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Short communication

Using whey proteins to detect the addition of bovine milk fat in buffalo cream destined for the butter-making process

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ABSTRACT

The addition of cow milk cream to buffalo cream, if not specified on the butter label, is a fraudulent practice. This adulteration is a common because cow cream has lower costs. A chromatographic method based on the detection of specific proteins (as described in DM 10/04/1996) was used to detect bovine cream in buffalo cream destined for the butter-making process.

The presence of cow proteins in buffalo cream at increasing percentages was confirmed by increased β-lactoglobulin (β-Lg) A levels (a variant absent in buffalo) and decreased Bx peaks (absent in cow).

β-Lg, an adulteration marker, allowed for the identification of 1% cow whey in buffalo whey.

The efficiency of the calibration curves was evaluated by analysing test samples prepared with known percentages of cow whey in buffalo whey (3, 8, 27 and 37%). The comparison between the empirical and theoretical values showed an error $\leq$3.1%.

The results obtained in this study demonstrate that this HPLC method is able to detect the addition of cow cream to buffalo cream. Therefore, it could be applied as a simple and rapid technique for the routine authentication of buffalo cream genuineness destined for the butter-making process.

1. Introduction

The addition of cow milk cream to buffalo cream during the butter-making process, if not specified on the product label, is a fraudulent practice. This adulteration is a common due to seasonal oscillations in buffalo milk availability and the lower price of cow milk. This illegal practice can promote negative consequences for individual health, specifically among those who are allergic to certain proteins (Woolfe & Primerose, 2004).

In recent years, a variety of methods have been developed to detect fraudulent milk mixtures from different species. These techniques include lipid (Saeed, Ali, Rahman, & Sawaya, 1989), protein (Addeo et al., 1995; Cozzolino, Passalacqua, Salemi, & Garozzo, 2002; Gonzàles-Córdova, Calderón de la Barca, Cota, & Vallejo-Córdova, 1998; Guarino et al., 2010) and DNA (Lanzilao, Burgalassi, Fancelli, Settimelli, & Fani, 2005; Saweyer, Wood, Shanahan, Gout, & McDowell, 2003) assays.

The official identification of cow milk in buffalo milk and cheese was reported in DM 10 April 1996. It was based on the detection of the specific whey protein β-lactoglobulin (β-Lg).

β-Lg is the major whey protein found in milk from ruminant species. It consists of a 162 amino-acid polypeptide with a molecular weight of approximately 18.3kD and is mainly present in a dimeric form under physiological conditions (Kontopidis, Holt, & Sawyer, 2004). β-Lg is characterized by genetic polymorphisms. Notwithstanding, 11 different genetic variants (A, B, C, D, E, F, G, H, I, J and W) have been identified; the A and B variants are associated with milk production performance and quality.

β-LG exhibits genetic polymorphisms in cows, though it is genetically invariant in Mediterranean water buffalo (Buffoni, Bonizzi, Pauciullo, Ramunno, & Feligini, 2011).

Two different genetic variants are characteristic of cow milk (A and B), while only one is characteristic of buffalo milk (B). Moreover, buffalo milk is characterized by a molecule called Bx, which is absent in cow milk (Pellegrino, De Noni, Tirelli, & Resmini, 1991, chap. 42).

No differences have been observed between buffalo and bovine β-Lg B (Zicarelli, 2004).

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The official method for detecting the addition of cow milk to buffalo milk and cheese was adapted in this study to identify a milk fatty derivative.

The objective of the study was to detect bovine cream in buffalo cream destined for the butter-making process by evaluating β-lactoglobulin levels.

2. Materials and methods

2.1. Sampling

Buffalo and cow creams of certain origins were sampled at Italian dairies.

Creams (30–42% fat) were obtained mainly from the milk skimming process. Approximately 1 kg of cream was collected from each dairy plant, and a total of 5 samples were sampled from species. The dairies were situated in different provinces of the Campania region. Cow and buffalo creams coming from different geographic areas were mixed separately to obtain representative samples of specific origins representing 100% pure cow cream and 100% pure buffalo cream.

2.2. Experimental plan

The 100% pure creams were subjected to whey extraction. Then, blends with increasing percentages of cow cream in buffalo cream were prepared as indicated in the experimental plan in Fig. 1.

2.3. Whey protein extraction

Whey was extracted with a simple and fast procedure.

Briefly, whey proteins were separated from cream by isoelectric precipitation of casein residues (at pH 4.2–4.6) and centrifugation at 8000 rpm for 5 min to remove also the lipid fraction.

