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# Adulteration of Argentinean milk fats with animal fats: Detection by fatty acids analysis and multivariate regression techniques

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ABSTRACT

The aims of the present study were to test the accuracy of the fatty acid ratios established by the Argentinean Legislation to detect adulterations of milk fat with animal fats and to propose a regression model suitable to evaluate these adulterations. For this purpose, 70 milk fat, 10 tallow and 7 lard fat samples were collected and analyzed by gas chromatography. Data was utilized to simulate arithmetically adulterated milk fat samples at 0%, 2%, 5%, 10% and 15%, for both animal fats. The fatty acids ratios failed to distinguish adulterated milk fats containing less than 15% of tallow or lard. For each adulterant, Multiple Linear Regression (MLR) was applied, and a model was chosen and validated. For that, calibration and validation matrices were constructed employing genuine and adulterated milk fat samples. The models were able to detect adulterations of milk fat at levels greater than 10% for tallow and 5% for lard.


## 1. Introduction

Bovine milk fat is regarded as one of the most complex fats containing a wide range of compounds. Lipids in bovine milk fat are present as globules emulsified in the aqueous phase of milk. The fat content varies as a result of changes in factors like breed of cow, diet and stage of lactation, but typically is in the range 3.5–4.7%. It is composed of triacylglycerols (TAG), diacylglycerols (DG), free fatty acids (FFA), phospholipids, and other minor components (MacGibbon & Taylor, 2006; Jensen, 2002). TAG are the most important components, accounting for about 98% of the total fat. They have a complex composition, due to the large number of possible fatty acids (FA) combinations on the glycerol backbone, more than 400 different FA have been identified. Most of them are present in very small quantities (<0.01%); however, about 15 FA are present at concentrations above 1.0% (Van Ruth, Villegas et al., 2010; MacGibbon & Taylor, 2006; Jensen, 2002).

Milk fat (MF) is one of the most expensive components of milk and therefore its characterization is an important issue in order to guarantee a constant well-defined quality. Detection of MF adulterations with other less expensive oils and fats has always been a challenge, because of the natural variability of milk fat chemical composition (Van Ruth et al., 2010; Lipp, 1996). Among the different ways of adulteration of milk fat, those made with animal fats are the most difficult to detect (Kumar, Lal, Seth, & Sharma, 2009; Lipp, 1996; Ulberth, 1995, 1994). Analytical techniques that have been proposed for this fact include the determination of physicochemical properties, constituents of unsaponifiable matter and water-soluble and insoluble volatile fatty acids (Van Ruth, Bremer, & Frankhuizen, 2010; Ulberth, 2000). Moreover, the gas chromatography (GC) analysis of TAG or FA profiles of MF in combination with multivariate statistical data processing have been used to detect adulterations in milk and dairy products with foreign fats (Van Ruth, Villegas et al., 2010; Fontecha, Mayo, Toledano, & Juárez, 2006; Goudjil, Fontecha, Fraga, & Juarez, 2003; Povolo, Bonfitto, Contarini, & Toppino, 1999; Fontecha, Díaz, Fraga, & Juárez, 1998; Ulberth, 1995, 1994; Precht, 1992).

Milk and dairy products production in Argentina is relevant; our country is located in the tenth position worldwide in milk production and in the second position in Latin America (FAO, 2014). In the central and east-central regions of Argentina dairy food manufacture is one of the major economic resources as it accounts for 70% of milk production (MinAgri – Argentina, 2015). Adulteration of MF with animal fats (as tallow or lard) is a serious problem that has not been solved yet. Despite this fact, there is scarce information related to genuine MF characterization and MF adulterations (Páez, Cuatrin, Taverna, Moretto, & Campos, 2006; Maritano de Correche, Oxley, & Fernández, 1985); statistical approaches reported about this topic are not available. The Argentinean Legislation (Código Alimentario Argentino CAA Art. 555 bis) establishes some specifications to characterize and detect adulterations

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of bovine milk fat, such as refractive, saponification, iodine, Reichert-Meissl and Polenske indices, fatty acids relations ($C_{10:0}/C_{8:0}$, $C_{12:0}/C_{10:0}$, $C_{14:0}/C_{12:0}$ and $C_{14:0}/C_{18:1}$) and sterols content (ANMAT, 2011) However, it is known that most of them are successful to detect only massive adulterations (Ulberth, 2000).

The aims of this work were to check the accuracy of the FA relations proposed by CAA and to obtain suitable mathematical regression models employing chromatographically determined fatty acid data, in order to improve the detection of adulterations of bovine milk fat with tallow and lard fats.

## 2. Materials and methods

### 2.1. Samples analyzed and treatment

Seventy genuine milk fats (MF) of fluid milks ($n = 20$) and butters ($n = 50$) were collected during a period of three years from important dairies located in the central dairy area of Argentina. Seventeen non-milk fats (NMF), ten from tallow and seven from lard, were obtained from local suppliers and used as adulterants.

Milk samples were centrifuged and thermal treated to release the cream fraction, which was beaten vigorously to obtain butter (Murphy, Mc Neill, Convolly, & Gleeson, 1990). All samples (butter, tallow and lard) were processed according to ISO 14156 (1999). For that, the samples were placed at an oven at 50–60 °C for 2 h to allow melting and fat separation, fats were then filtered at the same temperature in the presence of anhydrous $Na_2SO_4$, and stored at −18 °C until analysis.

