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Chromatography methods and chemometrics for determination of milk fat adulterants

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Abstract. Milk and milk-based products are among the leading food categories according to reported cases of food adulteration. Although many authentication problems exist in all areas of the food industry, adequate control methods are required to evaluate the authenticity of milk and milk products in the dairy industry. Moreover, gas chromatography (GC) analysis of triacylglycerols (TAGs) or fatty acid (FA) profiles of milk fat (MF) in combination with multivariate statistical data processing have been used to detect adulterations of milk and dairy products with foreign fats. The adulteration of milk and butter is a major issue for the dairy industry. The major adulterants of MF are vegetable oils (soybean, sunflower, groundnut, coconut, palm and peanut oil) and animal fat (cow tallow and pork lard). Multivariate analysis enables adulterated MF to be distinguished from authentic MF, while taking into account many analytical factors. Various multivariate analysis methods have been proposed to quantitatively detect levels of adulterant non-MFs, with multiple linear regression (MLR) seemingly the most suitable. There is a need for increased use of chemometric data analyses to detect adulterated MF in foods and for their expanded use in routine quality assurance testing.

1. Introduction

Milk fat (MF) is largely composed of triacylglycerols (TAGs) (these comprise about 98% of the total fat), while phospholipids account for only 0.8% of milk lipids. Sterols are also a minor component, comprising about 0.3% of the fat, cholesterol being the principal sterol [1]. The fat content of milk can vary from about 3.0% to 6.0%, but typically is in the range 3.5 to 4.7% [2]. In addition to the well-characterized differences among and within breeds of dairy cattle in MF content and fatty acid (FA) composition i.e. genetic factors, differences can also occur due to stage of lactation and diet [3-7].

MF is a good source of fat-soluble vitamins and essential FAs [8]. Fat is an essential component of the human diet and inclusion of MF as part of a balanced diet should be advantageous rather than detrimental. To date, no scientific study has produced evidence of any increased risk of disease associated with milk consumption [9]. Cow's milk is an important component of the human diet because of its high nutritional value. Its nutritionally balanced composition makes it one of the most complete foods available [10]. Ruminant MF contains butyric acid (C4:0), which is an important anti-cancer agent. However, longer-chain FAs can be problematic; e.g., myristic (C14:0) and palmitic acids (C16:0) are considered harmful, while stearic acid (C18:0) and short-to-medium-chain (C4–C10) FAs are deemed neutral [9]. Up to one-third of the FAs in MF have a chain-length of 14

carbons or less. These FAs are oxidized rapidly in the liver, have a lower energy value and are oxidized more readily than long-chain FAs. It follows that MF should contribute less to obesity than an equivalent amount of other dietary fats [11]. This reinforces the need for the dietetic community to reconsider current recommendations on dairy fat and human health on the basis of scientific evidence [2]. In conclusion, whereas future studies will help to elucidate the role of milk and dairy products in human health, their use within a balanced diet should be considered in the absence of clear recommendations [9, 11].

MF contains several compounds that have demonstrated anticancer activity in animal models [12]. The more important ones are rumenic acid (RA, *cis*-9, *trans*-11 conjugated linoleic acid, CLA) [13-14], a potent inhibitor of mammary tumorigenesis, sphingomyelin and other sphingolipids that prevent the development of intestinal tumors and butyric acid (C4:0), which prevents colon and mammary tumor development [4, 14-17]. Cows' diets have a major influence on the CLA content of MF, and these effects have been recently summarized [4, 14, 17-18]. Emerging evidence suggests that MF can prevent intestinal infections, particularly in children, prevent allergic disorders, such as asthma and improve the level of long-chain n-3 polyunsaturated fatty acids in blood [19].

This paper discusses analytical methods for detecting MF adulteration, with an emphasis on GC analysis of TAG and FA in combination with multivariate statistical data processing. Such data could be potentially useful in detecting foreign fats in the milk and dairy industries. Based on this preliminary investigation, the usefulness of this approach could be examined in the future for other foreign fats and oils, including vegetable and animal fat.

2. Adulteration of milk and dairy products

Milk adulteration is a current fraudulent practice to mask the quality parameters (e.g. protein and fat content) and increase the product shelf life. Milk and milk-based products are among the leading food categories according to reported cases of food adulteration [20]. Perhaps the most high-profile case involved the addition of melamine to high-protein feed and milk-based products to artificially inflate protein values in products that may have been diluted [21]. Melamine, an organic base, is widely used in plastics, adhesives, and other consumer products, and is known to pose a public health threat [22]. Adulterated milk could also be added into infant formula and other milk-based products. Baby formula is a common target for retail fraud, often by tampering with the sell-by codes to move expired product. The safety and integrity of dairy products is of particular interest, because these foods play an important role in feeding the population and are essential for certain groups of consumers, such as women, children and the elderly. Milk is a fairly expensive raw material, and from an economic point of view it could, therefore, be attractive to fraudulently modify its composition, replacing part of it with other dairy or non-dairy ingredients [23, 24].

