ORIGINAL RESEARCH

Changes in free fatty acid composition during storage of whole milk powder

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Changes in fat matter due to the microbial thermostable lipases are among the most significant deteriorations influencing the shelf life of whole milk powder. As a consequence, free fatty acids are produced, being the short-chain acids mainly responsible for the rancidity flavour. The aim of this work was to evaluate changes in different fatty acid concentrations during the storage of whole milk powder produced in two different seasons. Samples of whole milk powder produced in winter and summer and packed under inert atmosphere were used in the study. The samples were stored at 21°C and 40°C. Samples stored at 21°C were evaluated every 3 months, whereas samples stored at 40°C were evaluated monthly by free fatty acids profile and sensory analysis. Data were processed by principal component analysis and ANOVA. Results showed that changes in the free fatty acids of milk powders were correlated with the season of manufacture. Temperature and storage time had little influence on that profile.

Keywords Free fatty acids, Milk powder, Rancidity.

INTRODUCTION

Whole powder milk is the main dairy export from Argentina. This product is commonly exported to countries where temperatures near 40°C are common during handling, transport and storage. In these conditions, sensory properties of milk powder are affected by non-enzymatic browning, increase of free fatty acids (FFA) concentration and posterior transformation of these FFAs (Muir 1996; Renner 1988).

In particular, short- and medium-chain FFAs (C_4 to C_{12}) are responsible for certain flavours such as rancid, soapy, piquant and astringent. On the other hand, FFAs are precursors of oxidation reactions producing aldehydes and ketones, compounds responsible for flavours in milk powder such as oxidized, metallic and tallowy (IDF 1991; Deeth and Fitz Gerald 1994; Chen *et al*. 2003).

The bacteriological quality of fluid raw milk is of great importance in FFA production during the production and storage of milk powder as a consequence of bacterial thermostable lipases (Shamsuzzaman *et al*. 1987; Celestino *et al*. 1997; Chen *et al*. 2003).

The aim of this work was to evaluate changes in the concentration of FFAs of milk powder produced in two different seasons and stored at two different temperatures.

MATERIALS AND METHODS

Sampling and storage

The whole milk powder samples were provided by a dairy factory in the main Argentinian dairy area. The product was packed for export in bilaminated_boxes (PET 12 mic aluminized/PE 80 mic) of 400 g each, in an inert nitrogen atmosphere. These bilaminated boxes process act as a gas 'barrier'. For each sampling, packages were selected randomly from those manufactured in both winter and summer.

These packages were stored in controlled chambers at 21°C and 40°C. In Table 1, the characteristics of the sampling process are given.

Free fatty acid analysis

The FFA extraction and isolation were carried out according to the methodology of Deeth *et al*. (1983). The extraction was made with a diethyl hexane-ether mixture. The lipidic extract was injected into an extraction column with deactivated alumina (10%). The neutral lipids were then eluted by a diethyl hexane-ether mixture, whereas the FFAs were extracted with 6% formic acid in isopropilic ether. The analysis of the FFA fraction was carried out by gas chromatography, in an Autosystem XL–R 3,5 Perkin–Elmer equipment (Perkin–Elmer, Shellton, MA, USA), with PSS injector (Programmed Temperature Vaporiser), flow divider and flame ionization detector (FID). A 0.25 μm (30 m × 0.32 mm)

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Table 1 Characteristics of the sampling process

Year season	*Month of sampling*	*Sample storage temperature*	*Frequency of analysis*	*Total time in storage*
Summer	February	21°C	Every 3 months	15 months
		40°C	Every 10 days	4 months
Winter	June	21°C	Every 3 months	15 months
		40°C	Every 1 month	6 months

PE-FFAP fused-silica column was used. For the quantification, a mixture of valeric (C_5) and heptadecanoic acids (C_{17}) was used as internal standards. The results were expressed in mg/kg of milk powder or in mg/g of fat. The reproducibility of the technique was evaluated by injecting a mixture of standards and milk powder samples. The obtained variation coefficients were 4.14% and 3.67% in the standards and in the milk powder samples, respectively.