### 2.2. Fatty acid analysis by gas chromatography

The glycerides present in the MF and NMF samples were transesterified by acid-catalyzed ethanolysis according to IDF (IDF, 1999), with some modifications. Briefly, a volume (1 mL) of a solution of fat in *n*-hexane (10% w/v) was put into a screw cap tube, and 5% $H_2SO_4$–EtOH v/v (3.2 mL) was added. The reaction was performed at 70 ± 2 °C for 3 h. The fatty acid ethyl esters (FAEE) were extracted from the upper organic phase after addition of water (6 mL) and analyzed by GC/FID.

Gas chromatograph (Perkin Elmer model 9000, Massachusetts, USA) with a flame ionization detector and split/splitless injector was employed. FAEE were separated using a PE-Wax fused-silica capillary column (polyethylene glycol, 30 m × 0.25 mm × 0.25 μm). The column temperature was held at 50 °C for 4 min, increased at 10 °C/min to 150 °C, held at 150 °C for 3 min, increased at 10 °C/min to 230 °C, and held at 230 °C for 5 min. Nitrogen was the carrier gas with a flow of 3 mL $min^{-1}$ and a split ratio of 1/50. The temperatures of FID and injector were 275 and 220 °C, respectively.

Sixteen fatty acids ($C_{4:0}$, $C_{6:0}$, $C_{8:0}$, $C_{10:0}$, $C_{10:1}$, $C_{12:0}$, $C_{14:0}$, $C_{14:1}$, $C_{15:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:2conj.}$ and $C_{18:3}$) were quantified with heptanoic and margaric acids as internal standard (0.8 mL of $C_{7:0}$ and 4.0 mL of $C_{17:0}$ from individual standard solutions of 0.5 mg $mL^{-1}$; Sigma Aldrich, St. Louis, USA) added to the samples at the transesterification step.

The FID outsignal was recorded and the chromatograms were processed using Turbochrom v. 4. Software (Perkin Elmer Corp. Waltham, Massachusetts, USA). The concentration of each FA was reported as g per 100 g of anhydrous fat.

### 2.3. Preparation of adulterated milk fats

Adulterated milk fat samples (AMF) were simulated arithmetically employing the chromatographic profiles of milk fats (MF) and non-milk fats (NMF) and applying the following equation established by Timms (1980):

$$C_{iAMF} = X\,C_{iNMF} + (1 - X)C_{iMF}$$

where $C_{iAMF}$, $C_{iNMF}$, $C_{iMF}$ are the concentrations of the fatty acid "i" in the AMF, NMF and MF respectively, and X is the proportion of NMF in the adulterated milk fat (0.02, 0.05, 0.10, 0.15) which corresponds to 2%, 5%, 10% or 15% of adulteration. These adulteration percentages were defined taking into account that low levels of adulteration are difficult to detect with conventional methods, i.e. classical indices (Ulberth, 2000). In this way, to prepare samples adulterated with 2% of tallow, each tallow profile ($n = 10$) was numerically blended with each genuine milk fat profile ($n = 70$), giving a total of 700 AMF samples. Similarly, samples adulterated with 5%, 10% and 15% of NMF were calculated, having a total of 700 AMF samples for each level of adulteration.

For the case of lard, samples adulterated with 2%, 5%, 10% and 15% were obtained by numerically blending each lard profile ($n = 7$) with each genuine milk fat profile ($n = 70$), giving a total of 490 AMF samples for each level of adulteration.

### 2.4. Data processing

First of all, we applied an exploratory analysis on the 70 profiles of genuine MF using the principal component analysis (PCA), in order to visualize the grouping of samples and identify outliers (Di Anibal, Odena, Ruisánchez, & Callao, 2009; Purcell, Leonard, Óshea, & Kokot, 2005).

Subsequently, from the profiles of genuine and adulterated milk samples the fatty acids ratios established by CAA were calculated. In addition, the profiles were arranged in data matrices for each adulterant in order to study the adulteration of milk fat by applying Multiple Linear Regression (MLR).

The Unscrambler 7.6 (CAMO AS, Norway) and SPSS 17.0 (SPSS Inc., Chicago, USA) software packages were used.

#### 2.4.1. Principal component analysis (PCA)

PCA was carried out with the correlation matrix, in which variables were standardized giving them equal weighting. Therefore, it was possible to capture the effect of all the variables rather than the effect of a few variables with a comparatively large internal variance (Coker, Crawford, Johnston, Singh, & Creamer, 2005; Pripp, Stepaniak, & Sorhaug, 2000).

#### 2.4.2. Fatty acid ratios

Four FA ratios, $C_{10:0}/C_{8:0}$, $C_{12:0}/C_{10:0}$, $C_{14:0}/C_{12:0}$ and $C_{14:0}/C_{18:1}$, established by CAA to detect animal fat in milk fat, were calculated for MF and for all blended samples. The values obtained were compared with those fixed by the legislation.

#### 2.4.3. Multiple linear regression

MLR was applied to generate mathematical models based on the FA concentrations. The concentrations of sixteen fatty acids quantified for MF and calculated for AMF were considered as the independent variables and the percentage of adulteration of milk fat (A%) was defined as the dependent variable. A value of 0% was arbitrarily assigned to the genuine milk fat and values of 2%, 5%, 10% or 15% to corresponding AMF samples.

