Minor Fatty Acids composition (CLA) of Mozzarella Cheese obtained through chemical and biological acidification

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Abstract

Mozzarella cheese is one of the most important product obtained from cow milk by the coagulation of the casein. According to the preparation process it is possible to find two different curds: a chemical one by adding citric acid and a biological one obtained through the fermentation of lactic bacteria. During one year's period (oct. 2003 – sept. 2004) a significative mozzarella cheese samples were collected from the market in order to evaluate the effects of the acidification process on the CLA concentration. As result of the experimentation some characteristics of the mozzarella were analyzed and defined: content fat, tryglceridic composition and CLA concentrations of different isomers (besides the composition of fatty acids). By high resolution gas chromatography (HRGC) it was possible to identify 40 different fatty acids, included the "minor" category from C4 to C24.
In addition the CLA level of the mozzarella samples was contained in a range from 1.4 mg/g of fat (biological cheese) to 2.3 mg/g of fat (chemical cheese). In particular among the different identified isomers, chemical mozzarella cheese shows an higher presence of 11c, 13t-C18:2, and 11t, 13t-C18:2, while the "biological" mozzarella was richer in 9c,11t-C18:2.

Keywords: CLA , fatty acids, gascromatography, mozzarella cheese

Introduction

During the last period conjugated linoleic acid (CLA) has been subject of various studies on their nutritional effects. They naturally occurred with trans-fatty acid (TFA) in milk fat and dairy products fat. Trans and conjugated linoleic acids are both intermediates of biohydrogenation of PUFAs polynsatured fatty acids in the rumen (Kepler, 1967).
CLA can arise from a series of processes .The anaerobic bacterium Butyrivibrio fibrisolvens metabolites primarily linoleic acid (C18:2 c9c12), α- linolenic(18:3 c9c12c15) , and γ -linoleic acid (18:3 c6 c9c12) to rumenic acid and trans-vaccenic acid. In addition to rumenic acid , present at about 80% of total CLAs, a multiplicity of minor geometrical and positional isomers ranging from Δ6,8 to Δ13,15 are also formed in rumen (Griinari et al., 1999).
Isomer c9-t11, also called rumenic acid (Kremer JKG.1998) can be considered the main representative of CLAs and it is present primarily in dairy products.

Mozzarella Cheese and CLA

Cheese is the fresh or ripened product obtained from the acid, rennet or mixed coagulation of whole or partially skimmed milk. The process that transforms milk into cheese is called a technological process. Each cheese, and more in general, each dairy product is produced following a specific technological process. The following scheme represents a standard process of production of a Mozzarella cheese :

- Milk
- Coagulation
- Cutting
- Scalding
- Curd Maturation or acidification
- Stretching
- Shaping or Mozzatura
- Salting
- Ripening

In the classical technology curd maturation is due to lactic and acid bacteria fermentation and the pH value of the curd can be considered critical for the stretching fase. Steching attitude of the curd increase with pH decreasing value at 5 If one decides to use citric acid, lactic acid o r acetic acid, these will be added to obtain milk with an acidity of pH= 5.6. In this case the stretching immediately follows the cutting of the curd: the ripening phase is skipped; it is called immediate stretching.
In general cheeses made from cow's milk have about 0.4-0.6% (p/p) of CLA in fat composition . Feta cheese has more than 1.9% of CLA. Mozzarella di Bufala Campana cheese has CLA medium amount of'1.1% on total fatty acids (Fritsche S, Fritsche J.1998).
Fish and sea food contain only negligible amounts of CLA. Edible oils such sunflower, olive , rapeseeds, soybean, and peanut or coconut contain only a trace amounts of CLA(<0.01%)(Chin et al., 1991)
The aim of this work was the evaluation of the quantitative and qualitative CLA content and of minor acidic fraction composition, of Mozzarella cheese produced with different acidification process (biological or chemical).

Materials and Methods

A total of 80 Mozzarella cheese samples was collected during the period from May to October, twice a week. 40 samples were produced trough biological acidification and 40 trough chemical one. Samples of cheese were analized for chemical composition and fatty acids.

