Novel method to determine the lipid content of breast milk

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HIGHLIGHTS

- A positive relationship between the fat content and density of the milk powder solutions was found.
- The linear functional equation could be applied to predict the fat content of breast milk with a density.
- The absolute percentage error of fat content in 124 breast milk obtained by linear functional equation was less than 42.1% which was less than that of other methods.
- A Bland-Altman plot showed that the linear functional equation and gravimetric method were consistent (P < 0.0001).

ABSTRACT

The fat content of breast milk is important to establish the levels of organic pollutants in human being. Traditional liquid-liquid method was reliability, but time-consuming. In this study, a rapid method that predicts the fat content of breast milk on the basis of an accurate measurement of density is developed. 17 milk powder solutions were prepared, and the densities of these solutions were calculated after measuring its volume and weight. Based on the fat content and density, three equation models, a linear functional equation and two equations obtained by polynomial regression between fat content and density, were established and demonstrated a positive relationship between the fat content and density. The three equations were used to predict the fat contents of fresh milk and breast milk based on weight, volume, and density, respectively. Results showed that the linear functional equation of density and fat content produced a satisfactory result when the density was between 0.9975 and 1.0566 g mL^{-1}, with the predicted fat content matching well with the results from the gravimetric method. A Bland-Altman analysis also showed that the linear functional equation and gravimetric method were consistent when it was applied to measure the fat content of breast milk (n = 124, P < 0.0001). The absolute percentage error of the analytical results was less than 42.1% which is much less than other method. The weight or the density of milk samples should be measured with an accuracy of more than 0.001 g or 0.001 g mL^{-1}, respectively, to obtain a low relative error.

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1. Introduction

Breast milk contains 3%–7% fat (Jensen, 1989). Hence, lipophilic organic pollutants can be excreted with lipid in breast milk during lactation. Compared with blood, urine, fetal hair, and other bio-media, breast milk is more abundant and can be easily obtained and sampled non-invasively. Since the study of Laug et al. (1951), many others have determined the concentrations of lipo-soluble organic pollutants in breast milk and evaluated their residue levels to monitor the risk of exposure to humans of these compounds, especially persistent organic pollutants (POPs) (Kalra and Chawla, 1981; Hernandez et al., 1993; Okonkwo and Kampira, 2002; Poon et al., 2005; Tatsuya et al., 2006; Fujili et al., 2012). In order to accurately measure the level of POPs and evaluate the risk of exposure to POPs in human, the fat content of breast milk and the concentration of POPs in the fat (μg kg^{-1} fat or μg kg^{-1} lipid) have to be determined first.

Fat from breast milk is usually extracted through liquid–liquid extraction (LLE) and then weighed through gravimetry (Krauthacker et al., 1986; Burke et al., 2003; Yao et al., 2005; Rodas-Ortíz et al., 2008; Martins et al., 2013). LLE and gravimetric methods are precise in measuring fat content and target compounds but are

not always accurate (Pan et al., 2014). Normally, different extraction solvents were used for the extraction. However, the extraction efficiency of the fat for different solvent was not evaluated which may induce inaccuracy. Besides that, LLE and measurement of the fat content using gravimetric methods is rather time-consuming for dehydration and concentration, resulting in the loss of volatile target compounds. Previous studies did not measure fat content and insteadly an average fat content,3.5% (Stuetz et al., 2001), 3.6% (Ryan et al., 1993; Craan and Haines, 1998), or 4.0% (Antonio et al., 2008), were used in previous studies. The averaging method is time saving but usually leads to the poor accuracy of monitoring and evaluation.

The present study aims to determine the relationship and establish mathematical models that describe the correlation between the density and fat content of milk samples. The mathematical model is then applied to predict the fat content of breast milk rapidly, precisely, and accurately.

2. Materials and methods

2.1. Materials

Analytical-grade ammonia and anhydrous sodium sulfate were purchased from Beijing Chemical Reagent Co. (Beijing, China). Anhydrous sodium sulfate was dried in a muffle furnace at 600 °C for 6–8 h. Hexane, alcohol, and diethyl ether of chromatographic grade were purchased from J&K (CN) and Honeywell (USA). Deionized water with a resistivity above 18 Ω was used.