MLR is used to determine the relationship between multiple independent predictor variables and a dependent variable. At a first step, calibration is performed to build a mathematical model; then, the model is validated in a prediction step (Ragno, Ioele, & Risoli, 2004; Thomas, 1994). If there is collinearity between the predictor variables the regression coefficients may be poorly estimated as minor fluctuations in the data set have a major impact on the estimation. In this case, it is convenient to use variable

selection methods, which identify the independent variables (with a minimal correlation between them) that are highly correlated to the dependent variable (Coker et al., 2005). In the present study, for each adulterant, fifty samples were chosen by chance to serve as a calibration data matrix, and in the same way, twenty samples were chosen to construct a prediction data matrix. Backward, stepwise and best subset regression models methods were used and compared in order to find the best model. In this sense, the statistical significance of the screened models was judged in terms of mean square error (MSE or $S^2$) and square of the correlation coefficient ($R^2$). Also, the multicollinearity was evaluated by VIF (Variance Inflation Factor) and the partial correlations between prediction variables (Hair, Anderson, Tatham, & Black, 1999, chap. 4). Thus, for the selected model from each method, the root mean square error of calibration (RMSEC) was calculated. It is an indicator of the average error that infers how well the model fits to the data, and is defined by the following formula:

$$\mathrm{RMSEC} = \sqrt{\sum_{i=1}^{n} \frac{(\hat{y}_i - y_i)^2}{n}} = \sqrt{\mathrm{MSE}} = S$$

where $\hat{y}_i$ is the predicted percentage of adulteration in the calibration sample $i$, $y_i$ is the theoretical percentage in the calibration sample $i$ and $n$ is the number of calibration samples (Rodríguez-Nogales & Vázquez, 2007; Rodríguez-Nogales, 2006; Ragno et al., 2004; Hair et al., 1999; Massart, Vandeginste, Deming, Micote, & Kaufman, 1988, Chap. 13; Myers, 1986, Chaps. 3 and 7).

The predictive power of the model was evaluated by RMSECV (root mean square error of calibration of cross-validation), defined by:

$$\mathrm{RMSECV} = \sqrt{\sum_{i=1}^{n} \frac{(\hat{y}_{(i)} - y_i)^2}{n}}$$

where $\hat{y}_{(i)}$ is the predicted percentage of adulteration when the model is constructed without sample $i$, $y_i$ is the theoretical percentage in the calibration sample $i$ and $n$ is the number of calibration samples.

However, an external validation was also applied using the prediction matrix, as the real predictive ability of the model cannot be judged solely by using internal validation (Rodríguez-Nogales, 2006; Ragno et al., 2004). The root mean square error of prediction (RMSEP) was the criterion utilized to evaluate the suitability of the built models, which is defined by the following formula:

$$\mathrm{RMSEP} = \sqrt{\sum_{i=1}^{m} \frac{(\hat{y}_i - y_i)^2}{m}}$$

where $\hat{y}_i$ is the predicted percentage of adulteration in the prediction sample $i$, $y_i$ is the theoretical percentage in the prediction sample $i$ and $m$ is the number of prediction samples.In addition, the prediction intervals were calculated for the samples included in the prediction matrices applying the following expression (Myers, 1986):

$$\hat{y}_{(x0)} \pm t_{\alpha/2;n-k-1}\ \mathrm{SS}\,\hat{y}_{(x0)}$$

where $\hat{y}_{(x0)}$ is the percentage of adulteration calculated with the model selected for an individual prediction sample ($x0$); $t_{\alpha/2;n-k-1}$ is the value of the Student $t$ distribution with $n-k-1$ degrees of freedom with a $\alpha/2$ confidence level, and SS $\hat{y}_{(x0)}$ is the standard error of prediction for the model.

## 3. Results and discussion

### 3.1. Fatty acid profile and fatty acid ratios of genuine milk fats

The FA composition of the genuine milk fats ($n$ = 70) characterized by 16 FAs are shown in Table 1. The values obtained are comparable to those published by the Instituto Nacional de Tecnología y Agricultura (INTA) for Argentinean milk fat (Páez et al., 2006; Maritano de Correche & Oxley, 1985).

In Fig. 1 are shown, as an example, three typical chromatograms obtained for genuine milk fat, lard and tallow. The FA profiles are characteristic for each type of fat (Gunstone, Hardwood, & Dijkstra, 2007).

Besides, PCA method was applied to the profiles of 70 genuine milk samples to display variability. The results showed that 90.6% of the total data variance could be explained using five principal components (data not shown). It could be observed that samples were not separated definitely by season and no outliers were found.

On the other hand, the concentration ratios proposed by CAA to characterize milk fat were calculated for all samples and expressed as ranges; the values obtained were compared with normal ranges established by CAA (Table 2). As can be seen, the minimum and maximum values obtained for $C_{12:0}/C_{10:0}$ and $C_{14:0}/C_{18:1}$ ratios were included into the normal ranges for 100% samples; while for $C_{10:0}/C_{8:0}$ and $C_{14:0}/C_{12:0}$ ratios, more than 97.5% of samples were within normal ranges. Indeed, the majority of genuine milk fat samples analyzed in this work accomplished the values established by CAA. Pinto et al. (2002) and Ulberth (1994) analyzed genuine milk samples from Chile and Austria and calculated several FA ratios, establishing genuineness ranges to characterize their local milk FA profiles; however, the values obtained were not compared with legal values.