The major adulterants of MF are vegetable oils (soybean, sunflower, groundnut, coconut, palm and peanut oil) and animal fat (cow tallow and pork lard). Butter is made from milk, whereas butter substitutes, also called imitation butters, are generally manufactured from non-dairy fats or other suitable components to make butter-like products [25-27]. Dairy products have been traded for hundreds of years and make up a large proportion of the food industry trade. However, the adulteration of milk and butter is a major issue for the dairy industry. Adulteration of milk used to manufacture butter can result in an inferior final product that fails to meet consumer expectations. Adulterated milk and butter contain added substances such as water, neutralizers to mask acidity, salt or sugar to mask extra water or high solid contents, whey and hydrogen peroxide, among others [28]. Although many authentication problems exist in all areas of the food industry, adequate control methods are required to evaluate the authenticity of milk and milk products in the dairy industry. One method to detect adulteration of milk with water is measurement of osmolality [29]. Rezende *et al.* [30] stated that the refractive index method for water adulteration could adequate such as density and freezing point determinations. Mid infrared (MIR) spectroscopy combined with pattern recognition analysis were used to classify and quantify milk adulteration with whey, synthetic urea or hydrogen peroxide [28]. The addition of NaOH to milk, which aims to mask acid formation, is very easy to determine using

principal component analysis (PCA) to separate control and adulterated milks [31]. However, it is important to mention that the goal is not to identify a specific adulterant and its concentration, but the presence of a group of adulterants.

3. Methods for detecting the authenticity of milk and dairy products

Quality assurance (QA) and the methods used to authenticate foodstuffs are of great interest both from commercial and legal points of view [32]. In Europe, origin is one of the main authenticity issues concerning food. Determination of food authenticity is an important issue for both QA and food safety. Interest in Europe concerning food authentication is also shown by continuous funding of this topic, from FP 5 to the Horizon 2020 initiative. Authenticity testing is a quality criterion for food and food ingredients and is increasingly a result of legislative protection of regional foods. Thus, there is a pressing need for accurate, standardized food authentication techniques [32].

Over the last decade, several analytical procedures have been proposed for rapid screening or selective confirmation of the quality and authenticity of milk such as liquid chromatography (LC) and GC, especially coupled with mass spectrometry (MS). The studies are often supported by a chemometric approach allowing reliable qualitative (classification) and quantitative (multivariate calibration) procedures. GC flame ionization detection is exploited for analysis of MF because the milk FAs and TAGs can be monitored and compared with reference standards. This procedure can be used to discriminate the source of adulteration. GC separation of the TAG classes in MF according to their carbon number (CN) (C24–C54) has been used to determine milk origins and the potential adulteration of dairy products with foreign fats [25, 33]. Adulteration of expensive oils and fats, such as MF, has always been a serious problem because of the economic advantages of replacing high-priced fats and oils with low-priced oils, including soybean oil or corn oil, without labeling the product accordingly [25, 27]. Consequently, the European Union made the GC methodology official, converting it to a reference method for the detection of foreign fat in MF by TAG analysis using short capillary column GC (more polar polysiloxane phases containing a higher proportion of phenyl groups (50–65%) and low blending at temperatures as high as 370-400°C) [34]. Because of the wide variety of FAs contained in MF, the characterization of TAGs in MF is a complex and difficult task [35]. Before quantitative analysis, TAGs must be grouped on the basis of some of their common characteristics (molecular weight, degree of unsaturation, etc.). Moreover, the GC analysis of TAG or FA profiles of MF in combination with multivariate statistical data processing has been used to detect adulterant fats in milk and dairy products [25, 27, 36, 37, 39]. GC analysis of TAG was also used to detect goat cheese adulterated with cheaper cow`s milk [40].