Lipid extraction

Cheese lipid fraction (10g) were extracted according to the principle of Schimid-Bondzynsky – Ratzlaff(NF VO 4, 1985).The extracted lipid fraction was diluted with n-hexane (2%) and analyzed with HRGC

Determinazione degli acidi grassi minori (CLA)

The analysis of FAMEs was carried out using a Perkin Elmer Autosystem XL gascromatograph fitted with a flame ionization detector A 50% Cyanopropil Methil Silicone mod. SP 2380 (100m, 0,25mm ID 0,20µm film thickness. Supelco Bellefonte, PA), was used.

With the help of this technique (Kramer JKG et al., 2001) as with the silver ion high performance liquid chromatography (Ag + HPLC)analysis CLA region was well identified.

The GC-operating condition were: carrier gas Hydrogen flow 20cm/sec; Detector temperature 260°C.

The coloumn was operated at 70°C for 5 minutes, then temperature programmed at 3°C/min. to 165°C for 10 minutes, then temperature programmed at 3°C/min to 260, held there for 28 minutes. Fatty acid minor component was analyzed after derivatization with 2N KOH solution in methanol (AOAC methods,1990; Iupac methods, 1979).

According with recent study on difficulties of finding pure CLAs standard (Spiros et al., 2002),peaks identification was gained using an external standard (Supelco TM 37 components FAME MIX). Unidentified peaks of CLAs, were determined trough the comparison of retention time with literature examples in the same working condition.

Data were analyzed by Statistical Analysis System (SAS).trough T-student test.

Result and Discussion

In Table 1 was showed milk fatty acid composition analyzed in previous sperimentation (from 1984 to 2005). The GC analysis using 100m polar columns allowed the study of milk and mozzarella cheese "Minor" acidic component and the detection of α and γ isomers of Oleic, linoleic and linolenic acids. (Yurawecz et al, 2001; Robert et al., 1991).

Table 1. Different fatty acids composition of milk (%) analyzed from1987 to 2004

Fatty acids	Bondi 1987	Piva 1989	Palmquist 1991	Jakobsen 1999	Robert 1999	Wiking 2003	Collomb 2004
C4	3.3	2.7	2.4	3.4	2.6	2.3	3.1
C6	1.6	2.3	2.3	2.1	1.9	1.7	2.2
C8		1.2	1.6	1.2	1.0	1.0	1.4
C10	3.0	2.6	4.1	2.6	1.8	2.3	3.6
C11		-	-	-	-	-	-
C12:0	3.1	3.2	2.4	2.9	2.0	2.6	4.5
C12:1	-	-	-	-	-	-	-
C13:0	-	-	-	-	-	-	-
iso/anteisoC14:0	-	-	-	-	-	-	-
C14:0	9.5	9.7	13.3	9.5	0.4	10.5	12.0
C14:1	-	-	0.9	-	0.7	-	-
C15:0	-	-	1.5	-	2.6	-	1.4
iso/anteisoC16:0	-	-	-	-	-	-	-
C16:0	26.3	26.2	37.2	25.3	30.2	26.0	31.1
C16:1 cis	-	-	-	-	1.9	1.5	1.4
C17:0	-	-	-	-	-	-	-
iso/anteiso C 18:0	-	-	-	-	-	-	-
C18:0	14.6	10.8	9.1	14.68	15.6	13.1	5.4
C18:1(t6,t9,t10,t11)	-	-	-	-	-	-	2.0
C18:1n-9c	-	-	17.2	-	28.9	31.7	11.9
C18:2 9c,12c	2.4	4.2	2.2	-	1.7	5.3	1.8
C20:0	-	-	1.5	-	0.5	-	0.4
C18:3	-	-	0.2	-	1.1	-	0.7
Σ CLA	-	-	-	-	-	-	0.7
C20:2	-	-	-	-	0.1	-	-
C22:0	-	-	-	-	0.2	-	-
C20:3 n-6	-	-	-	-	-	-	0.1
C20:3 n-3	-	-	-	-	-	-	-
C20:4 n-6	-	-	-	-	0.1	-	0.1
C23:0	-	-	-	-	-	-	-
C22:2	-	-	-	-	-	-	-
C24:0	-	-	-	-	0.1	-	-

A typical GC profile of Mozzarella Fatty acids composition is illustrated in figure 1 Minor acidic fraction zone is showed in figures 2-4.