The aqueous phase between the fat and casein that contained the whey proteins was filtered with Whatman paper and collected in 50 ml tube. The whey was then centrifuged at 8000 rpm for 5 min, and the supernatant was diluted 1:10 in ultra-pure water. The diluted whey was filtered through a 0.45 μm membrane. An aliquot was stored at −18 °C until HPLC analysis, and another aliquot was used for the blends preparation.

Cow/buffalo blends were prepared by mixing increasing percentages of cow cream with buffalo whey from 1 to 50% w/w.

The extracted wheys were analysed by HPLC-DAD to determine the whey proteins present.

For the quantification of cow whey in buffalo whey, the areas and heights of β-Lg and Bx peaks obtained from the blended samples were measured.

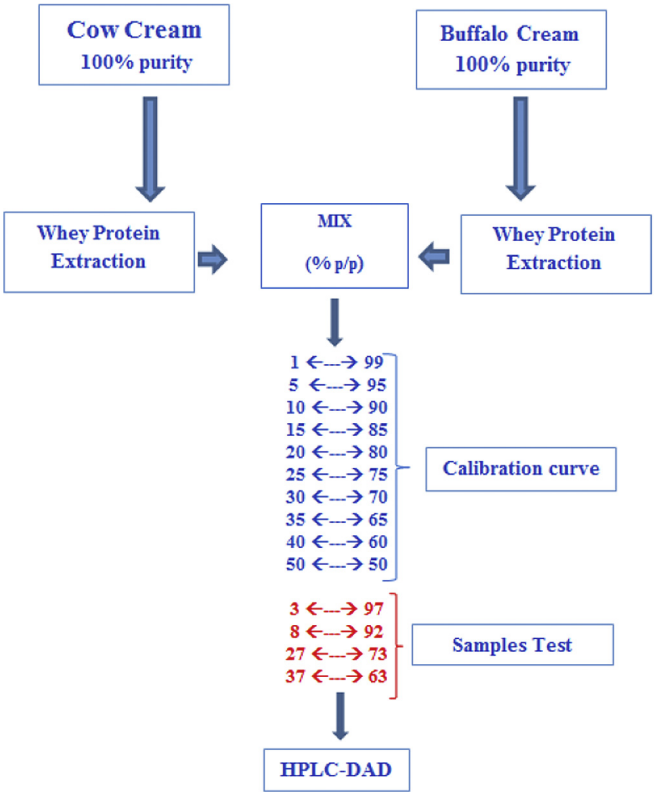

Fig. 1. Experimental plan.

2.4. Analytical method

The method described by Enne et al. (2005) with some modification was used. Briefly, an HPLC system (Agilent technologies, 1100) equipped with two pumps, a manual injector (20-μl loop) and an UV detector was used.

The separation was performed on a Jupiter C4 column (250 mm × 4.6 mm, 300 Å, 5 μm) (Phenomenex, Torrance, CA, USA) kept at room temperature. The detection wavelength was set at 205 nm. Mobile phases were A: Water +0.1% trifluoroacetic acid (TFA) and B: acetonitrile + 0.1% TFA.

The elution gradient was set as follows: 0–1 min 35% B, 1–8 min 35–38% B, 8–16 min 38–42% B, 16–27 min 42–46% B, 27–29 min 46–90% B, 29–30 min 90% B, 30–35 min 90–35% B, 35–40 min 35% B at a flow rate of 1 ml/min.

All reagents were of analytical and chromatographic grade and were supplied by Sigma Aldrich (St Louis MO).

Purified cow standard proteins of β-Lg (50% β-Lg A and 50% β-Lg B, Sigma Aldrich, St. Louis, MO, USA) was used to identify the chromatographic peaks.

For the quantification of cow whey in buffalo whey, the areas and the heights of β-Lg and Bx peaks obtained for blends samples were measured.

2.5. Method validation

The method was validated for its repeatability, reproducibility, linearity and efficiency.

Two different calibration curves were plotted for the increasing amounts of cow whey in buffalo whey; the first one for the addition of 1–15% cow whey and the second for the addition of 20–50% cow whey.

The β-Lg A/Bx height ratios were calculated from the addition of 1–15% cow whey in buffalo whey, and the β-Lg A/β-Lg B areas ratios were calculated from the addition of 20–50% cow whey in buffalo whey.

2.6. Statistical analysis

All determinations and experiments were performed in triplicate, and the presented results are the average values from three replicates. The coefficient of linearity (R^2) was obtained using the Microsoft Excel 2000 program (Microsoft Corporation, Redmond, WA, USA). To validate the method, a standard analysis with β-Lg was repeated 5 times. Analysis of variance (ANOVA) was carried out using XLSTAT 2006, version 2006.6 (ADDINSOFT, Paris, France) to compare the obtained data.