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Time resolved fluorescence of cow and goat milk powder

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ABSTRACT

Milk powder is an international dairy commodity. Goat and cow milk powders are significant sources of nutrients and the investigation of the authenticity and classification of milk powder is particularly important. The use of time-resolved fluorescence techniques to distinguish chemical composition and structure modifications could assist develop a portable and non-destructive methodology to perform milk powder classification and determine composition. This study goal is to differentiate milk powder samples from cows and goats using fluorescence lifetimes. The samples were excited at 315 nm and the fluorescence intensity decay registered at 468 nm. We observed fluorescence lifetimes of 1.5 ± 0.3, 6.4 ± 0.4 and 18.7 ± 2.5 ns for goat milk powder; and 1.7 ± 0.3, 6.9 ± 0.2 and 29.9 ± 1.6 ns for cow's milk powder. We discriminate goat and cow powder milk by analysis of variance using Fisher's method. In addition, we employed quadratic discriminant analysis to differentiate the milk samples with accuracy of 100%. Our results suggest that time-resolved fluorescence can provide a new method to the analysis of powder milk and its composition.

1. Introduction

Milk of different ruminant species is a great source of nutrients and milk. Bovine milk is the most common for human consumption around the world. However, cow milk allergy is very common, particularly among children [1]. Goat milk is similar to cow milk in composition, but it lacks beta-carotene and agglutinin, has smaller fat globules and different casein types, which makes it more easily digested [1]. In addition, goat's milk stability during heat treatments is lower than that of cow milk [2,3].

Milk powder represents most of the international dairy commodity tradable volumes [3]. It is made from dried milk solids, has prolonged shelf life, is easy to store and transport, and does not need to be refrigerated due to its low moisture content [5–8].

Given the international trade of goat and cow milk powders, the investigation of the authenticity and classification of milk powder is particularly important. The studies on rapidly, lower cost, and nondestructive detection methods for composition/adulterants is becoming essential for food safety and public health. Milk powder contains numerous intrinsic fluorophores such as vitamin A, tryptophan, tyrosine, phenylalanine and riboflavin [9,10]. Fluorescence techniques has been applied to detect structural changes in milk induced by heat treatments [9–11], to evaluate riboflavin [12,13], to measure Maillard reaction products in milk [14,15], to detect adulterations [16], to monitor changes during storage [17], etc. However, very few studies analyzed goat dairy products using fluorescence spectroscopy [13,18,19].

A prospective alternative to chemistry-based laboratory methods is the use of fluorescence-based techniques, given the potential of fluorescence for identifying chemical components [14–16]. Time resolved fluorescence offers some advantages for the characterization of food products when compared with infrared spectroscopy because measures the time dependence of the fluorescence emission. Lifetimes are determined from the fluorescence intensity decay and does not depend on intensity of excitation, duration of light exposure and method of measurement. Moreover, photo bleaching does not affect the fluorescence lifetime, and it is independent of the fluorescence intensity and fluorophore concentrations [20,21].

In this study, we investigate static fluorescence and time resolved fluorescence as an alternative and fast screening method for the qualitative discrimination of goat and cow's milk powders.

2. Materials and Methods

2.1. Sample Preparation

We acquired distinct batches of the only two goat's milk powder products available in local markets, and acquired distinct batches of two cow's milk powder in local markets from different producers. Table 1 describes each product ingredients and its contents of carbohydrates, proteins and fat.

For each batch, we made triplicate samples with 130 mg approximately, totalizing 24 samples. The samples were hard-pressed on a hydraulic press using 3 tons for 1 s, creating a disc with 1 cm of diameter and thickness of 1 mm. We performed the measurements of the samples directly after the preparation.

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Table 1
Ingredients and contents of carbohydrates, proteins and fat for each milk product acquired.

Milk powder	Carbohydrates (g in 100 g)	Proteins (g in 100 g)	Fat (g in 100 g)	Ingredients
Cow milk A	36.92	25.77	27.31	Cow milk powder.
Cow milk B	38.08	26.15	26.92	Cow milk powder, iron, zinc and vitamins (A, C, D).
Goat milk A	30.77	26.92	32.31	Goat milk powder, vitamin (B9), and soy lecithin.
Goat milk B	30.81	26.90	30.74	Goat milk powder.

We also measured Sigma's casein from bovine (CAS: 9000-71-9; composition: α-s1 Casein, α-s2 Casein, β-Casein, and κ-Casein) and anhydrous lactose powder (CAS: 63-42-3; PubChem Substance ID 24850720) to evaluate the contribution of these components in the fluorescence intensity and lifetime.

2.2. Fluorescence Spectroscopy

The static fluorescence were measured with excitations at 270 nm, and 315 nm and the emission recorded between 300 and 600 nm with 1 nm step. The excitation source was an Edinburgh nF900 ultrafast nanosecond flashlamp filled with hydrogen gas. The acquisitions were performed with the Edinburgh FL920 spectrometer with a blue sensitive photomultiplier to detect the emitted photons. The dwell time was defined to 0.1 s and each emission was the sum of three repetitions.