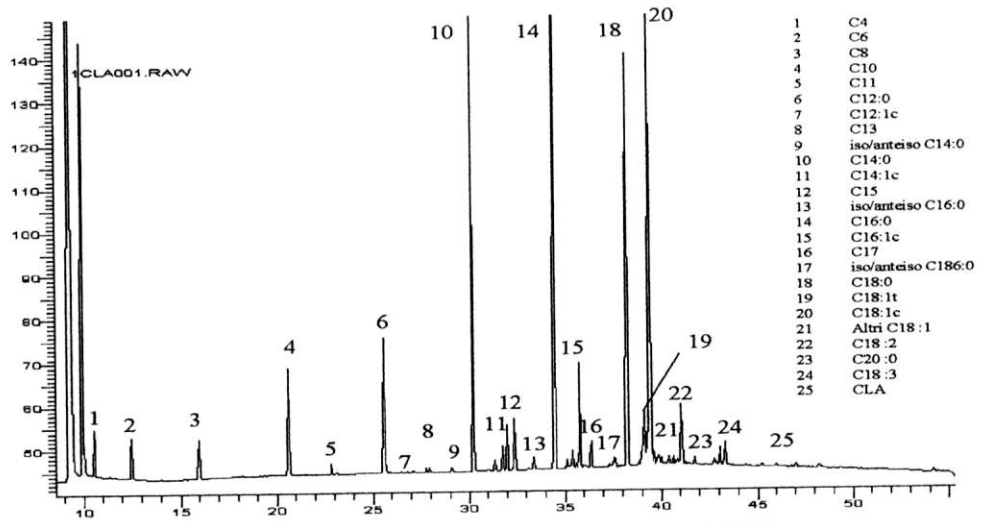

Figure 1- Gascromatographic profile of mozzarella cheese FAMEs

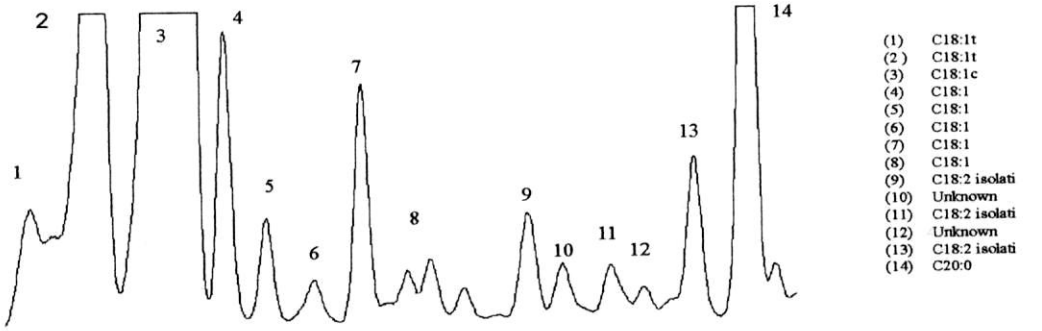

Fig.2 C18:1, C18:2 and isomers zone in gascromatographic profile of mozzarella.

