Detection of hydrogenated vegetable oils (vanaspati) in ghee using infrared spectroscopy

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ABSTRACT

Infrared spectroscopy, widely used for the characterization of cis- and trans-isomers of oils and fats, was used for the detection of adulteration in ghee with hydrogenated vegetable oils (Vanaspati) as the latter contains significantly higher levels of trans isomers formed during hydrogenation process used for vanaspati manufacturing. The average content of trans isomers (expressed as % trielaidin) in buffalo ghee, cow ghee and vanaspati as determined using infrared (IR) absorption at 10.36 µ (965.25/cm) was 5.41, 6.82 and 37.05%, respectively. On the basis of increased level of trans isomers in ghee, as low as 5% addition of vanaspati to ghee could be detected easily.

Key words: Adulteration, Ghee, Hydrogenated vegetable oils, IR spectroscopy, Vanaspati

MATERIALS AND METHODS

Collection of milk and preparation of butter: Milk used for the preparation of ghee samples was collected from the Institute’s cattle yard. Cow milk was a mixture of the milk obtained from the herd of Karan Swiss, Karan Fries, Sahiwal and Tharparkar breeds. Buffalo milk used was also the herd milk from Murrah breed only. Cows and buffaloes were maintained under identical conditions of feeding and management. Soon after the collection of milk, it was warmed to 40°C and separated into cream, using mechanical cream separator. The cream was pasteurized at 77°C for 5 min, cooled to room temperature and then kept in a refrigerator (5 to 10°C) for 3 to 5 h for ageing. Butter was

Table 1. Trans isomers content in pure ghee (buffalo and cow), vanaspati (hydrogenated vegetable oils) and ghee adulterated with vanaspati.

<table>
<thead>
<tr>
<th>Type of fat</th>
<th>Level of vanaspati (%)</th>
<th>Trans content (% Trielaidin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffalo ghee</td>
<td>5</td>
<td>5.41±0.51</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>6.82±0.23</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>7.34±0.14</td>
</tr>
<tr>
<td>Cow ghee</td>
<td>5</td>
<td>37.05±1.02</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9.23±0.12</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>11.33±0.21</td>
</tr>
<tr>
<td>Vanaspati</td>
<td>5</td>
<td>8.74±0.28</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10.61±0.14</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>12.42±0.15</td>
</tr>
<tr>
<td>CD (P&lt;0.05)</td>
<td>0.61</td>
<td></td>
</tr>
</tbody>
</table>

Values bearing different superscripts in column differ significantly (P<0.05). Values are given as Mean±SE (n=3).
Fig. 1. IR absorption spectra of pure buffalo ghee (A) and pure cow ghee (B).

Fig. 2. IR absorption spectra of vanaspati (A) and reference standard of Trielaidin (B).

Fig. 3. IR absorption spectra of buffalo ghee adulterated with vanaspati at 5% (A), 10% (B) and 15% (C) levels.

Fig. 4. IR absorption spectra of cow ghee adulterated with vanaspati at 5% (A), 10% (B) and 15% (C) levels.
prepared under standard conditions (9°C in summer and 13°C in winter) by churning the cream using hand churn.  

Collection of adulterants: The vanaspati (hydrogenated vegetable oils) ghee was collected from the local market.  

Preparation of adulterated ghee samples: The vanaspati ghee was added individually to ghee (buffalo as well as cow) at the butter stage on the basis of its fat content at 5, 10 and 15% levels. The butter samples admixed with the adulterants were clarified on direct flame in a stainless steel vessel under continuous stirring at temperature of 120°C/flash and finally filtered through Whatman No. 4 filter paper. Simultaneously, pure ghee sample (control) was also prepared under similar conditions from the same lot of butter without adding any of the adulterants.  

For the measurement of trans-unsaturation, infra-red (IR) spectroscopy of pure ghee as well as ghee samples added with hydrogenated vegetable oils (Vanaspati) was done according to Firestone and Villadelmar (1961) using trielaidin as a standard.  

Whole fat samples melted at 35°C were scanned through IR range of 4000 to 600/cm using Fourier Transform Infra-Red (FTIR) spectrophotometer in the Department of Pharmaceutical Chemistry at National Institute of Pharmaceutical Education and Research (NIPER), Mohali (Punjab). The experimental conditions used were as follows:  

- **Range**: 4000 to 600/cm  
- **Beam splitter**: KBr  
- **Detector**: DTGC (Deuterated Triglycine Sulphate)  
- **Laser**: Helium /Neon (He/Ne) with ATR attachment  
- **Make**: Nicolet  
- **Model**: Impact-410  

### RESULTS AND DISCUSSION  

Infra-red (IR) spectroscopy has been widely applied in the analysis of oils and fats, especially for the characterization of cis- and trans- isomers. In order to observe as to how far IR spectroscopy can be helpful in detecting adulteration of ghee with hydrogenated fats, the samples of pure ghee (buffalo and cow), vanaspati and ghee samples adulterated with 5, 10 and 15% vanaspati were analysed, in triplicates, for the determination of trans isomers content in terms of % trans as trielaidin (reference standard triglyceride containing all the three fatty acids as trans-oleic acids).  

Samples in the melted form were scanned through an IR range of 4000 to 600/cm to observe the differences in their absorption spectra. Upward peaks were considered as negative since they represented the regions in which the absorbance is less than that of reference while downward peaks were considered as positive. The results obtained are presented in Table 1 and depicted in Figures 1 to 4.  

The reference standard triglyceride (trielaidin) showed an absorption peak of choice (i.e. for trans isomers) at 964.79/cm. The average content of % trans isomers (as trielaidin) in vanaspati, cow ghee and buffalo ghee were 37.05, 6.82 and 5.41%, respectively (Table 1), indicating the significantly higher (P<0.01) content of trans component in vanaspati as compared to cow and buffalo pure ghee. Cow ghee showed significantly higher (P<0.05) trans content than buffalo ghee.  

Addition of vanaspati at 5, 10 and 15% to both buffalo and cow ghee resulted in the proportional increase in the trans isomers content (Figs 1 to 4). Further, analysis of variance revealed that the increase in trans content in buffalo ghee as well as cow ghee due to the addition of vanaspati even at 5% level was significant (P<0.01).  

Keeping in view the overall range of trans content of cow and buffalo ghee into consideration, it may be revealed that detection of adulteration of ghee with vanaspati using IR absorption at 965.25/cm (10.36 µ) may be possible even at 5% level. Firestone and Villadelmar (1961) has reported the % trans content in butter oil as 7.5% (as trielaidin) which is in close agreement with the observation made in the present study on cow and buffalo ghee. The higher content of % trans content as trielaidin in vanaspati observed in the present study may be due to the conversion of cis isomers to trans isomers during the hydrogenation process carried out to convert the vegetable oils into hardened fat (Fox and McSweeney 2015) and such fats are reported to contain as high as 50% trans fatty acids. Few workers (Firestone and Villadelmar 1961, Chahine et al. 1958) reported that the hydrogenated cottonseed oils and margarine contained 35.0 to 41.7% trans isomers as trielaidin which corroborates our findings.  

Milk fat contains small amounts of trans fatty acids (about 5%), which arises due to the biohydrogenation process occurring in rumen, where the dietary lipids containing the cis-configurations are partially converted enzymically into trans isomers. In the present study, buffalo and cow pure ghee samples were found to contain 6.82 and 5.41% trans isomers, respectively. On the basis of increase in the trans unsaturation of pure ghee, it was possible to detect adulteration of ghee with vanaspati at a level as low as 5%.  

### ACKNOWLEDGEMENTS  

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### REFERENCES  