4. Chemometrics as a tool to identify milk fat adulteration

Chemometrics is an interdisciplinary research field that involves multivariate statistics, mathematical modelling and computing especially applied to chemical data, and is required for food authentication or identity confirmation. It must be combined with suitable database infrastructure and uses appropriate mathematical tools [41]. Chemometrics has been useful in evaluating the quality and identity control of processing parameters for dairy products [41-44]. For this approach, a large set of analyses and many pattern classification procedures, such as PCA, linear discriminant analysis (LDA), hierarchical cluster analysis (HCA), soft independent modelling of class analogies (SIMCA), partial least squares regression (PLS), canonical variate analysis (CVA), and artificial neural network (ANN) can be utilized. Pattern analyses are applied to a dataset to compare similarities or differences of sample data with original data. GC analysis of the FA profile is widely used to detect adulteration of MF with foreign fats. For example, Rebechi *et al.* [37] artificially adulterated MF with 0, 2, 5, 10 and 15% tallow or lard. Their multiple linear regression (MLR) detected adulterations of MF at levels greater than 10% for tallow and 5% for lard. Gutiérrez *et al.* [25] adulterated raw MF with 0, 5, 10, 15, and 20% non-MFs. When LDA was used, the global percentage of satisfactory classification was 94.4%; consequently, LDA was effective in detecting adulterations at levels <10%. Kim *et al.* [27] used specific bio-markers (FAs, TAG and cholesterol) which enabled the detection of adulteration as

low as 10% of non-MFs in MF. The validity of the classification rule was also tested by 206 gravimetrically prepared fat mixtures. These data can be potentially useful in detecting foreign fats in butter products. In the work of Lipp [36], GC analysis of TAG was further analyzed by PLS and ANN to identify mixtures of butter fat with foreign fat. While ANN was most suitable for classification, quantitative results were obtained by PLS. Souza *et al.* [42] identified groups of adulterants, including formaldehyde, starch, urine, hydrogen peroxide and chlorine, by physicochemical analysis and application of PCA and HCA.

The results obtained in this research should contribute to a proposal for a national standard to verify MF authenticity in milk and dairy products. Theoretically, after multivariate analysis, and taking into account many analytical factors, adulterated MF should form singular groups that can be easily distinguished from authentic MF.

5. Conclusion

Cow's milk is an important component of the human diet because its nutritionally balanced composition makes it one of the most complete foods available. Milk and milk-based products are among the leading food categories according to reported cases of food adulteration. Although many authentication problems exist in all areas of the food industry, adequate control methods are required to evaluate the authenticity of milk and milk products in the dairy industry. This paper discusses analytical methods for detecting MF adulteration with an emphasis on using GC to analyze TAG and FA in combination with multivariate statistical data processing. Using multivariate statistical methods such as MLR, adulterations of MF at levels as low as 10% of non-MF can be detected. With respect to aduteration, chemometrics is a powerful data reduction tool used to qualitatively group or classify unknown MF samples with similar characteristics and to quantitatively determine levels of adulterant analytes in MF. The results obtained in this research should contribute to a proposal for a national standard to verify MF authenticity in milk and dairy products.

References

[1] Mac Gibbon A K H and Taylor M W 2006 *Advanced Dairy Chemistry, Volume 2, Lipids,* ed P F Fox and P L H McSweeney (New York, Springer, USA) pp 1-42
[2] Lock A L and Bauman D E 2011 *Proc. Cornell Nutr. Conf. for Feed Manuf.* (New York, Cornell University Ithaca) pp 126-35
[3] White S L, Bertrand J A, Wade M R, Washburn S P, Green Jr J T and Jenkins T C 2001 *J. Dairy Sci.* **84** 2295-301
[4] Kelsey J A, Corl B A, Collier R J and Bauman D E 2003 *J. Dairy Sci*. **86** 2588-97
[5] Auldist M J, Johnston K A, White N J, Fitzsimons W P and Boland M J 2004 *J. Dairy Res.* **71** 51-7
[6] Kay J K, Weber W J, Moore C E, Bauman D E, Hansen L B, Chester-Jones H, Crooker B A and Baumgard L H 2005 *J. Dairy Sci.* **88** 3886- 93
[7] Soyeurt H, Dardenne P, Gillon A, Croquet C, Vanderick S, Mayeres P, Bertozzi C. and Gengler N 2006 *J. Dairy Sci.* **89** 4858-65.
[8] Jensen R G, Ferris A M and Lammi-Keefe C J 1991 *J. Dairy Sci*. **74** 3228–43
[9] Visioli F and Strata A 2014 *Adv. Nutr.* **5** 131-43
[10] Palmquist D L 2006 *Advanced Dairy Chemistry, Volume. 2, Lipids, ed* P F Fox and P L H McSweeney (New York, Springer, USA) pp 43-80
[11] Parodi P W 2004 *Aust. J. Dairy Technol*. **59** 3-59
[12] Parodi P W 1997 *J. Nutr.* **127** 1055–60
[13] Ip C, Banni S, Angioni E, Carta G, McGinley J, Thompson H J, Barbano D and Bauman D 1999 *J. Nutr.* **129** 2135-42
[14] Griinari J M and Bauman D E 1999 *Advances in Conjugated Linoleic Acid Research, Vol. 1, ed* M P Yurawecz, M M Mossoba *et al*. (Champaign, IL, AOCS Press, USA) pp 180-200

