PAPER

INVESTIGATION OF CHEESE-MAKING FACTORS EFFECT ON RIPENING OF A SEMI-HARD CHEESE FOR PRODUCT QUALITY ENHANCEMENT

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ABSTRACT

The work is devoted to understand how cheese making factors can influence the quality of cheese along ripening. The study is focused on a semi-hard cheese (Toma). Three parameters (type of milk, of curd and of starter bacteria) were studied by a full factorial experimental design, providing a set of eight cheese samples characterised, after one and two months of ripening respectively, by compositional parameters and the content of biogenic amines. Principal Component Analysis and the regression models built for one and two months relating cheese making factors to quality parameters, allowed to point out important relationships existing between cheese making conditions, ripening time and quality and led to suggest best practices in the cheese making procedure of Toma cheese, according to the improvement in both food safety and quality.

The approach is intended as a guideline for product optimization taking into account the technological features of the product, food safety issues and the receipt constraints.

- Keywords: Experimental Design, food quality, food safety, biogenic amines determination, cheese ripening, Principal Component Analysis -

INTRODUCTION

The ripening process is responsible for the positive features of cheese involving flavour, texture and aroma; on the other hand, during ripening the proteolytic process can lead to the formation of compounds that are potentially toxic for humans (KALIT *et al.*, 2005). The entire process leads both to changes in the chemical-physical properties of cheese (pH, moisture, etc.) and to the formation of biogenic amines (BAs) due to the decarboxylation of free amino acids.

BAs are organic bases characterised by a biological activity as they play psycho- and vasoactive effects on humans: the consumption of large amounts of BAs can in fact cause troubles of different entity, from light illnesses as headache and nausea to more dangerous diseases as hyper- and hypo-tension, cardiac palpitation and anaphylactic shock syndrome (NOVELLA-RODRIGUEZ, 2000).

Many different factors have been studied to characterise cheese and also investigate variations during cheese ripening processes: moisture, salt, proteins, D+L lactic acid, nitrogen fractions soluble in water and acid, free amino acids content, BAs profile and fatty acid methyl ester content, protein fractions (ATTAIE, 2005; BARILE *et al.*, 2006; GENNARO *et al.*, 2003; KEBARY *et al.*, 1999; KUCHROO and FOX, 1982; PINTADO *et al.*, 2008; PUERTO *et al.*, 2004; WATKINSON *et al.*, 2001).

During ripening, changes are evident in moisture, amount of proteins, salt, pH, nitrogen soluble fractions and amount of BAs; all these parameters are influenced by cheese making procedures and storage conditions (GENNARO *et al.*, 2003; KEBARY *et al.*, 1999; KUCHROO and FOX, 1982; MICHAELIDOU *et al.*, 2003; NIKOLAOU *et al.*, 2002; NOVELLA-RODRIGUEZ *et al.*, 2004; SKEIE and ARDO, 2000; VARLIK and UGUR, 2002; WALLACE and FOX, 1997; WATKINSON *et al.*, 2001).

In terms of food safety, a previous work (GENNARO *et al.*, 2003) carried out by our research group showed the correlation between amount of BAs and production conditions: this kind of knowledge can suggest optimal production and storage conditions to provide the minimization (and/or decrease) of the BAs content in cheese.

The aim of this work is deepening the knowledge of the effect played by cheese making parameters on the quality parameters of the final product along with ripening, providing in the meantime information about the best practices in cheese production according to the improvement of food quality and safety. The study is focused on a semi-hard cheese, namely "Toma" by full factorial experimental design and Principal Component Analysis in order to point out relationships existing between cheese making conditions, ripening time and quality of the final products.

The approach can be intended as a guideline for product optimization that allows to contemporarily consider the technological features of the product, food safety issues and the existence of receipt constraints.