### 3.2. Fatty acid ratios for adulterated milk fat

The four concentration ratios were also calculated for all adulterated milk samples and the percentages of samples that were outside CAA ranges are shown in Table 2. For tallow and lard adulterations, admixtures from 2% to 15% AMF could not be detected by the ratios $C_{10:0}/C_{8:0}$ and $C_{12:0}/C_{10:0}$. For $C_{14:0}/C_{12:0}$ and $C_{14:0}/C_{18:1}$ ratios, the percentages of samples detected outside the normal ranges increased as the level of adulteration increased from 5% to 15%. In particular, the percentages of detected samples adulterated with tallow and lard ranged from 1.8% to 20.0% and 6.9% to 34.3%,

**Table 1**
Fatty acid composition of 70 genuine milk fat (g FA/100 g milk fat).

| Fatty acids | Mean | Minimum | Maximum |
|---|---|---|---|
| $C_{4:0}$ | 3.56 | 2.83 | 4.30 |
| $C_{6:0}$ | 2.13 | 1.86 | 2.37 |
| $C_{8:0}$ | 1.11 | 0.91 | 1.31 |
| $C_{10:0}$ | 2.38 | 1.74 | 3.03 |
| $C_{10:1}$ | 0.29 | 0.22 | 0.35 |
| $C_{12:0}$ | 2.96 | 2.14 | 3.89 |
| $C_{14:0}$ | 10.20 | 8.69 | 11.27 |
| $C_{14:1}$ | 0.86 | 0.65 | 1.01 |
| $C_{15:0}$ | 1.36 | 1.14 | 1.66 |
| $C_{16:0}$ | 24.10 | 22.78 | 25.63 |
| $C_{16:1}$ | 1.21 | 0.87 | 1.42 |
| $C_{18:0}$ | 10.71 | 9.61 | 12.19 |
| $C_{18:1}$ * | 25.78 | 23.05 | 28.87 |
| $C_{18:2}$ | 1.68 | 1.28 | 2.19 |
| $C_{18:3}$ | 0.93 | 0.49 | 1.43 |
| $C_{18:2conj.}$ ** | 1.45 | 0.85 | 2.03 |

* Mostly 9cis (includes a small percentage of other fatty acids $C_{18:1}$ that overlap in the chromatographic runs).
** Mostly 9c – 11t (rumenic acid).