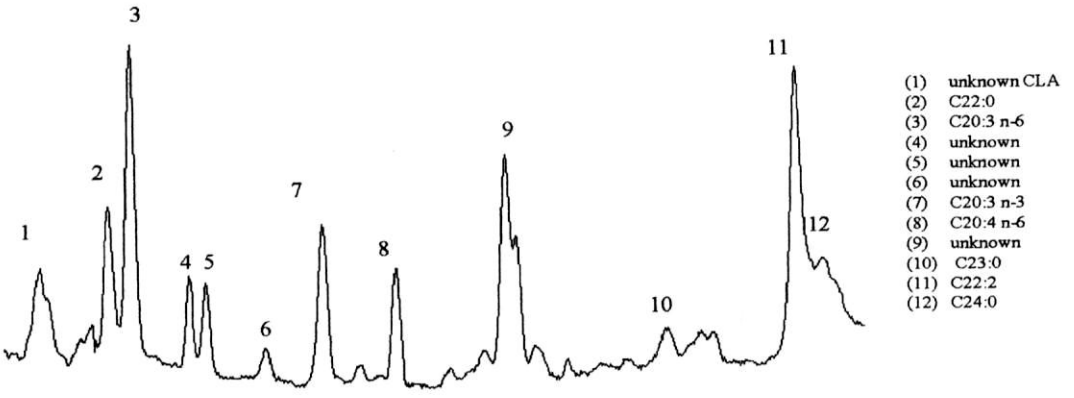

Fig.3 Unsatured high molecular weight fatty acids zone of mozzarella FAMEs profile.

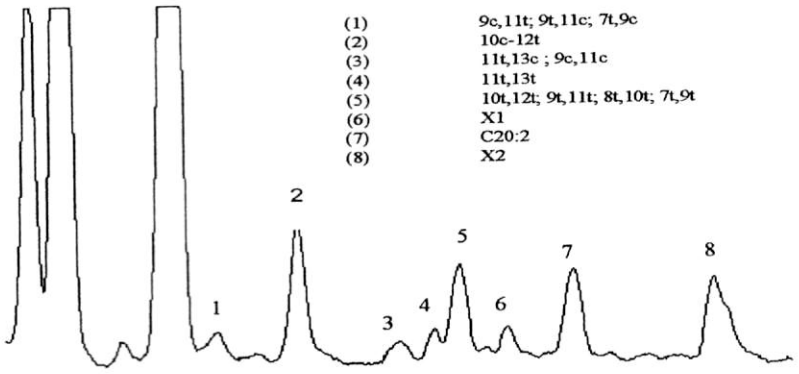

Fig.4 CLA Zone.

Results were summarized in table 2. It was compared the average fatty acids composition of mozzarella cheese made with biological and chemical acidification . It was possible to underline the identification of 40 fatty acids and two unidentified isomers in CLA zone.
The Low, medium and high molecular weight fraction didn't show significant differences.
Analysing every fatty acids of singular categories, it was revealed significant differences between the two acidification process as regard the fatty acids composition of mozzarella.
In particular there was a positive trend (5,9 e 6,7%)for C11 and C12:1 respectively. Their presence was more evident in citric mozzarella. This behaviour was followed also by C16:1cis and C14:1. The analysis of high molecular weight component (C17:0; C24) gave important information by technological and nutritional point of view.

Table 2. Comparison between FAMEs composition of Mozzarella cheese obtained with biological and chemical acidification.

	Biological		Chemical		
FAMEs	**mean**	**ds**	**mean**	**ds**	**Δ % to biological one**
C4	3,45	0,60	3,38	0,53	1,86
C6	1,90	0,21	1,83	0,39	3,52*
C8	1,09	0,16	1,07	0,16	1,59
C10	2,47	0,21	2,53	0,14	-1,42
C11	0,23	0,02	0,24	0,02	-5,91*
C12:0	3,22	0,24	3,18	0,13	1,20
C12:1	0,06	0,01	0,07	0,005	-6,69*
Σ LMW	12,43		12,31		0,97
C13:0	0,09	0,01	0,08	0,01	3,90*
iso/anteisoC14:0	0,12	0,01	0,11	0,01	2,26
C14:0	8,85	0,42	8,75	0,17	1,13
C14:1	0,83	0,04	0,86	0,16	-4,02*
C15:0	0,90	0,04	0,92	0,02	-2,14
iso/anteisoC16:0	0,26	0,02	0,24	0,01	5,41*
C16:0	23,85	0,67	23,79	1,39	0,25
C16:1 cis	1,28	0,07	1,38	0,07	-7,91*
Σ MMW	36,17		36,14		0,07
C17:0	0,52	0,02	0,53	0,01	-2,33
iso/anteiso C 18:0	0,19	0,01	0,21	0,01	-9,29*