Sensory analysis

The sensory evaluation of the samples was performed by a group of seven selected and trained panellists. The method used for the evaluation was a descriptive one with a 10 cm unstructured scale, anchored in the extremes, with 0 representing the absence of defect, and 9 the intensity that makes the sample unacceptable. The grade assigned by the panellists to the evaluated characteristics was called 'grading' or 'score' in the present work. The panellists became familiarized with the rancid descriptor in order to agree on the characteristics of this sensory defect. The panel average threshold for this descriptor was also determined. This allowed technicians to record a score indicating when the descriptor was perceptible, generating a sensory defect in the product. At each testing moment, a stored package was opened at the corresponding temperature. The sensory evaluation was made on samples of reconstituted milk powder at 13% total solids, being then used as fluid milk. Each milk powder sample was analysed by duplicate.

Statistical analysis

The concentrations of FFAs were evaluated by means of principal component analysis (PCA). This is a multivariate technique that tries to explain, according to a linear model, an extensive set of observable variables by means of a reduced number of hypothetical variables called 'components'. The first component always detects the maximum variability of the system. Normally, this analysis is represented by two-dimensional plots, meaning that the two first components are visualized. In this work, 'eigenvalue' criterion = 1 was used as the cut-off point to determine the number of components to be analysed. The correlations between the original variables and the components are called 'loadings'. With the number of components determined, the 'score' of the samples for each component was calculated. These 'scores' represent the sample in the new space of principal components. This analysis was executed using SPAD version 3.5 (CSIA-CERESTA, Saint Maude, France). A variance analysis was also performed with repeated measures in time (Proc Mixed with a matrix of variance and of compound symmetry covariance), and correlation analysis using the SAS statistical package (SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

Taking into account the FFA composition data obtained during the entire period of storage from the samples of whole milk powder elaborated in winter and summer and maintained at 21°C and 40°C, PCA was carried out. From this analysis, the two first components, PC1 and PC2, were selected, as they explained 89.6% of the total variability of the system. In equations (1) and (2), the relationships between these two components and the original variables with their respective coefficients or 'loadings' are shown together with the percentage of variance for each component.

$$PC1 = -0.69C_4 - 0.85C_6 - 0.90C_8 - 0.94C_{10} - 0.73C_{12} - 0.69C_{14} - 0.85C_{16} - 0.67C_{18} - 0.82C_{18:1} \quad (1)$$

Percentage of variation associated with the PC1 63.8%

$$PC2 = 0.65C_4 + 0.49C_6 + 0.34C_8 + 0.03C_{10} - 0.14\,C_{12} - 0.24C_{14} - 0.45C_{16} - 0.64C_{18} - 0.14C_{18:1} \quad (2)$$

Percentage of variation associated with the PC2 16.5%

On the other hand, with the FFA values of all the samples, the numerical values of PC1 and PC2—also called 'scores'—were calculated. In Figure 1, the graph representation in two axes, PC1 and PC2, of 'loadings' and 'scores' is presented.

Concerning 'loadings', it can be said that they are all significant and of equal sign for PC1, and it can thus be inferred that this component discriminates the samples according to the total of FFAs. For the

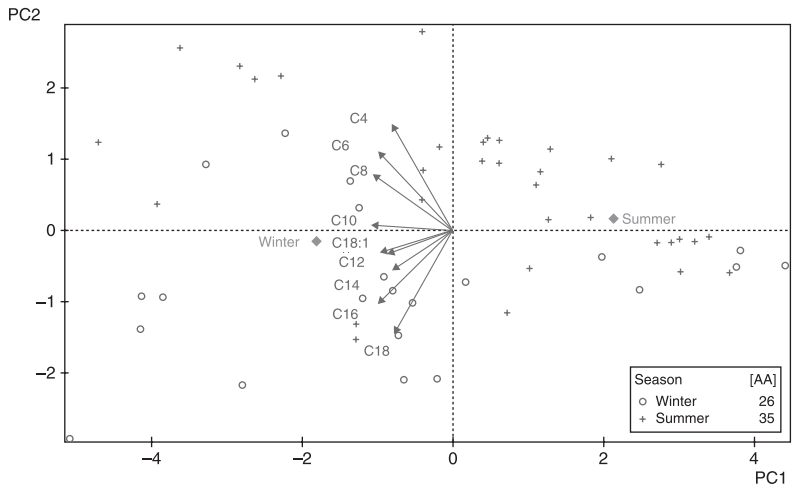

Figure 1 Distribution of whole milk powder samples in a PCA biplot according to free fatty acid composition.