MATERIALS AND METHODS

Sample preparation

The cheese samples were prepared by varying three cheese making conditions according to a two-level full factorial design (BOX and HUNTER, 1978; CARLSON, 1992; DEMING and MORGAN, 1993): a) the milk pre-treatment (raw or pasteurised), MILK; b) the starter bacteria (thermophile or mesophile), STRAIN; c) the treatment of the curd (raw or heated curd), CURD. The experiments required are 8 and three replicates of the overall experimental plan were produced, thus providing a total of 24 cheese samples. Factorial design theory is not further deepened here since it is widely described elsewhere (BOX *et al.*, 1978; CARLSON, 1992; DEMING and MORGAN, 1993).

Chemical and physico-chemical parameters were determined on each independent replication after one and two months ripening. Each set of cheese samples was obtained from separate batches of 50 litres of bovine whole milk, coagulation was obtained at 35°C by means of liquid bovine rennet (1:10000). In the case of "heated curd", a temperature of 44°C was applied for 12 min. In all cases, curd was put in cylindrical moulds (diameter 25 cm) then salted in a 21% NaCl w/w brine for 12 h and ripened at 10°C and 85% R.H. up to two months.

Bacterial cultures were two different commercial starters, with different composition:

- *Mesophilic strains: Lactococcus lactis* (subspecies *lactis*), *Lactococcus lactis* (subsp. *cremoris*), *Lactococcus lactis* (subsp. *biovar*), *Lactococcus lactis* (subsp. *diacetylactis*) and *Leuconostoc mesenterioides* (subsp. *cremoris*);
- *Thermophilic strains: Streptococcus thermophilus* and *Lactobacillus delbreuckii* (subsp. *Bulgaricus*).

Reagents

Ultrapure water from Milli-Q system (Millipore), acetonitrile, HCl 0.1 M and acetone HPLC grade were purchased from Merck. Histamine dihydrochloride, tyramine hydrochloride, putrescine dihydrochloride, cadaverine dihydrochloride, sodium hydrogen carbonate and dansyl-chloride were Sigma-Aldrich reagents.

Instrumentation

An HPLC Lachrom, Merck-Hitachi (Tokyo, Japan), equipped with a quaternary pump L-7100, an UV detector L-7400 and an interface L-7000

were used; the chromatographic column was a Merck LiChrospher 100 RP-18 250 x 4 mm (5 μm) coupled with a guard column of the same material. A homogeneiser UltraTurrax T25 (IKA, Warke, Germany) and a centrifuge 5804 (Eppendorf, Hamburg, Germany) were used. A Crison pH2001 pH-meter equipped with a combined glass-calomel electrode was employed for the pH measurements.

Methods

Amine identification and quantification were made by HPLC, after extraction and dansylchloride derivatization, performed according to the protocol from MORET and CONTE (1996). The mobile phase was a water/ACN mixture in the following gradient elution: 0-5 min water/ACN 35/65, 5-20 min water/ACN 25/75. Flow-rate was 0.8 mL/min and UV detection was performed at 254 nm. pH and moisture determinations were performed according to FENELON and GUINEE (1999); water and acid soluble nitrogen were measured by the method reported by KUCHROO and FOX (1982), while total nitrogen content was estimated by the AOAC standard method 2001.14. D+L Lactic acid was determined by a Boehringer-Mannheim enzymatic kit and the total aminoacids quantity was evaluated as in POLYCHRONIADOU (1988).

Principal Component Analysis, regression models and all graphical representations were performed by Statistica 7.1 (Statsoft Inc., USA) and Excel 2003 (Microsoft Corporation, USA).

Data analysis

Principal Component Analysis (PCA) (MASSART *et al.*, 1988; VANDEGINSTE *et al.*, 1998) was carried out here to provide a dimensionality reduction; PCA was applied with a Varimax rotation of the relevant PCs (i.e. the maximisation of the variances of all the original variables on each PC), to obtain a clearer insight of the investigated phenomena (MASSART *et al.*, 1988; VANDEGINSTE *et al.*, 1998). The Varimax rotation is particularly suitable for this application to identify the macro-variables hidden in the data structure, e.g. the ripening time or the content of biogenic amines.