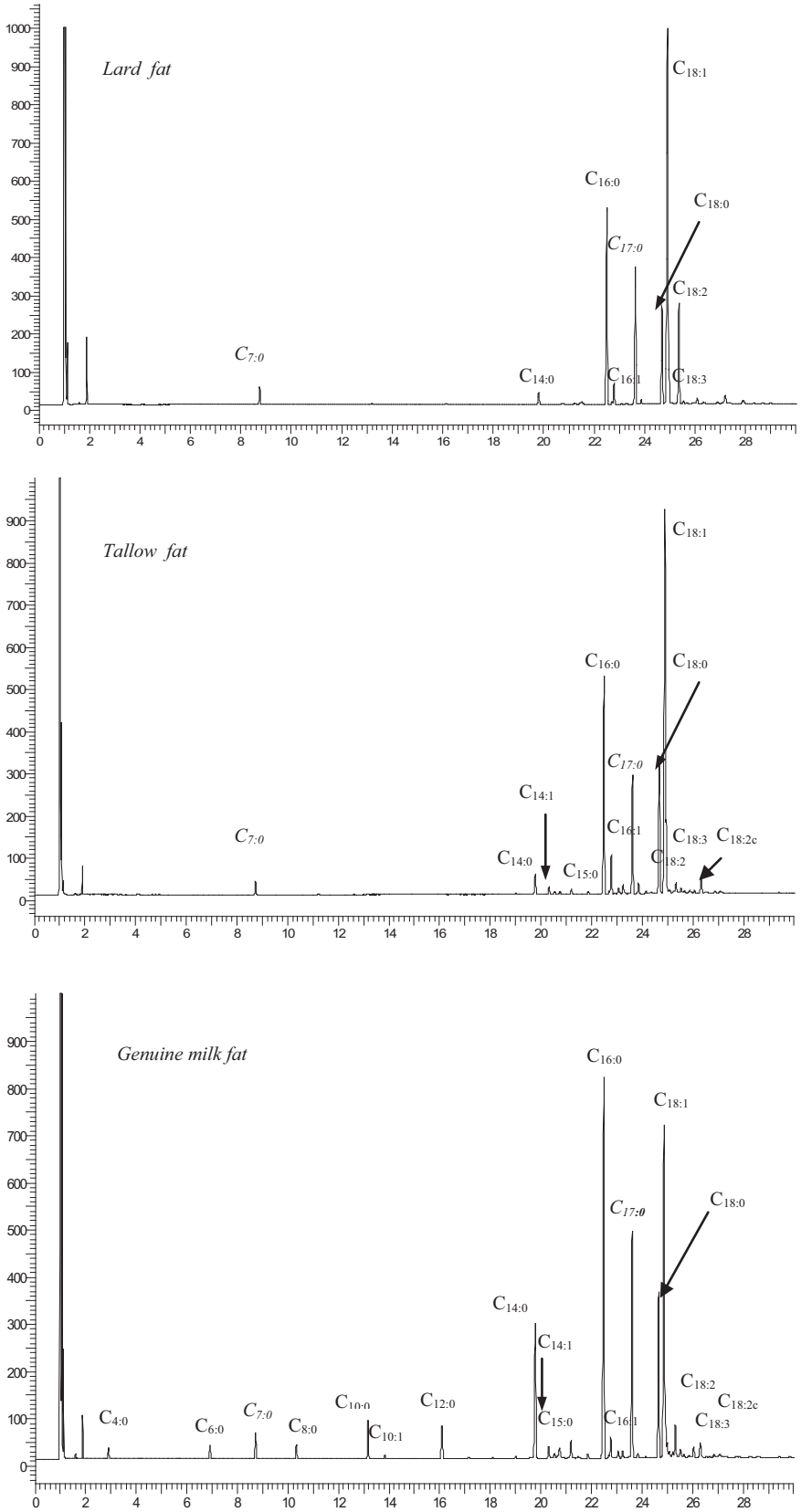


**Fig. 1.** Chromatographic profiles of genuine milk fat, tallow fat and lard fat.

respectively. Indeed, the FA ratios established by CAA were not a useful tool to detect adulterations with lard and tallow at the adulterations levels studied; being tallow adulterations the worst situation observed.

Our results are in concordance with those reported in the bibliography available about the use of FA ratios approaches to identify MF samples adulterated with tallow and lard. Ulberth (1994) tested several FA ratios (including $C_{10:0}/C_{8:0}$, $C_{12:0}/C_{10:0}$ and

**Table 2**
Concentration ranges of the FA ratios for genuine milk fats and adulterated milk fats with tallow and lard.

| | | Fatty acid ratios | | | |
|---|---|---|---|---|---|
| | | $C_{10:0}/C_{8:0}$ | $C_{12:0}/C_{10:0}$ | $C_{14:0}/C_{12:0}$ | $C_{14:0}/C_{18:1}$ |
| Normal ranges (CAA)* | | 1.85–2.30 | 0.95–1.30 | 3.00–4.10 | >0.30 |
| Genuine milk fats | 0% | 1.91–2.39 | 1.13–1.29 | 2.89–4.22 | 0.31–0.48 |
| MF adulterations with tallow | 2% | 1.91–2.39 (–) | 1.13–1.29 (–) | 2.91–4.26 (–) | 0.30–0.47 (–) |
| | 5% | 1.91–2.39 (–) | 1.13–1.29 (–) | 2.92–4.33 (1.8) | 0.29–0.45 (3.9) |
| | 10% | 1.91–2.39 (–) | 1.13–1.29 (–) | 2.96–4.47 (7.6) | 0.27–0.42 (10.0) |
| | 15% | 1.91–2.39 (–) | 1.13–1.29 (–) | 3.00–4.61 (11.9) | 0.25–0.40 (20.0) |
| MF adulterations with lard | 2% | 1.91–2.39 (–) | 1.13–1.29 (–) | 2.90–4.23 (–) | 0.31–0.48 (0.00) |
| | 5% | 1.91–2.39 (–) | 1.13–1.29 (–) | 2.90–4.26 (–) | 0.29–0.44 (6.9) |
| | 10% | 1.91–2.39 (–) | 1.13–1.29 (–) | 2.91–4.32 (1.6) | 0.27–0.41 (11.5) |
| | 15% | 1.91–2.39 (–) | 1.13–1.29 (–) | 2.93–4.37 (2.3) | 0.25–0.38 (34.3) |

() Percentages of samples that are outside normal ranges (CAA).
* Values established by CAA.

$C_{14:0}/C_{12:0}$) and concluded that they failed to identify non-MF/MF blends containing tallow and lard at a level less than 10%. In particular, for tallow adulteration, the author found that $C_{4:0}/C_{6:0}$ ratio was useful for detecting 63.4% of the samples with 10% tallow, and 14.8% samples with 5% tallow. In addition, he detected all cases of 10% lard adulteration by means of ratios $C_{14:0}/C_{18:2}$ and $C_{18:2}/C_{8:0}$; these ratios were also effective in 61.6 and 71.9 at 5% of adulteration. Toppino, Contarini, Traversi, Amelotti, and Gargano (1982) calculated various FA ratios (including $C_{12:0}/C_{10:0}$ and $C_{14:0}/C_{12:0}$) and found that four ratios ($C_{18:0}/C_{8:0}$, $C_{14:0}/C_{18:0}$, $(C_{6:0} + C_{8:0} + C_{10:0} + C_{12:0})/C_{18:0}$, $C_{18:1}/C_{18:0}$) were useful to detect adulterations made with 10% added tallow in 83% of cases.

### 3.3. Regression analysis

#### 3.3.1. Obtaining the regression models

Backward, stepwise and best subset regression models were applied to the calibration matrix for each adulterant studied in order to develop models that could be employed to detect adulterations. It is very difficult to generalize the superiority of one method over another, because the relative performance of the methods often depends on the particular data set analyzed (Ragno et al., 2004).