PC2 component, however, it is observed that the low-molecular-weight FFAs, C_4 to C_{10}, have a different sign from the long-chain ones, and it can therefore be postulated that this component discriminates by chain length. Considering the 'scores' bidimensional representation, it is possible to imagine that they form two 'classes' or groups that partially respond to the season. According to the class analysis performed, class 1 includes 76% of the winter samples, whereas class 2 includes 71% of the summer samples. This grouping shows that most of the changes in the FFA profile are associated with the season, and they could also have a relationship with the changes in the characteristics of the fat matter related to the season and to feeding. On the other hand, Evers *et al*. (2000) have established a close relationship between the FFA level in raw milk and the FFA in powder milk.

To complete the analysis, a variance analysis was applied with repeated measures in time in order to evaluate the effect of the season in which the sample was elaborated, storage time and the interaction of both in each FFA. This analysis was repeated for each temperature, evaluating the composition at the beginning of storage and in the 15th and third months for the samples stored at 21°C and 40°C, respectively (Tables 2 and 3).

Table 2 Free fatty acid (FFA) composition of whole milk powder at the beginning and in the 15th month of storage at 21°C

Time (months)	*0*		*15*		*Effect*		
Season FFA							
(mg/kg powder)	*Winter*	*Summer*	*Winter*	*Summer*	*Season*	*Time*	*Season*time*
4:0	17.61	25.24	15.19	21.48	*		
6:0	13.05	17.17	10.25	13.33		*	
8:0	10.77	13.23	8.43	10.9		*	
10:0	21.43	24.55	16.68	15.46		*	
12:0	33.24	27.78	26.43	16.36			
Σ short-chain FFA	**96.1**	**107.97**	**76.98**	**77.53**			
14:0	54.63	84.29	38.59	40.25	*	*	
16:0	201.52	305.52	134.19	131.71	**	**	*
18:0	189.36	323.19	133.59	72.92	*	**	**
18:1	313.97	257.6	180.39	204.36		**	*
Total FFA	**951.68**	**1186.54**	**640.72**	**604.3**			

*$P < 0.1$, **$P < 0.05$.

Table 3 Free fatty acid (FFA) composition of whole milk powder at the beginning and in the 3rd month of storage at 40°C

Time (months)	*0*		*3*		*Effect*		
Season FFA							
(mg/kg powder)	*Winter*	*Summer*	*Winter*	*Summer*	*Season*	*Time*	*Season*time*
4:0	17.61	28.52	21.51	20.61			
6:0	13.05	17.57	16.71	13.35			**
8:0	10.77	13.26	14.08	9.09			*
10:0	21.43	25.71	24.92	17.11			
12:0	33.24	32.39	40.37	23.88			
Σ short-chain FFA	**96.1**	**117.45**	**117.59**	**84.04**			
16:0	201.52	308.56	199.07	132.74	*	*	*
18:0	189.36	298.48	210.95	86.5			
18:1	313.97	281.16	269.79	203.6			
Total FFA	**951.68**	**1202.24**	**960.99**	**628.92**			

*$P < 0.1$, **$P < 0.05$.

At 21°C, butyric (C_4) was the only short-chain FFA that displayed seasonal differences, whereas caproic (C_6), caprylic (C_8) and capric (C_{10}) acids displayed a significant change due to storage time. In the case of the long-chain acids, miristic (C_{14}), palmitic (C_{16}), estearic (C_{18}) and oleic ($C_{18:1}$), their behaviour in time differs according to the season.

In the 15th month of storage at 21°C, a 33% reduction in the winter samples and a 49% reduction in the summer samples of the total FFA with respect to the initial values were registered. The short-chain FFA ($C_{4:0}$ to $C_{12:0}$) represented 10% of the total winter samples and 9.1% of the summer ones at the beginning of the storage, whereas in the 15th month, this proportion was 12% in winter samples and 12.8% in the summer ones.

In samples of whole milk powder stored at 40°C (Table 3), the caproic (C_6), caprylic (C_8) and palmitic (C_{16}) acids displayed different behaviours in time according to the season. This was reflected in the short-chain FFA, which increased 22% in the winter samples and decreased 28% in the summer samples after 3 months of storage at the above-mentioned temperature. In the third month in storage at 40°C, the short-chain FFAs represented 12.2% of the total winter samples and 13.4% of the summer ones. Considering the total FFA content, a minimum change was observed in the winter samples in the third month in relation to the initial concentration. In contrast, in the summer samples, there was a 48% reduction in the total FFA after 3 months of storage, similar to the result in the samples stored at 21°C after 15 months. The only FFA that displayed a noticeable seasonal change independently of the storage temperature was the C_{16}.