The effect played by the factors investigated (MILK, CURD and STRAIN) was studied by a two-level full factorial design that allows the evaluation of the effect of the principal factors and of their interactions (two- and three-way interactions) on the investigated responses. Eight cheese samples corresponding to the experiments deriving from a 2^3 full factorial design were performed (replicated three times to provide 24 final products). The samples obtained were ripened for two months and analyzed after one and two months respectively by the compositional parameters (moisture, pH, lactic acid, aminoacids, nitrogen fraction soluble in water and in trichloroacetic acid (TCA)) and the BAs content (cadaverine, histamine, putrescine and tyramine).

The Ordinary Least Squares (OLS) regression model that can be investigated by the 2^3 full factorial design applied in this case is:

$$y = b_0 + b_1 \cdot MILK + b_2 \cdot STRAIN + b_3 \cdot CURD + \\ + b_{12} \cdot MILK \cdot STRAIN + b_{13} \cdot MILK \cdot CURD + \\ + b_{23} \cdot STRAIN \cdot CURD \\ + b_{123} \cdot MILK \cdot STRAIN \cdot CURD$$

where the coefficients b_i measure the effect of the corresponding variable and of their interactions on the final response y. The variable y can be either a direct experimental measurement (e.g. the concentration of investigated analytes) or a variable taking into account the correlation structure of the data (score on a significant PC). Here, the regression models were built for each PC calculated by PCA after Varimax rotation.

Data analysis consisted therefore in a multistep procedure:

1) Principal Component Analysis.

PCA was first applied to the overall dataset, provided in Supplementary Information 1, in which values lower than the LOD were set to 0. After autoscaling, the Varimax rotation was applied to the first four PCs calculated, explaining about 85% of the total variance. This first step provides a reduced set of macro-variables.

2) Evaluation of the experimental error.

For each regression model, the experimental error was obtained calculating the pooled variance as:

$$s^2_{pw} = \frac{\sum_{i=1,N} \left(v_i \cdot s^2_i \right)}{\sum_{i=1,N} v_i}$$

where:

$v_i = n_i - 1$ is the number of degrees of freedom of each estimate of the experimental error s^2_i of the *i-th* replicated experiment (n is the number of replications; here $n_i=3$ for each s^2_i). The sum runs on the $N=8$ experiments of the experimental design.

3) Calculation of regression models

Regression models were then built relating each rotated principal component to the investigated cheese making factors (MILK, STRAIN, CURD) and their interactions. Statistically relevant coefficients of factors and/or interactions to be included in the model were identified as in (BOX and HUNTER, 1978), comparing the coefficients to the corresponding standard error:

$$StdError = \frac{S_{pe}}{\sqrt{N}}$$

where N is the number of experiments (here, N=24).

Thank to the availability of several replicated experiments, each model containing only the relevant factors and interactions could be tested for lack-of-fit. The test was carried out according to ANOVA (ANalysis Of VAriance), by a Fisher F-test (BOX and HUNTER, 1978) comparing the residual information due to lack-of-fit, MSS_{lof} (not accounted for by the), model to the residual information due to pure experimental error (MSS_{pe}):

$$F_{lof,calc} = \frac{MSS_{lof}}{MSS_{pe}}$$

If the $F_{lof,calc}$ is smaller than the F-Fisher tabulated for the corresponding degrees of freedom of MSS_{lof} and MSS_{pe}, at a significance level =0.05, no lack-of-fit is detected.

The analysis of the regression models provides general information about the effect played by each factor on the general characteristics of cheese along ripening and allows the identification of best practices in cheese making procedures. Independent regression models for one and two months ripening times were built, in order to identify possible changes in the effect played by the cheese making conditions.

RESULTS AND DISCUSSION

Principal Component Analysis

PCA was applied to the overall dataset after autoscaling and Varimax rotation of the first 4 factors, explaining about 85% of the total variance.

PC_1 accounts for the largest amount of total variance (about 31%) and the corresponding loading plot (Fig. 1a) shows large positive weights of the variables related to the BAs content and at a lower extent, to the fraction of N_2 extracted in water. This PC therefore mainly accounts for the overall BAs content (more positive scores on PC_1 correspond to a larger BAs content).