[15] Parodi P W 1999 *J. Dairy Sci.* **82** 1339–49
[16] Bauman D E, Baumgard L H, Corl B A and Griinari J M 1999 *Proc. Amer. Soc. Anim. Sci.* (Champaign, IL USA) pp1-15
[17] Bauman D E, Corl B A, Baumgard L H and Griinari J M 2001 *Recent Advances in Animal Nutrition*, ed P C Garnsworthy and J Wiseman (Nottingham, Nottingham University Press, UK) pp 221-50
[18] Bauman D E and Lock A L 2006 *Advanced Dairy Chemistry, Volume. 2, Lipids, ed* P F Fox and P L H McSweeney (New York, Springer, USA) pp 93-136
[19] Parodi P W 2006. *Advanced Dairy Chemistry, Volume 2 Lipids,* ed P F Fox and P L H McSweeney (New York, Springer, USA) pp 601-39
[20] Johnson R 2014. *Food Fraud and "Economically Motivated Adulteration" of Food and Food Ingredients* (Washington D.C, Congressional Research Service, Library of Congress, USA) pp 1-40
[21] Sharma K and Paradakar M 2010 *Food Secur.* **2** 97–107
[22] Bottemiller H 2011 Food Safety News, April 29, (Available at: http://www.foodsafetynews.com/2011/04/chinese-authorities-seize-more-melamine-tainted-dairy/#.WMfu5G8rLcs)
[23] De La Fuente M A and Juárez M 2005 *Crit. Rev. Food Sci.* **45** 563–85
[24] Karouri R and Baedemaeker J 2007 *Food Chem.* **102** 621–40
[25] Gutiérrez R, Vega S, Díaz G, Sánchez J, Coronado M, Ramírez A, Pérez J, González M and Schettino B 2009 *J. Dairy Sci.* **92** 1846–55
[26] Molkentin J 2007 *Eur. J. Lipid Sci. Technol.* **109** 505-10
[27] Kim J M, Kim H J and Park J M 2015 *J. Food Sci.* **80** C1945- 51
[28] Santos P M, Pereira-Filho E R and Rodriguez-Saona L E 2013 *Food Chem.* **138** 19–24
[29] Büttel B, Fuchs M and Holz B 2008 *Chem. Cent. J.* **2** 6-12
[30] Rezende P S, Carmo G P and Esteves E G 2015 *Talanta* **138** 196–202
[31] Santos P M, Wentzell P D and Pereira-Filho E R 2012 Scanner digital images combined with color parameters: A case study to detect adulterations in liquid cow's milk. *Food Anal. Method* **5** 89–95
[32] Danezis G P, Tsagkaris A S, Camin F, Brusic V. and Georgiou C A 2016 *TrAC Trends Anal. Chem.* **85** Part A 123–32
[33] Fontecha J, Ríos J J, Lozada L, Fraga M J and Juárez M 2000 *Int Dairy J.* **10** 119-28
[34] Commission Regulation (EC) No 273/2008 *Official Journal of the European Union* 2008, *Annex XX, Article 16,* L88/97-L88/106
[35] Mottram H R and Evershed R P 2001 *J. Chromatogr.* A **926** 239–253
[36] Lipp M 1996 *Food Chem.* **55** 389-395
[37] Rebechi S R, Vélez A, Vaira S and Perotti M C 2016 *Food Chem.* **192** 1025–32
[38] Fraga M J, Fontecha J, Lozada L and Juárez M 1998 *J. Agr. Food Chem.* **46** 1836– 43
[39] Vieitez I, Irigaray B, Callejas N, González V, Gimenez S, Arechavaleta A, Grompone M and Gámbaro A 2016 *J. Food Compos Anal.* **48** 95–101
[40] Bertacchini L, Cocchi M, Vigni M L, Marchetti A, Salvatore E, Sighinolfi S, Silvestri M and Durante C 2013 *Chemometrics in Food Chemistry,* ed F Marini (Oxford, Elsevier, UK) pp 371-410
[41] Faye B, Konuspayeva G, Messad S and Loiseau G 2008 *Dairy Sci. Technol.* **88** 607–17
[42] Souza S S, Cruz A G, Walter E H M, Faria J A F, Celeghini R M S, Ferreira M M C, Granato D and de S. Sant'Ana A 2011 *Food Chem.* **124** 692–5
[43] Sacco D, Brescia M A, Sgaramella S, Casiello G, Buccolieri A, Ogrinc N and Sacco A 2009 *Food Chem.* **114** 1559–63
[44] Sola-Lanarranga C and Navarro-Blasco I 2009 *Food Chem.* **112** 189–96