To evaluate the storage temperature effect, a variance analysis with measures repeated in time was applied. The results of analysis are observed in Table 4. At the evaluated times (0, 3 and 6 months), no significant differences were detected in the composition of FFA due to the effect of storage temperature. Nevertheless, there were differences in the short-chain FFA sum and in lauric (C_{12}), palmitic (C_{16}) and oleic ($C_{18:1}$) acids produced by the storage time effect.

The evolution with time of the short-chain and total FFA content in milk powder elaborated in winter as well as in summer and stored at different temperatures is observed in Figures 2–5.

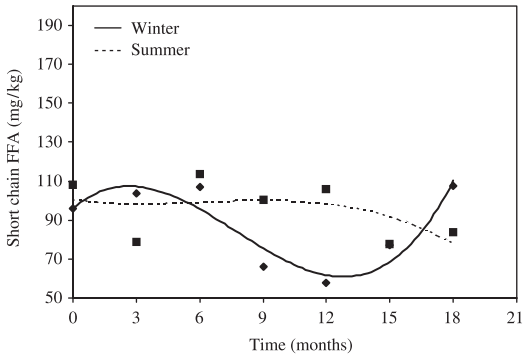

Figure 2 Short-chain free fatty acid evolution in samples stored at 21°C.

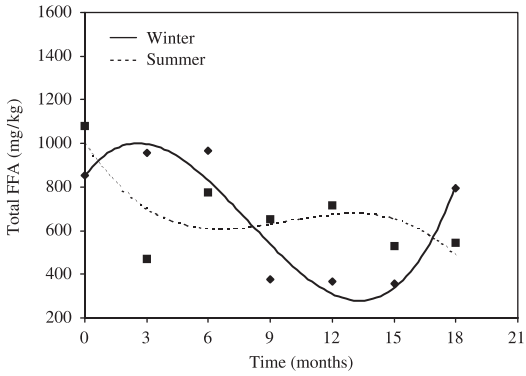

Figure 3 Total free fatty acid evolution in samples stored at 21°C.

Table 4 Composition of free fatty acid (FFA) in whole milk powder stored at 21°C and 40°C at different times

Time (months)	*0*		*3*		*6*		*Effect*		
Temperature FFA (mg/kg powder)	*21*	*40*	*21*	*40*	*21*	*40*	*Temp*	*time*	*Temp*time*
4:0	21.4	23.1	17.3	20.4	23.5	24.5			
6:0	15.1	15.3	12.1	13.9	15.5	16.7			
8:0	12.0	12.0	10.0	10.2	11.7	11.8			
10:0	23.0	23.6	19.7	19.0	22.5	25.7			
12:0	30.5	32.8	32.0	29.9	37.1	48.6		*	
Σ short-chain FFA	**102.0**	**106.8**	**91.3**	**93.8**	**110.3**	**127.5**		*	
16:0	253.5	255.0	179.7	177.9	203.4	194.8		*	
18:0	256.3	243.9	196.6	207.8	207.1	257.1			
18:1	285.8	297.6	202.6	227.4	290.2	294.6		**	
Total FFA	**1069.1**	**1077.0**	**803.6**	**842.1**	**972.3**	**1041.8**			

*$P < 0.1$, **$P < 0.05$.

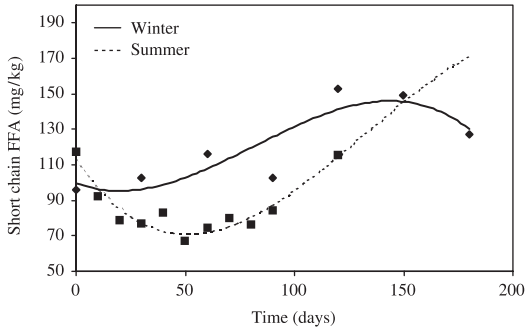

Figure 4 Short-chain free fatty acid evolution in samples stored at 40°C.

