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Rapid quantification of cholesterol in dairy powders using Fourier transform near infrared spectroscopy and chemometrics

J. Chitra*, M. Ghosh, H.N. Mishra

Department of Agricultural and Food Engineering (AgFE), Indian Institute of Technology, Kharagpur, West Bengal 721302, India

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ABSTRACT

A rapid method for the quantification of cholesterol in the standardized dairy powders was developed using Fourier transform near-infrared (FT-NIR) spectroscopy coupled with appropriate chemometric techniques. Different spectral preprocessing methods were investigated for the partial least-squares (PLS) regression model development. The results showed that the second derivative PLS model in the spectral region of 6101.9–5446.2 cm^{-1} was the most robust with the best performance indicators (r^2 validation = 0.9998, RMSECV = 1.05 mg cholesterol/100 g, rank = 6 and RPD > 8). Functional band assignment of the major spectral peaks in the cholesterol spectrum was also possible. Statistical evaluation with the HPLC method proved that the developed NIR–chemometric method has good reproducibility and satisfactory accuracy profile. The comparable relative standard deviation (RSD) along with good precision accuracy (95.9–101%) of the proposed FT-NIR method, demonstrate its suitability for the rapid and routine analysis of cholesterol content in the dairy powders.

1. Introduction

Cholesterol (5-cholesten-3β-ol) is a polycyclic steroid compound present exclusively in foods of animal origin including meat, dairy and poultry (Xu et al., 2002). It is an important functional constituent of cellular membrane functions and precursor of important endogenous substances such as corticosteroids, sex hormones, and bile acids (Bragagnolo, 2010, pp. 187–220). However, dietary cholesterol has been associated with numerous adverse health effects. Several animal model studies to determine the effects of dietary cholesterol, cholesterol esters and cholesterol oxides on the development of atherosclerosis have revealed the role of the same in increasing the serum cholesterol and triglyceride levels significantly (Hur, Min, Nam, Lee, & Ahn, 2013; Liu et al., 2015). In human follow-up studies, dietary cholesterol intake was positively associated with the risk of total stroke and cerebral infarction in women (Larsson, Virtamo, & Wolk, 2012). Also, there was a dose-related increase in all-cause mortality and atherosclerotic events with both cholesterol intake and egg consumption in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study population (Spence, Judd, Howard, Safford, & Howard, 2015). Systematic meta-analysis study conducted showed positive association between egg consumption (>220 mg cholesterol/egg) and the risk of CVD and diabetes (Li, Zhou, Zhou, & Li, 2013). However, the studies on the link between dietary cholesterol intake and plasma cholesterol level have been mostly inconclusive (Berger, Raman, Vishwanathan, Jacques, & Johnson, 2015).

Hence, the monitoring of cholesterol content in processed/unprocessed foods has become vital due to apparent reasons viz. (i) the regulatory aspects of nutritional labeling obligatory in many countries, (ii) escalating health concerns due to its alleged correlation with atherosclerotic and coronary heart disease, especially in the diabetic and obesity susceptible population subgroups, and (iii) to gain nutritional information before and after implementation of any dietary manipulation or intervention (Hwang, Wang, & Choong, 2003; Mestre Prates, Gonçalves Quaresma, Branquinho Bessa, Andrade Fontes, & Mateus Alfaia, 2006; Dinh, Blanton, Brooks, Miller, & Thompson, 2008; Borkovcová, Janoušková, Dračková, Janštová, & Vorlová, 2009; Larsson et al., 2012; Li et al., 2013).

The current Association of Analytical Chemists (AOAC) recommended procedure for cholesterol analysis in the dairy products involves lipid saponification, derivatization of sterols to form trimethylsilyl (TMS) ethers, followed by purification cum concentration, and gas liquid chromatographic (GC) quantification of cholesterol (AOAC, 2000; Dinh et al., 2008; Fletouris, Botsoglou,

* Corresponding author.
E-mail addresses: jchitra86@gmail.com (J. Chitra), mousumi199118@gmail.com (M. Ghosh), hnm@agfe.iitkgp.ernet.in (H.N. Mishra).

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Psomas, & Mantis, 1998). The silyl or methyl derivatization process is cumbersome and the derivatizing agents are highly volatile which sometimes affects the accuracy and repeatability of the GC quantification. Also, cholesterol can be thermally decomposed in the GC column (Yang & Choong, 2001). A UV-HPLC method for cholesterol estimation requires the use of large quantities of corrosive chemicals and extensive sample cleanup by Reverse Phase (RP) cartridge or Sep-Pak (Hurst, Aleo, & Martin, 1983). Conventional cholesterol analysis methods including HPLC and GC that, although highly reliable, are time consuming, expensive, tedious and unsuitable for a real-time response and for analyzing large volumes of samples (Fletouris el al. 1998; Larsen, 2012). In addition to problems such as low speed and high cost, wet-chemistry methods are destructive and require extensive sample preparation, including saponification, extraction with organic solvents and extensive cleanup using membrane filters. The recent focus thereby lies on the development of rapid, accurate, non-destructive and simplified method for the routine analysis of cholesterol.