For tallow adulteration, statistical parameters ($R^2$, adjusted $R^2$, $S^2$) and the existence of multicollinearity for the best MLR models obtained by the three methods were summarized in Table 3. In particular, backward method gave five models. From the five models, a model with twelve predictor variables was chosen ($C_{4:0}$, $C_{8:0}$, $C_{10.0}$, $C_{10:1}$, $C_{12:0}$, $C_{14:0}$, $C_{14:1}$, $C_{16:0}$, $C_{16:1}$, $C_{18:2}$, $C_{18:3}$ and $C_{18:2conj.}$), as it had the highest adjusted $R^2$ and the lowest $S^2$ with a significant linear regression. On the other hand, only one FA, $C_{16:1}$, was necessary to predict the percentage of adulteration by stepwise method. Finally, a model of five predictor variables ($C_{10:0}$, $C_{10:1}$, $C_{14:0}$, $C_{14:1}$ and $C_{16:1}$) was selected by the best subset regression models according to the lowest $S^2$ and the highest $R^2$ with a significant linear regression. As can be seen, the backward and best subset models were superior to the stepwise model as judged by the $R^2$, adjusted $R^2$ and $S^2$; however, as the backward model presented multicollinearity, the best subset regression models was selected. For the latter, full cross-validation was applied and a value of 2.54% for the RMSECV was obtained.

**Table 3**
Statistical parameters of different regression models obtained for tallow and lard.

| | | $R^2$ | Adjusted $R^2$ | $S^2$ | Multicollinearity |
|---|---|---|---|---|---|
| *Regression models for tallow* | | | | | |
| MLR | Backward | 0.909 | 0.871 | 3.79 (1.95)* | Yes |
| | Stepwise | 0.591 | 0.581 | 12.34 (3.51)* | – |
| | Best subset | 0.827 | 0.803 | 5.80 (2.41)* | No |
| *Regression models for lard* | | | | | |
| MLR | Backward | 0.961 | 0.943 | 1.69 (1.30)* | Yes |
| | Stepwise | 0.923 | 0.915 | 2.50 (1.58)* | No |
| | Best subset | 0.924 | 0.916 | 2.49 (1.58)* | No |

* Values in parentheses are the RMSEC values in %.

A similar approach was adopted for lard adulteration: Table 3 shows the statistical parameters for the best MLR models. Four models from the backward method were obtained. A model with thirteen predictor variables was selected ($C_{4:0}$, $C_{6:0}$, $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{16:1}$ $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ and $C_{18:2conj.}$), which showed the best adjusted $R^2$ and the lowest $S^2$ with a significant linear regression. The stepwise method yielded four models, the chosen model had four predictor variables ($C_{4:0}$, $C_{8:0}$, $C_{16:1}$ and $C_{18:2}$). Furthermore, a model with four variables was selected ($C_{16:0}$, $C_{16:1}$, $C_{18:1}$ and $C_{18:2}$) by best subset models method. In all cases the adjusted $R^2$ was higher than 0.9. Even though slightly better results ($R^2$ and $S^2$) were obtained by the backward model, it showed multicollinearity. For this reason, stepwise and best subset models were selected and validated by full cross-validation. Similar values of RMSECV (1.70%) were obtained for both models.

It is important to notice that the RMSECV values for tallow and lard obtained are very good as they are lower than 3%. In particular, the RMSECV for lard adulteration was lower than that obtained for tallow. This fact is closely related to the higher similarities between milk fat and tallow FA profiles in comparison to milk fat and lard FA profiles (Ulberth, 1995).

#### 3.3.2. Application of the selected models to the prediction set

The effectiveness of the regression models for future predictions was checked by means of the prediction matrix for each adulterant; dimensions of tallow and lard matrices were $340 \times 5$ and $260 \times 4$ respectively.

For tallow, when the proposed model was applied to the prediction samples ($m = 340$), a RMSEP of 2.80% was obtained. This value is very good, as we can expect an average uncertainty in new samples of less than 3% in the percentage of adulteration.

Furthermore, prediction intervals for each tallow sample were calculated and plotted in Fig. 2. It can be seen that the prediction intervals of all genuine milk fat samples ($m = 20$) contained the axis of the abscissa, which corresponds to 0% level of adulteration. Besides, within the group of 2% adulterated milk fat ($m = 80$), all prediction intervals contained the theoretical level of adulteration and the majority also included the level of 0%; so it was only possible to detect 12% of the adulterated samples (Fig. A1). The