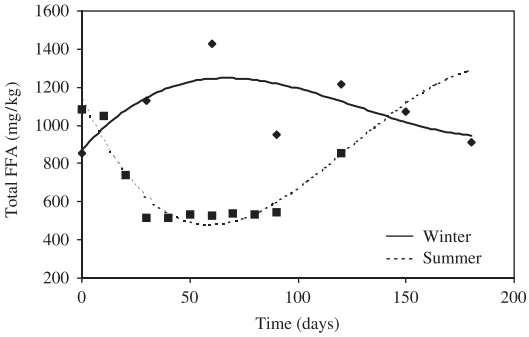

Figure 5 Total free fatty acid evolution in samples stored at 40°C.

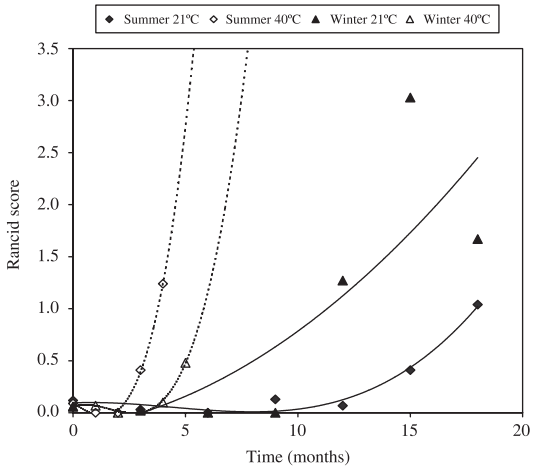

Figure 6 Rancid score evolution in whole milk powder samples stored at 21°C and 40°C.

The total FFA displayed behaviour typical of a reaction intermediary, independently of the season and of the storage temperature (Figures 3 and 5). The FFA are generated in the first storage stages and then become available substrate for or precursors of oxidation development, with the corresponding formation of compounds that generate intense flavour such as ketoacids and methyl ketones.

Figure 2 shows an increased tendency towards short-chain FFA in winter samples stored at 21°C starting in the 12th month. In contrast, the short-chain FFA in whole milk powder elaborated in summer did not present significant changes during storage. An identical increased trend in the short-chain FFA was observed from the second month, in the summer and winter samples stored at 40°C (Figure 4).

The values obtained in the sensory evaluation of samples are presented in Figure 6. The rancid flavour detection by the trained specialists took place in the fourth month in the summer samples stored at 40°C and in the 12th month in the winter samples stored at 21°C.

In order to find some possible relationship between the FFA profile and the rancidity of whole milk powder, a correlation analysis was performed between the rancid 'score' and each one of the FFA, including the short-chain and the total FFA obtained in each season at the two storage temperatures. It was only found in the summer samples that the rancidity correlates in a positive way with $C_{18:1}$ content ($r = 0.42$; $P = 0.01$). The difficulties in finding more consistent relationships between the rancidity and the different FFA could be due to the changes that take place in the profile during the different process stages, mainly in relation to losses in the short-chain FFA, which are principally responsible for the appearance of undesirable flavours (Páez *et al*. 2002).

At the storage times in which the rancidity was detected, both at 21° and at 40°C (Figure 6), the concentration of short-chain FFAs (C_4 to C_{12}) oscillated between 90 and 125 mg/kg of milk powder, respectively. These concentrations are equivalent to 11.7 and 16.3 mg/L of reconstituted whole milk powder at 130 g/L. These short-chain FFA concentrations are below the reported flavour threshold in milk of 55 mg/L (Chen *et al*. 2003). From the results obtained, it is deduced that volatile FFA concentration in reconstituted milk powder samples with slight rancidity is almost four times smaller than the theoretical threshold concentration in milk. These results coincide with those published by Jeon (1994) who found smaller concentrations of volatile FFA in UHT milk than the published threshold values.

CONCLUSIONS

There are noticeable seasonal changes in the FFA composition of whole milk powder that elaborated in summer having a greater initial content of total FFA. The FFA profile during storage maintained seasonal changes independently of the storage time and temperature. The FFA acts as a reaction intermediary, thus making it difficult to find a relationship with the 'rancidity' flavour.

The short-chain FFA levels found in reconstituted whole milk powder samples and identified by the sensory specialist group as 'slightly rancid ones', are approximately four times smaller than the thresholds values published for milk.

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