PC_2 (about 28% explained variance) shows a positive contribution (positive weight; Fig. 2a) of the amount of nitrogen extracted by TCA, amino acids and pH; a lower but still positive contribution is also present for nitrogen extracted in water. The amount of nitrogen extracted by TCA is usually related to non protein nitrogen, while N extracted in water is commonly considered to have a protein origin (KALIT *et al.*, 2005; KUCHROO and FOX, 1982) [1, 7]. The amount of variance accounted for by water soluble nitrogen is thus separated into two contributions: one related to proteolysis and one related to the BAs content. From this point of view, the generation of BAs can be looked at as a secondary product of proteolysis and an increase of pH and of the amount of N in TCA can be related to proteolysis working-out (WATKINSON *et al.*, 2001) [10], i.e. to a correct ripening from the technological point of view. An increase of the scores on PC_2 can therefore be related to the presence of a proteolyc process.

PC_3 (13% explained variance) accounts for the information on moisture, showing a large negative weight (loading plot in Fig. 3a); more negative scores on this component are therefore related to a larger moisture.

The fourth PC, explaining about 12% of total variance, accounts for the contribution of lactic acid (large negative weights). Since PC_4 does not show systematic variations among the samples, it is not discussed further here since no relevant models could be built.

Regression models

Table 1 reports the regression models calculated for each PC after 1 and 2 months ripening: coefficients in parenthesis were not included in the final model (p-level > 0.05). R^2 and F_{lof} values refer therefore to the final model excluding not significant parameters and/or interactions. As it can be noticed good fitting abilities for all models were obtained (R^2 values always > 0.92), with no evidence of lack-of-fit ($F_{lof,\,calc} < F_{lof,\,tab}$).

Hereafter, the models obtained are discussed in more detail for each PC independently to extrapolate the effect played by each cheese making factor on the evolution of the physical-chemical characteristics of the samples along ripening. Models are discussed on the basis of surface response graphics showing the corresponding PC score along the z-axis and the variation of two parameters at a time on the x and y axes respectively. All response surfaces are represented on a colour scale from red (large positive scores) towards blue (large negative scores).

- **PC_1: content of biogenic amines**

For what regards the 1st month ripening, the effect played by the cheese making conditions must be discussed on the basis of the three-way interaction, that appears relevant in the model (Table 1). Fig. 1b represents the response surfaces illustrating the three way interaction. Passing from raw to pasteurised milk, BAs decrease (more negative scores) independently from the type of strain and curd. However, with heated curd, the effect of the type of milk is larger (steeper curve) with thermophile than for mesophile strains.

Therefore, the use of pasteurised milk, after one-month ripening, smoothes the effects of the other two variables on the BAs content. Either