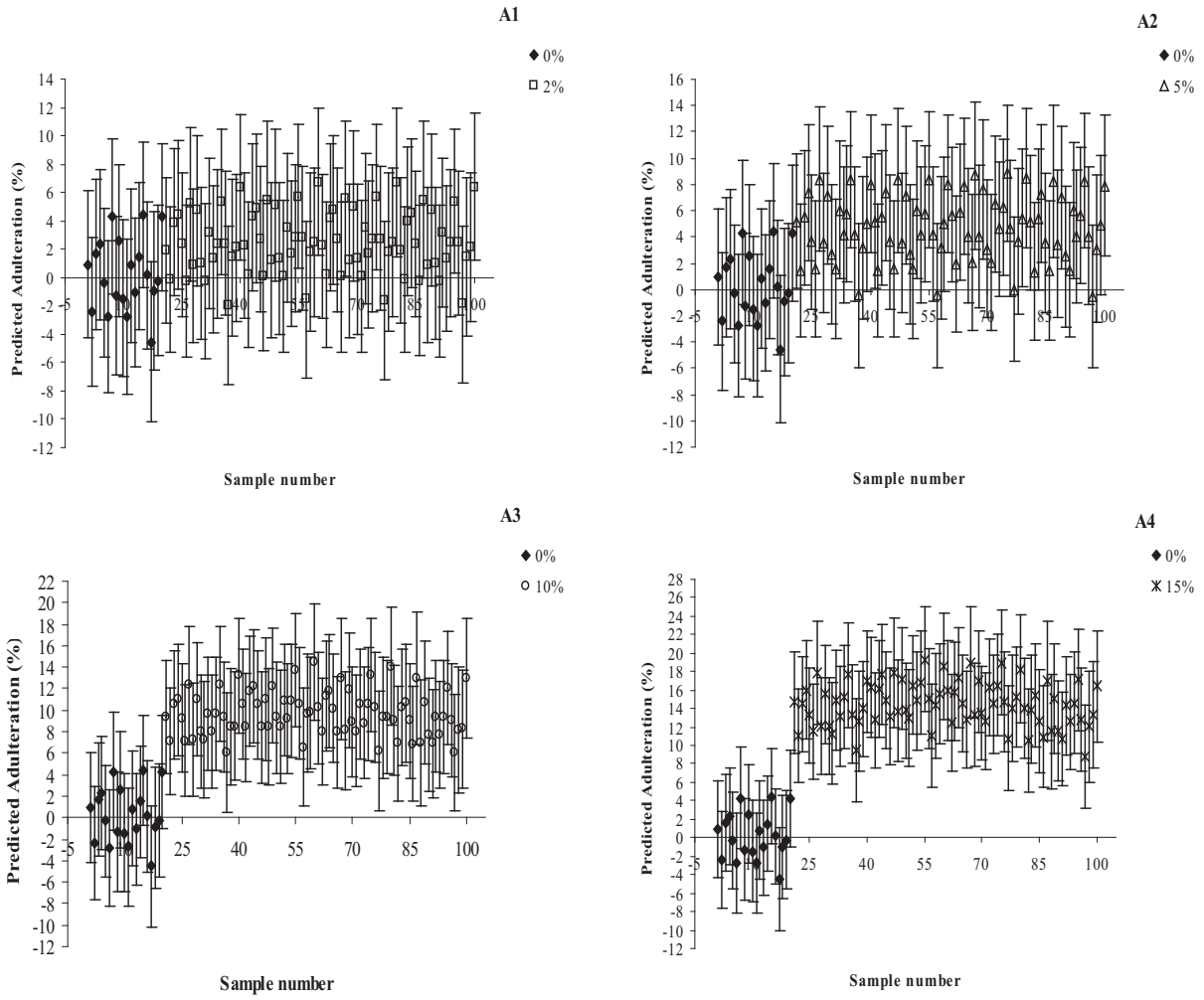


**Fig. 2.** Prediction intervals for adulterated milk fat with tallow. (A1) prediction intervals of genuine and 2% adulterated milk fat, (A2) prediction intervals of genuine and 5% adulterated milk fat, (A3) prediction intervals of genuine and 10% adulterated milk fat and (A4) prediction intervals of genuine and 15% adulterated milk fat.

prediction intervals of all 5% adulterated milk fat samples ($m = 80$) contained the theoretical adulteration level, but some samples also included the level of 0%, so it was only possible to detect 46% of samples adulterated (Fig. A2). Fig. A3 and A4 show the prediction intervals for 10% and 15% adulterated milk fat samples ($m = 80$ for each other). In all cases they contained their respective theoretical adulteration levels and none included the level of 0%. Indeed, 100% of the adulterated milk fat samples were detected in both groups.

For lard, when the two calibration models selected were applied to the prediction set ($m = 260$), the lowest RMSEP (1.77%) was obtained for best subset models. For this reason, this model was selected and employed for the construction of prediction intervals (Fig. 3).

The prediction intervals of all genuine milk fat samples ($m = 20$) included 0% level of adulteration. The intervals for 2% adulterated milk fat samples ($m = 60$) included the theoretical adulteration level but most of them also included the level of 0%; so the adulteration was detected only in 22% of cases (Fig. B1). For 5% adulterated milk fat samples ($m = 60$), Fig. B2 shows that the intervals from all samples contained the theoretical adulteration levels, being possible to detect 97% of adulterated milk fat samples. Fig. B3 and B4 reveal that all milk fat samples adulterated ($m = 60$ for each other) at levels of 10% and 15% were detected.

According to our knowledge, there is scarce information about the study of milk fat adulteration based on MLR analysis of FA profiles. However, as mention above, other multivariate statistical techniques have been applied to TAG and FA profiles in order to analyze milk fat genuineness or adulterations. Ulberth (1994) applied Linear Discriminant Analysis (LDA) to FA data and classified more than 95% of admixtures containing $\geqslant$3% of tallow and lard. Likewise, Ulberth (1995) tested MLR and MLR stepwise, among others methods, to FA profiles obtaining an RMSEP of approximately 1.5% tallow in milkfat. Thus, the percentage of adulteration detected and the average error were lower than the obtained in our work, probably because differences in the dimension of data set matrices and/or the statistical technique applied. More recently, Van Ruth, Villegas et al. (2010) predicted the identities of milk, cow and pig fats applying partial least square discriminant analysis (PLS-DA) to FA profiles, TAG profile and both profiles combined. They found that the identity of each type of fat samples was most successfully predicted (100%) using the combined FA and TAG data set compared to the individual FA and TAG data sets.