Powdered milk, Similac® 2 (Abbott Nutrition, Auckland, New Zealand) containing 23.5% fat, and fresh milk samples were all purchased from a supermarket. The following Chinese brands of fresh milk were included: Sanyuan® fresh milk (fat content 3.6%), Mengniu® pure milk (36.0 g L^{-1}), Yili® fresh milk (3.4%), Sanyuan® breakfast milk (3.1%), Sanyuan® tepin fresh milk (37.0 g L^{-1}), and Mengniu® high-calcium milk (60.0 g L^{-1}). A total of 124 breast milk samples were collected from donors living in Beijing between 2010 and 2013. The participants in the study had lived in Beijing for at least the last 3 years. All donors completed questionnaires about age, body weight, dietary habits, lifestyles, as well as date of give birth, current and former area of residence. In brief, 20 mL of each sample was collected in individual glass bottles by manual or breast pump. The bottles were capped, transported to the laboratory, and then stored at −20 °C until analysis.

2.2. Establishing of mathematical models

Samples of powdered milk and pure water were weighed (Sartorius, BSA 124S, d = 0.1 mg) accurately to ± 0.0001 g and then mixed to obtain 17 powdered milk solutions with fat contents ranging between 0.46% and 8.83% (the fat content of breast milk generally ranges from 3% to 7%). The solution was occasionally shaken or stirred to dissolve the powder in water completely and evenly. Two 5000 μL samples of each powdered milk solution were measured accurately by pipette (Brand, Transferpette® S, D-5000). Their weights were recorded to calculate the average density of each of the 17 solutions on the basis of two weight measurements. Three mathematical models were established using Microsoft Office Excel® software (version 2007), with density and fat content as the independent and dependent variables, respectively, to describe the relationship between the density and fat content of the powdered milk solutions.

2.3. Sample analysis

Three mathematical models were developed to predict the fat

contents of the fresh milk and breast milk samples. The fat content of the breast milk samples was also measured in accordance with the gravimetric method described by Song et al. (2013). In brief, each milk sample was removed from the refrigerator and then warmed up to ambient temperature before analysis. Then, 10 mL of the sample (2 × 5000 μL samples) was weighed. The fat from the sample was extracted twice using a mixture of ammonia solution, ethanol, diethyl ether, and hexane. The organic phase extracted was evaporated to dryness for the gravimetric determination of milk fat content [f (%)] (Yao et al., 2005; Song et al., 2013), which can be expressed as follows:

$$f(\%) = (m_1/m) \times 100, \tag{1}$$

where m_l is the lipid weight in the sample, and m_s is the sample weight.

Finally, the predictions by the three models were compared with the values marked on the packaging (for fresh milk) or with those obtained by the gravimetric method (for breast milk).

3. Results and discussion

3.1. Comparison of three models

The equations and R^2 of the three mathematical models (Fig. 1) can be summarized as Eqs. (2)–(4), as follows:

$$f = 43.19\bar{\rho} + 41.403 \quad R^2 = 0.9364 \tag{2}$$

$$f = -177.84\bar{\rho}^2 + 425.21\bar{\rho} - 245.99 \quad R^2 = 0.9649 \tag{3}$$

$$f = 3774\bar{\rho}^3 - 12312\bar{\rho}^2 + 13411\bar{\rho} - 4872.2 \quad R^2 = 0.9924, \tag{4}$$

where $\bar{\rho}$ is the average density from two 5000 μL samples.

As showed in Fig. 1, the fat content of the milk powder solutions highly positively correlated with density in the three mathematical models. Eq. (2), which is a linear function, deviated from the experimental data over the whole range of densities. The deviations were positive in the low-density region (0.9915 g mL^{-1} < ρ < 1.0238 g mL^{-1}), negative in the medium-density region (1.0238 g mL^{-1} < ρ < 1.0507 g mL^{-1}), and positive in the high-density region (1.0507 g mL^{-1} < ρ < 1.1596 g mL^{-1}). The curve for Eq. (3), which is a quadratic polynomial regression equation, provided a good degree of fit to the experimental data in the low-density region (0.9915 g mL^{-1} < ρ < 1.0238 g mL^{-1}) but showed a negative deviation from the experimental data when the density was between 1.0238 and 1.1596 g mL^{-1}. The curve from Eq. (4), which is a cubic polynomial regression equation, provided almost a perfect match to the experimental data over the entire range of densities with an R^2 value of 0.9924. Similar to Eqs. (2) and (3), the highest deviations were observed in the medium-density region (1.0238 g mL^{-1} < ρ < 1.0507 g mL^{-1}) for Eq. (4), but their values were obviously lower than those of Eqs. (2) and (3).

3.2. The analytical results of fresh milk with different equations

The actual fat contents of the six fresh milk samples, marked on the packaging, were similar, ranging from 3.10% to 3.82% (Table 1). The densities were calculated after the volume and weight of the fresh samples were measured. The three equations were then used to predict the fat content of the six fresh milk samples on the basis of density. The analytical results and relative errors are listed in Table 1.

Table 1 shows that the densities of the six fresh milk samples