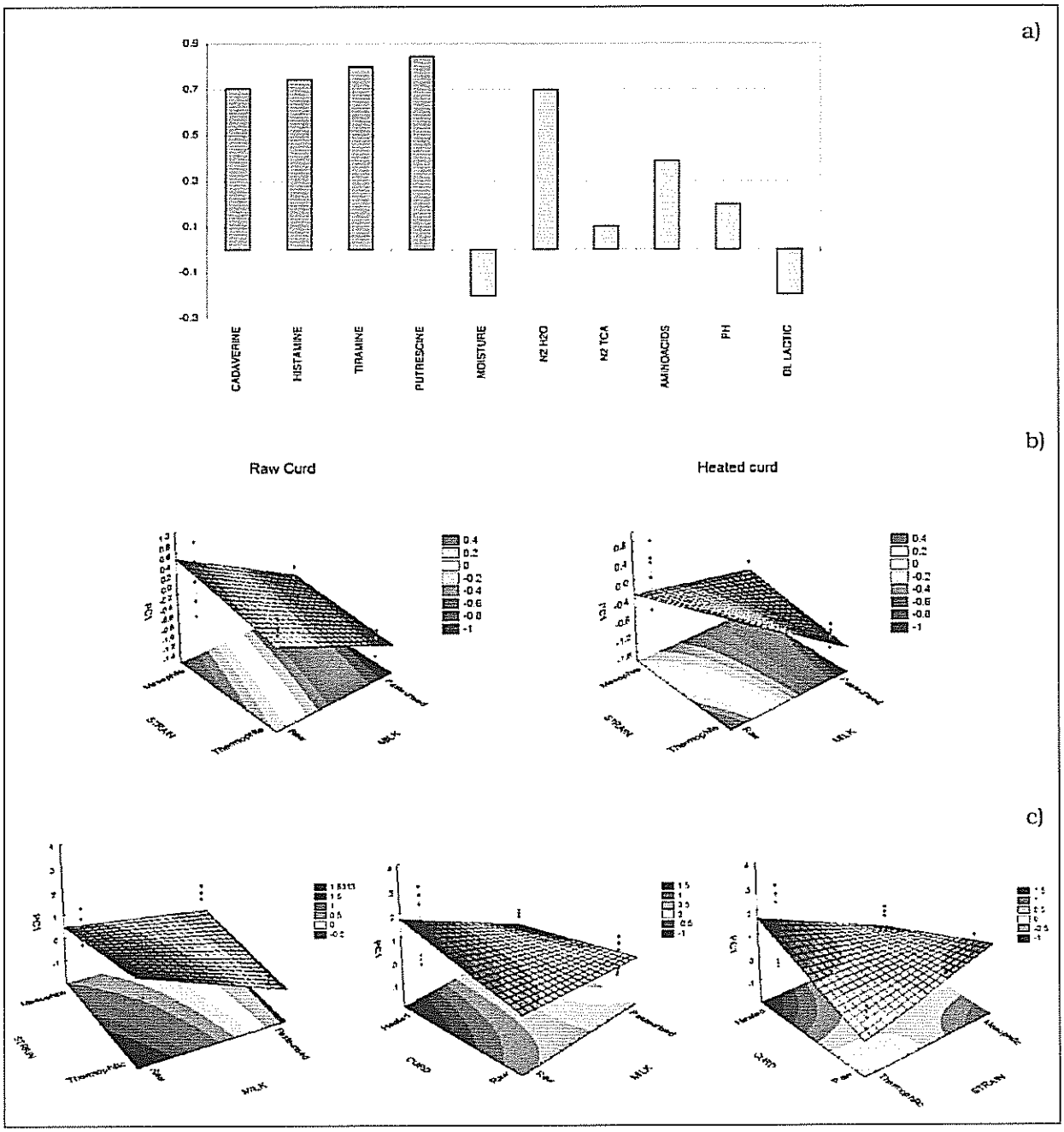

Fig. 1 - Results obtained for PC_1 (Biogenic Amines): loading plot (a); surface plots for the three-way interaction for raw and heated curd separately for the model after one month ripening (b); surface plots for the two-way interactions for the model after two months ripening (c).

heating or not heating the curd, it is useful to adopt pasteurised milk to obtain a minor content of BAs: this is true for both types of strain. The smallest possible concentration of BAs is obtained with pasteurised milk, heated curd and mesophile strain.

If raw milk has to be used, instead, the interaction between curd and strain becomes relevant: thermophile strains have to be used with raw curd and mesophile strains if curd is heated.

For what regards the 2nd month regression model, the effects of the factors are discussed on the basis of the surface plots of all the relevant two-way interactions (Table 1 and Fig. 1c):

- *MILK * STRAIN* Interaction. With pasteurised milk, the change of the type of strain does not produce effects on the BAs content (they are in general at low levels), while the starter bacteria plays an effect with raw milk: passing from thermophile to mesophile strains, BA decrease. Passing from raw to pasteurised milk produces a decrease of the BAs content with both thermophile and meso-

phile strains but the effect is larger with the first ones. The smallest amount of amines is detected with pasteurised milk and thermophile strains.