## 4. Conclusions

The results obtained in this work revealed that the FA ratios ranges proposed in the CAA were not successful in identifying less than 15% of adulterations of milk fat with tallow and lard.

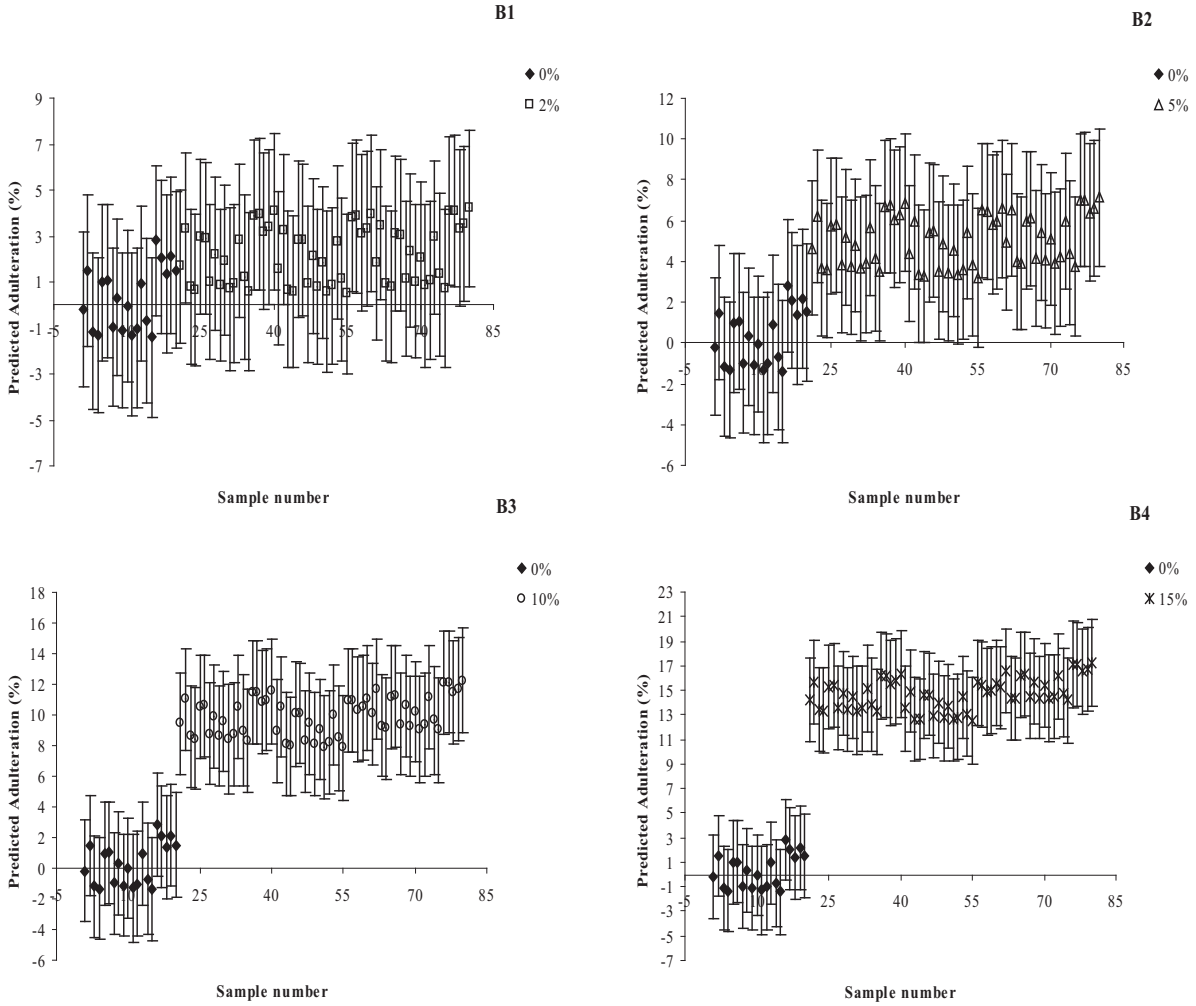


**Fig. 3.** Prediction intervals for adulterated milk fat with lard. (B1) prediction intervals of genuine and 2% adulterated milk fat, (B2) prediction intervals of genuine and 5% adulterated milk fat, (B3) prediction intervals of genuine and 10% adulterated milk fat and (B4) prediction intervals of genuine and 15% adulterated milk fat.

On the other hand, Multiple Linear Regression applied to FA profiles proved to be a valid statistical tool for the evaluation of adulterations of milk fat. One model for each adulterant was proposed, which were able to detect adulterated milk fat samples at levels greater than 5% for lard and 10% for tallow. The superior performance of the prediction model for lard adulteration was also observed in the RMSEP values. Indeed, the study shows that tallow is the most challenging situation in the adulteration study.

Finally, the results reached in this work with the applications of MLR to FA profiles represent an important advance in the knowledge of milk fat adulterations in Argentina.

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