RP-HPLC Method for the Detection of Adulteration of Ghee with Vegetable Oils/Fats

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Objective

Ghee is one of the costliest fat due to its pleasant taste and immense health benefit. Due to its high cost, some fraud businessmen use to adulterate ghee with vegetable oil, for making of more money. Though there are so many techniques based on physico-chemical parameters or fatty acids are already available, but all of these techniques have some backdrops like less precession as well as accuracy, time consuming etc. It is definite that detection of vegetable oil in ghee is like that differentiation of tap water with river water. So considering all facts in our present study we had developed a simple HPLC based protocol to differentiate genuine ghee with adulterated ghee (with vegetable fat) based on β-sitosterol (main sterol in vegetable oil) peak and retention time analysis.

Methodology

Extraction of Unsaponifiable Matter (USM) from the fat samples

Unsaponifiable matter from fat samples was extracted as per the method standardized by Samridhi (2012). One gram of fat sample was saponified with 5% methanolic KOH. The unsaponifiable matter was extracted with hexane and dried. The dried unsaponifiable matter obtained was then dissolved in chloroform and methanol. This sample was then filtered through 0.22 µm Millipore filter paper and were subjected to RP-HPLC analysis. Along with this, the reference standards of cholesterol and β-sitosterol were also run on RP- HPLC and peak detection was made at 205 nm using UV-Visible detector.

Result and Discussion

RP- HPLC conditions for the separation of standard sterols as described by Oh *et al.* (2001) were adopted and standardized for the profiling of sterols in unsaponifiable matter of ghee, vegetable oils, specific adulterant oil and adulterated samples. The results showed that the cholesterol and β-sitosterol had a wide gap in their retention times and resolved well under the standardized RP- HPLC conditions. On the basis of these results the method was selected and used in the present study. It was evident from the chromatogram (figure 1) that the unsaponifiable matter of pure ghee samples showed one prominent peak corresponding to the retention time of standard cholesterol, indicating that the major sterol in pure ghee was cholesterol. In case of vegetable oils, a prominent peak of β-sitosterol was observed along with a small peak corresponding to the retention time of cholesterol was also observed. As this study was based on the fact that cholesterol is the major sterol in ghee whereas, phytosterols are predominant in vegetable oils; therefore the appearance of peak of β-sitosterol has been used as a marker for detecting the adulteration of ghee with the adulterant oils. The height of the peak for β-sitosterol increased as the level of addition of adulterant oils in ghee increased.

Conclusion

Our study showed that during adulteration of vegetable oil in ghee presence of β-sitosterol (which was not present in pure ghee) found and its concentration corresponding β-sitosterol peak in chromatogram was also depends on the level of vegetable oil adulteration, even at very low level of vegetable oil adulteration β-sitosterol peak was observed in chromatogram. So our study proved that even at very low concentration of vegetable oil in ghee could be detected efficiently using β-sitosterol as an efficient marker and there

was no chance for false result as we found no peak of β-sitosterol in pure ghee sample even procured from cotton tract area of Gujarat.

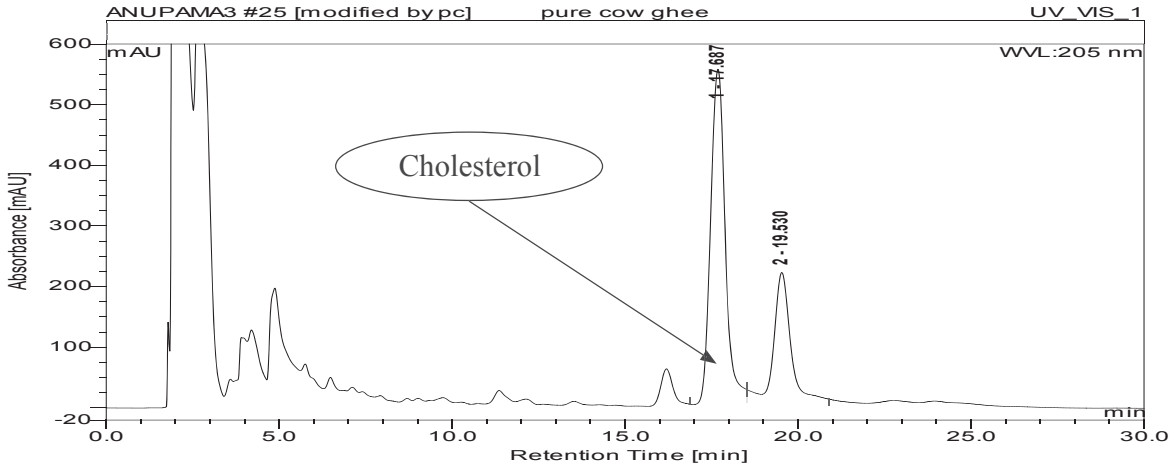
References

Oh, H.I., Shin, T.S., Chang, E.J. 2001. Determination of cholesterol in milk and dairy products by high-performance liquid chromatography. *Asian-Australian J. Animal Sci.***14**: 1465-1469.

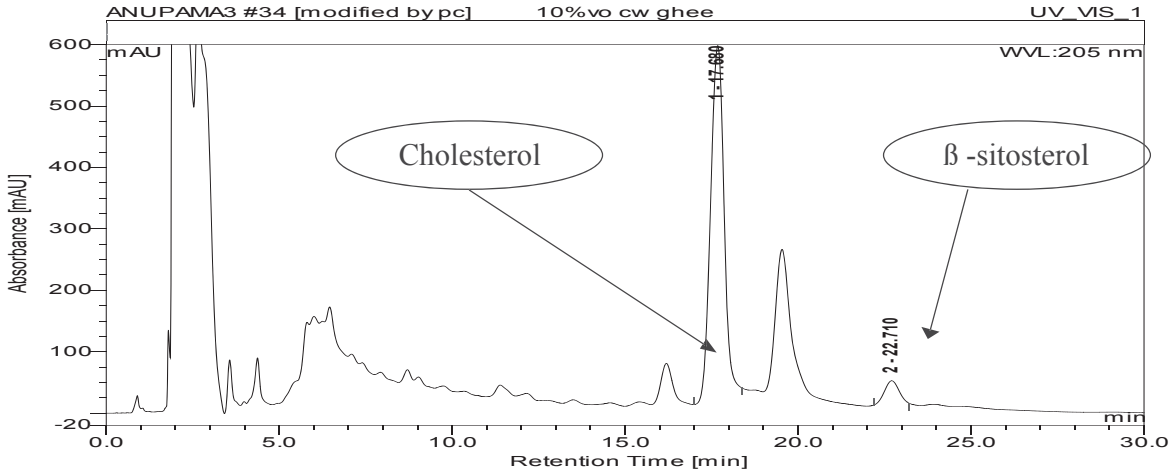
Samridhi. 2012. Evaluation of chromatographic methods for the detection of milk fat adulteration with vegetable oils. M.Sc. *Thesis* submitted to National dairy Research Institute (Deemed University), Karnal, India.

Figure 1 (a) HPLC chromatogram of pure cow ghee

(b) HPLC chromatogram of cow ghee adulterated with vegetable oil.



(a)



(b)