Recently many infrared and vibrational spectroscopic techniques including infrared (IR) and near infrared (NIR) spectroscopy have come to focus owing to their accuracy, high throughput and real time analysis (Ferreira, Galão, Pallone, & Poppi, 2014). The near infrared region (NIR), discovered by Herschel in 1800, covers the range of the electromagnetic spectrum between the range of 780–2526 nm corresponding to the wave number range 12820–3959 cm^{-1} as per American Society of Testing and Materials (ASTM) (Pasquini, 2003; Reich, 2005). The advantages of FT-NIR spectroscopy are well recognized. It requires minimal or no sample preparation, does not require costly reagents, nor produces any waste. Other advantages include non-destructive nature and high speed of the analysis, affordable and flexible instrumentation, and ease of multi-component analysis from a single spectrum along with good precision when calibrations are well developed (Bevilacqua, Bucci, Materazzi, & Marini, 2013; Gaspardo et al., 2012; Tripathi & Mishra, 2009). Since its first analytical inception in 1960's, FT-NIR has been applied to study various quality attributes and internal compositional analysis in products of agriculture and food industries (Ferreira et al., 2014; Sinija & Mishra, 2009).

In the dairy industry, most of the surplus dairy milk is converted in the form of skim milk powder (SMP), whole milk powder (WMP) or cream powders (CP) due to the ease of transportation, handling and storage. Currently, these powders constitute a significant share (61%) in the dairy ingredient sector (includes milk powder; whey ingredients; lactose & derivatives; casein & caseinates and milk protein concentrate & isolate) with the global production of 9.534 Million Metric Tons in 2015 (Markets & Markets, 2015; USDA Foreign Agricultural Service, 2016). Paradkar and Irudayaraj (2002) determined cholesterol content by FT-NIR in certain dairy products. However, the quantification was done paradoxically, after the time consuming chemical based extraction of cholesterol, which negates the advantages of FT-NIR as being fast and non-destructive method. Hence, the present research was undertaken with the objective of exploring the possibility of coupling FT-NIR spectroscopy with chemometrics for the development of robust, rapid and reliable method for the direct assessment of cholesterol in these powders without any sample preparation. As a reference method, high performance liquid chromatography (HPLC) technique was employed.

2. Materials and methods

2.1. Preparation of calibration and validation standards

WMP and CP with varying concentrations of fat were prepared by lyophilization of the standardized milk (adjustment of milk fat and milk solid not fat content) in the laboratory, while complying with legal requirements of 26–42% and >42% milk fat contents respectively (CODEX STAN 207-1999). A laboratory-scale freeze-dryer, Lyo 0555 (Lyodel Delvac, India) was used for freeze-drying. Manual adjustment of cholesterol content is not possible in these samples during standardization, as it is dependent upon the fat content. Usually WMP (of 26% fat) contains 90–110 mg/100 g cholesterol (Indyk, 1990; Paradkar & Irudayaraj, 2002). Hence, to increase the variability of cholesterol quantities, many WMP were subjected to supercritical fluid extraction in the pressure range of 150–250 bar at 40–80 °C for 2 h, which lowered the cholesterol content considerably (Applied Separations, the *Spe-ed* SFE model 7070, USA) (Chitra, Deb, & Mishra, 2015). SMP contains very low levels of fat (<1.5%) and hence sparing levels of cholesterol content too. To increase the range of cholesterol in SMP, pure cholesterol powder was dry blended to SMP in various amounts. The particle size distribution of commercially available fat filled powder ranges from 200 to 250 μm, which also confer rapid dispersion (Sharma, Jana, & Chavan, 2012). Hence, the dehydrated powders (<4% moisture content) were size graded using ASTM standard mesh sieves and the powders with particle size distribution between 200 and 250 μm were collected and used for FT-NIR analysis.

2.2. Chemicals and reagents

Cholesterol standard (>99% purity) was procured from Sigma–Aldrich (Sigma, St. Louis, MO). HPLC grade acetonitrile, methanol and iso-propanol were procured from Merck Chemicals, India. All other chemicals and solvents used were of analytical reagent grade.

2.3. FT-NIR instrumentation

FT-NIR MPA™ – Multipurpose analyzer (Bruker Optics, Germany) equipped with a quartz beam splitter; an integrated Michelson interferometer; highly sensitive PbS 12.800–3.600 cm^{-1} detector, multiple NIR measurement accessories for different sampling techniques combined with OPUS/QUANT 5.5 software was used for spectral acquisition. For the acquisition, the sample was densely packed into the sample vial and subjected to FT-NIR under diffuse reflectance mode combined with macrosample integrating sphere measurement channel. The absorption spectra was generated over a full spectral range of wave numbers from 12,500 cm^{-1} to 3600 cm^{-1} at a resolution of 8 cm^{-1} at speed of 10 KHz and each spectra was the average spectrum of 32 scans. The spectra were interpreted based on the overtones of different functional groups in the product. A background spectrum was recorded prior to the sample run using an empty vial. To minimize noise in spectral data, the averaged spectrum of three replicates per sample was analyzed by rotating the sample vial by 120°at three different points. The temperature was controlled at 30 ± 1 °C throughout the spectral scanning process.

2.4. Chemometrics

Chemometrics involves the use of regression techniques coupled with spectral preprocessing (mathematical and statistical methods) for improving the extraction of relevant information from complex NIR spectral data (dos Santos, Lopo, Páscoa, & Lopes, 2013). Multiple Linear Regression (MLR), Principal Component Regression (PCR) and Partial Least Square Regression (PLS) are the most commonly used chemometric tools; other techniques include neural network modelling etc. (Pasquini, 2003). Use of chemometrics allows the use of multivariate data for allocation of peaks/absorption bands to specific functional groups and quantification of chemical components. Mathematical transformations i.e.,