

Ghee is one of the important dairy products in India and is normally prepared by using cow milk or buffalo milk. Ghee prepared from cow milk has wide consumption all over the country and is mentioned in ayurvedic texts as an ingredient of many formulations/additives as well. Cow ghee is often adulterated with vegetable oils/fats and animal body fats. Detection of adulterants and their estimation is a key concern over many years. Adulterating ghee with animal fat materials like tallow emerges as a serious concern, especially due to cultural and religious background in India, which includes vegetarianism as well as motherly attitude toward cows. Moreover, consumption of tallow induces risk of increase in serum cholesterol and triglycerides levels, which can lead to many further complications.

Detection of tallow adulteration in ghee has always challenged the scientific community and there are hardly any techniques, which could be reliable as well as feasible to be performed easily. Different values such as Reichert-Meissl value, butyro-refractometer reading etc., which have been included as parameters for quality evaluation, are many a times overlapping, for pure and adulterated ghee materials. The saponification value, percent unsaponifiable matter and free fatty acid content in case of cow ghee are not more than (NMT) 225, 1.25 and 3% respectively, whereas for tallow the same values go as 190-202, NMT 1.2% and NMT 1.25%, respectively. Butyro-refractometer reading (at 40°C) for ghee has to be in 40-45 range while refractive index (at 40°C) for tallow lies between 1.448 and 1.460, which corresponds to butyro-refractometer reading of 33.8-51. It is obvious from the values that readings of these parameters for mixture of ghee and tallow will also lie in the same range and will be of little help in mapping differences between pure ghee and that adulterated with tallow. Methods such as ultraviolet (UV) fluorescence technique, paper chromatography, gas chromatography (GC),
GC-mass, combination of preparative thin layer chromatography and GC are reported for detection of tallow adulteration in ghee. However, UV fluorescence and paper chromatography techniques are not that much sensitive while the other methods require sophisticated instrumental facilities such as GC, Mass spectrometry, which due to their very high cost and costly analytical process, are available in limited laboratories and are also time consuming. There is a need to develop a sensitive, rapid, cost-effective method. Direct spectrophotometric determination of multi-ingredient sample is often complicated by interference from ingredients itself and spectral overlapping. Derivative spectrophotometry is a useful means of resolving such problem. In the present communication, an easy, sensitive, rapid and cost-effective derivative spectrophotometric method for detection of tallow adulteration in cow ghee is reported.

MATERIALS AND METHODS
Cow ghee was prepared in-house from pure cow milk. Tallow was prepared in the laboratory from fat rich beef (flesh surrounding kidneys mainly comprising of adipose tissue). These samples were used for the study.

Sample preparation
One gram of pure ghee and tallow were taken separately, melted by warming, 10 ml chloroform (analytical grade from Merck India) was added, sonicated for 15 min to make it homogenous and were used as stock solution. Mixture of ghee and tallow in different proportions ranging from 9:1 to 1:9 were prepared by using the stock solutions of ghee and tallow. 10% v/v solution in chloroform of different samples, i.e. cow ghee, tallow as well as cow ghee containing different proportion of tallow, as prepared above, were used for the present study.

UV spectrophotometry
The normal absorption spectra of ghee and tallow of all the samples were recorded in the range 200-400 nm using a PerkinElmer UV-visible recording spectrometer (Model – Lambda25). Data was analyzed (using 149 data points each for slope calculation) through PerkinElmer Data processor and viewer software to obtain second order derivative absorption spectra.

RESULTS AND DISCUSSION
The normal UV spectra of cow ghee and tallow [Figure 1] did not reveal sufficient information that can be useful for detection of tallow adulteration in cow ghee. Hence, the second order derivative UV spectra of the samples were recorded [Figure 2]. The two absorption peaks, one at about 238 nm (Peak 1) and the other around 297 nm (Peak 2) are seen in all the samples. The details of these two peaks, absorption wavelength and the corresponding absorbance is presented in Table 1. The absorbance of the Peak 1 remains almost same in all the samples while in Peak 2, the absorbance is the highest in ghee and steadily decreases with the increasing concentration of tallow in ghee and is the lowest in tallow. Between these two peaks in each of the spectra, one remains unchanged while the absorbance of another peak changes with the changing proportion of tallow in ghee. It was felt that the ratio of absorbance of these two peaks may be useful in quantifying the tallow adulteration in ghee and the ratio of absorbance (Peak 2 absorbance/Peak 1 absorbance, i.e., B/A) was calculated [Table 1]. A graph was prepared by plotting B/A value against the concentration of tallow in ghee to see the linearity. The decrease in this ratio is quite linear ($R^2 = 0.989$), i.e., proportionate to the concentration of adulteration [Figure 3]. Hence, it will be useful in detecting and quantifying adulteration of tallow in cow ghee.

The data reveals that there are clear differences in the second order derivative spectra of tallow and cow ghee. The intensity of absorption around 297 nm is about four times in cow ghee as compared with that of tallow. This intensity decreases with
**Table 1: Details of second order derivative spectra of ghee and tallow samples**

| Sample               | Peak 1 (wave length, nm) | Peak 1 absorbance (A) | Peak 2 (wave length nm) | Peak 2 absorbance (B) | B/A   |
|----------------------|--------------------------|-----------------------|-------------------------|-----------------------|-------|
| Ghee                 | 238.50                   | 0.0267                | 293.36                  | 0.0189                | 0.7078|
| Ghee+tallow (90+10)  | 238.19                   | 0.0281                | 296.60                  | 0.0170                | 0.6050|
| Ghee+tallow (80+20)  | 238.26                   | 0.0270                | 296.78                  | 0.0155                | 0.5741|
| Ghee+tallow (70+30)  | 238.08                   | 0.0276                | 297.04                  | 0.0136                | 0.4927|
| Ghee+tallow (50+50)  | 238.09                   | 0.0273                | 297.62                  | 0.0101                | 0.3699|
| Ghee+tallow (30+70)  | 238.12                   | 0.0274                | 298.66                  | 0.0070                | 0.2554|
| Ghee+tallow (20+80)  | 238.21                   | 0.0275                | 299.24                  | 0.0057                | 0.2072|
| Ghee+tallow (10+90)  | 238.25                   | 0.0278                | 300.73                  | 0.0046                | 0.1654|
| Tallow               | 238.31                   | 0.0275                | 302.47                  | 0.0040                | 0.1454|

Figure 3: Correlation of tallow concentration in ghee with the ratio of absorbance

applicability for quantitative estimation of tallow in cow ghee.

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