C18:0	7,91	0,55	7,56	0,31	4,34*
C18:1(t6,t9,t10,t11)	1,78	0,28	2,02	0,33	-14,01**
C18:1n-9c	17,96	0,63	17,90	0,49	0,30
Altri c18:1	1,36	0,19	1,32	0,16	2,50
C18:2(ISOLATI)	0,68	0,03	0,89	0,04	-31,46
C18:2 9c,12c	2,79	0,45	2,35	0,47	15,54**
C20:0	0,14	0,01	0,13	0,02	4,83*
C18:3	2,33	0,31	2,81	0,71	-20,26**
9c-11t,8t-10c,7t-9c(CLA)	0,03	0,02	0,02	0,01	11,84**
X1	0,01	0,01	0,01	0,01	24,79**
11c,13t(CLA)	0,04	0,01	0,05	0,02	-33,69**
11t-13c,9c11-c(CLA)	0,02	0,01	0,03	0,01	-11,43**
11t-13t(CLA)	0,02	0,01	0,03	0,02	-37,34**
10t-12t,9t-11t,8t-10t,7t-9t(CLA)	0,06	0,01	0,05	0,01	9,47*
X2	0,01	0,01	0,02	0,01	-100,28**
C20:2	0,06	0,01	0,06	0,01	-4,01*
C22:0	0,05	0,01	0,05	0,01	-6,84*
C20:3 n-6	0,23	0,03	0,19	0,03	13,26*
C20:3 n-3	1,02	2,80	0,51	0,11	49,65**
C20:4 n-6	0,02	0,01	0,01	0,01	20,60**
C23:0	0,03	0,02	0,04	0,02	-32,14**
C22:2	0,05	0,01	0,06	0,01	-26,28**
C24:0	0,04	0,01	0,04	0,01	-4,93*
Σ HMW	37,30		36,90		1,06

There were significant differences($p<0,001$) between linoleic acid isomer. Estimated percentage variation were of 33,7%; 11,4% e 37,3% for11c-13t; 11t-13c and 9c-11t; 11t-13t: isomers respectively, found with high amounts in citric mozzarella.
The 9c-11t, 8t-10c, 7t-9c 10t-12t, 9t-11t, 8t-10t, 7t-9t isomers were present at much higher concentration in biologic mozzarella.
This behaviour could be explained trough the analysis of chemical synthesis of this components. CLA increase in citric mozzarella seems to be correlated with decreasing values of C18:2 9c-12c , wich is their precursor.
From table 2 it was clear the positive trend , about 20%, of linolenic acid also for citric mozzarella ($p<0,001$).
As regards high molecular weight fatty acids from C20:0 to C24:0 , the main variations were relatives to C20:3 n-3, C20:3 n-6, C20:4 n-6, C23:0 e C22:2 ($p<0,001$). Although, could be underlined a percentage mean of –5% for C20:2, C22:0 e C24:0 in citric mozzarella respect
Odd number fatty acids showed a statistical variation of -6% ($p<0,05$) for C11 instead C13, C15 and C17 had a percentage mean of 3%. C23:0 showed a statistical significant variation of -32%, in citric mozzarella cheese.

Conclusion

HRGC analysis performed with a 100m capillary coloumn allowed the identification and quantification of 40 different fatty acids, many of them present in traces, in fat composition of Citric and Biologic mozzarella cheese.
This minor component (odd number fatty acids, iso/anteiso compounds, conjugated linoleic acids isomers, unsatured fatty acids with a chain of more than 20 carbon atoms)

These results showed that the main differences between the two acidification process can be revealed trough the variation of minor acidic component of mozzarella cheese.
Gas-chromatographic analysis has evidenced meaningful differences between various fatty acids. In particular the fat extracted from citric mozzarella presents an higher amount of C12:1, C14:1, C16:1, C18:3, C20:2, C22:2 rather than of conjugated linoleic acids isomers and odd number fatty acids. Biologic mozzarella cheese showed an high level of C18:0, C18:2 , C20:3 n-6, C20:3 n-3, C20:4 n-6.

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