2.3. Time Resolved Fluorescence Spectroscopy

The fluorescence intensity decay was measured on basis of time-correlated single photon counting (TCSPC). The excitation source was an Edinburgh nF900 ultrafast nanosecond flashlamp filled with hydrogen gas. The acquisitions were performed with the Edinburgh FL920 spectrometer with a blue sensitive photomultiplier to detect the emitted photons. The full width at half maximum (FWHM) of the instrument response function (IRF) was typically 2 ns and time resolution was 0.1 ns per channel. We observed no significant background in the experiments and the software F900 provided by Edinburgh Instruments was used for fitting of the individual decays into multi-exponential curves. Eq. (1) describes the fluorescence intensity decay on time, where β_i are the pre-exponential factors and τ_i are lifetimes.

$$I(t) = \sum_i \beta_i \exp(-t/\tau_i) \quad (1)$$

Samples were excited at 315 nm and the fluorescence intensity decay registered at 468 nm. The decay profile intensity was fitted to a one, two, three and four exponential decay using deconvolution with the instrument response function via the software F900 provided by Edinburgh. We analyzed the quality of the fit by the statistical parameter reduced-χ^2 (reduced chi squared) and by examining the residuals distribution. The average value of the reduced chi square for one lifetime decay was 87.50, for two lifetimes decay was 8.45, for three lifetimes decay was 1.23, and for four lifetimes decay was 1.21. We decided to use three lifetimes (three exponential decay fitting) because it presented an even residual distribution, the chi-squared with fewer terms suggested that it did not fit to data sufficiently, and models with more terms increases the risk of fitting-to-noise. The software also provides the pre-exponential factors β_1, β_2 and β_3, and the normalized pre-exponential factors β_{n1}, β_{n2} and β_{n3} (which sum equals to 1).

2.4. Data Processing

From the results of the fit of experimental decays for both excitation wavelengths, we calculated the mean amplitude lifetime, using the definition [22]: $\langle\overline{\tau_A}\rangle = \sum_i \beta_i\tau_i / \sum_i \beta_i$.

In addition, from the results of the fit of experimental decays for both excitation wavelengths, we calculated the mean intensity lifetime, using the definition [22,23]: $\langle\overline{\tau_I}\rangle = \sum_i \beta_i\tau_i^2 / \sum_i \beta_i\tau_i$.

We analyzed the differences in the decay values τ_1, τ_2, τ_3, $\langle\overline{\tau_A}\rangle$ and $\langle\overline{\tau_I}\rangle$ between different milk powders (goat and cow) by one-way analysis of variance (ANOVA) using Fisher's LSD method. We perform the quadratic discriminant analysis using Minitab 16 Statistical® software, which computes the Mahalanobis distances using individual class covariance matrices. The cross-validation routine used omits each observation one at a time, recalculating the classification function using the remaining data, and then classifying the omitted observation.

Principal component analysis (PCA) is frequently used to reduce the dimensionality of data with correlated variables, by displaying the maximum amount of variation in a data profile within a few principal components. The principal components (PCs) are a simple linear combination of the original variables. Pairwise score plots resulting from PCA are advantageous to identify similarities and contrasts between samples, while the loadings vectors describe the importance of each original variable on the PCA model. We performed principal component analysis (PCA) on the normalized, mean centered, emission spectra from cow and goat milk powder samples to study the combinations of variables and comparison of the spectral patterns for cow and goat milk samples. We performed all PCA calculations on MATLAB® software by singular value decomposition (SVD) algorithm and analyzed the first 10 principal components.

3. Results

Fig. 1 presents typical reflectance fluorescence spectra for cow and goat milk powders, bovine milk's casein powder and anhydrous lactose powder on the excitation wavelengths of 270 nm (Fig. 1a) and 315 nm (Fig. 1b). For excitation at 270 nm, all milk powders samples presented a peak centered roughly on 335 nm and similar spectral form. Purified casein powder present spectra similar to milk powders with a peak centered at 333 nm. However, anhydrous lactose powder present two peaks at 355 and 410 nm and two shoulders at 310 and 480 nm (Fig. 1a).

The emission spectra of cow's and goat's milk powders for excitation at 315 nm present two peaks centered roughly on 410 and 468 nm and similar spectra form (Fig. 1b). Purified casein powder present spectra related to milk powders with peaks centered at 350 and 466 nm and a shoulder at 420 nm. However, anhydrous lactose powder present a distinct fluorescence with three peaks at 396, 467 and 557 nm.

We performed PCA on the fluorescence spectra of all milk powder samples for excitations at 270 nm (fluorescence acquired from 300 to 500 nm) and 315 nm (fluorescence acquired from 340 to 600 nm). PCA applied on the spectral data resulted in 10 principal components (PCs), which first four relative eigenvalues are detailed on Table 1. The first two PCs explained >80% of the total variation on all excitation wavelengths, which suggest a strong connection between spectral variables.

Fig. 2 shows the fluorescence spectra for all milk powder samples at excitation 270 nm (Fig. 2a), the PCA score plots for PC1 and PC2 (Fig. 2b), PC1 and PC3 (Fig. 2c), PC1 and PC4 (Fig. 2d), the PCA loadings for PC1 and PC2 (Fig. 2e), and the PCA loadings for PC3 and PC4 (Fig. 2f) calculated for excitation 270 nm.