- *MILK * CURD* Interaction. Passing from raw to heated curd, BAs decrease with pasteurised milk and increase with raw milk. With both raw and heated curd, milk pasteurisation decreases amines but the effect is more evident if the curd is heated. The most different situations can be detected with heated curd: with pasteurised milk the content of amines is the smallest, with raw milk their content is the largest.
- *STRAIN * CURD* Interaction. Passing from raw to heated curd, BAs decrease with mes-

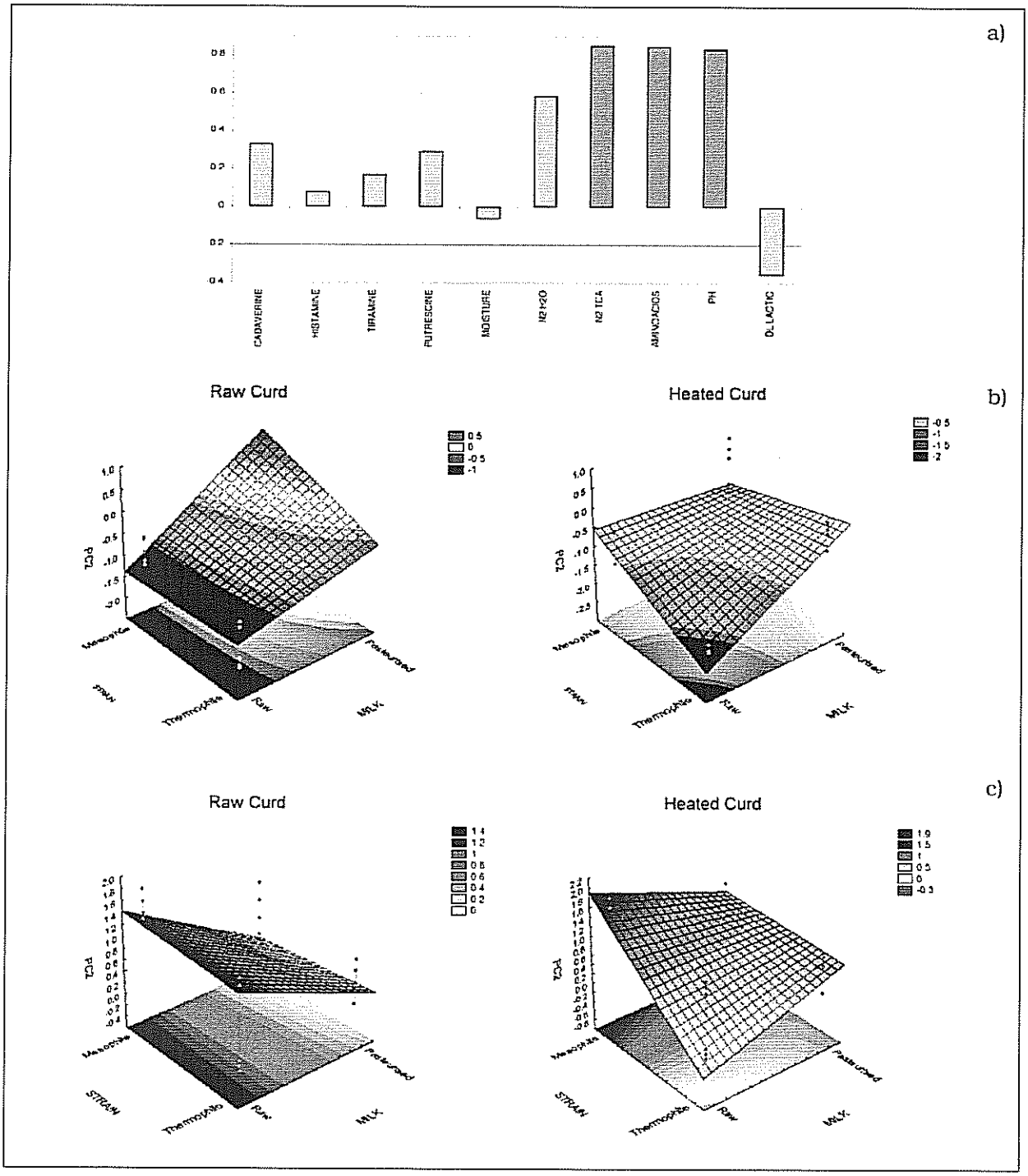

Fig. 2 - Results obtained for PC_2 (Proteolysis): loading plot (a); surface plots for the three-way interaction for raw and heated curd separately for the model after one month ripening (b); surface plots for the three-way interaction for raw and heated curd separately for the model after two months ripening (c).

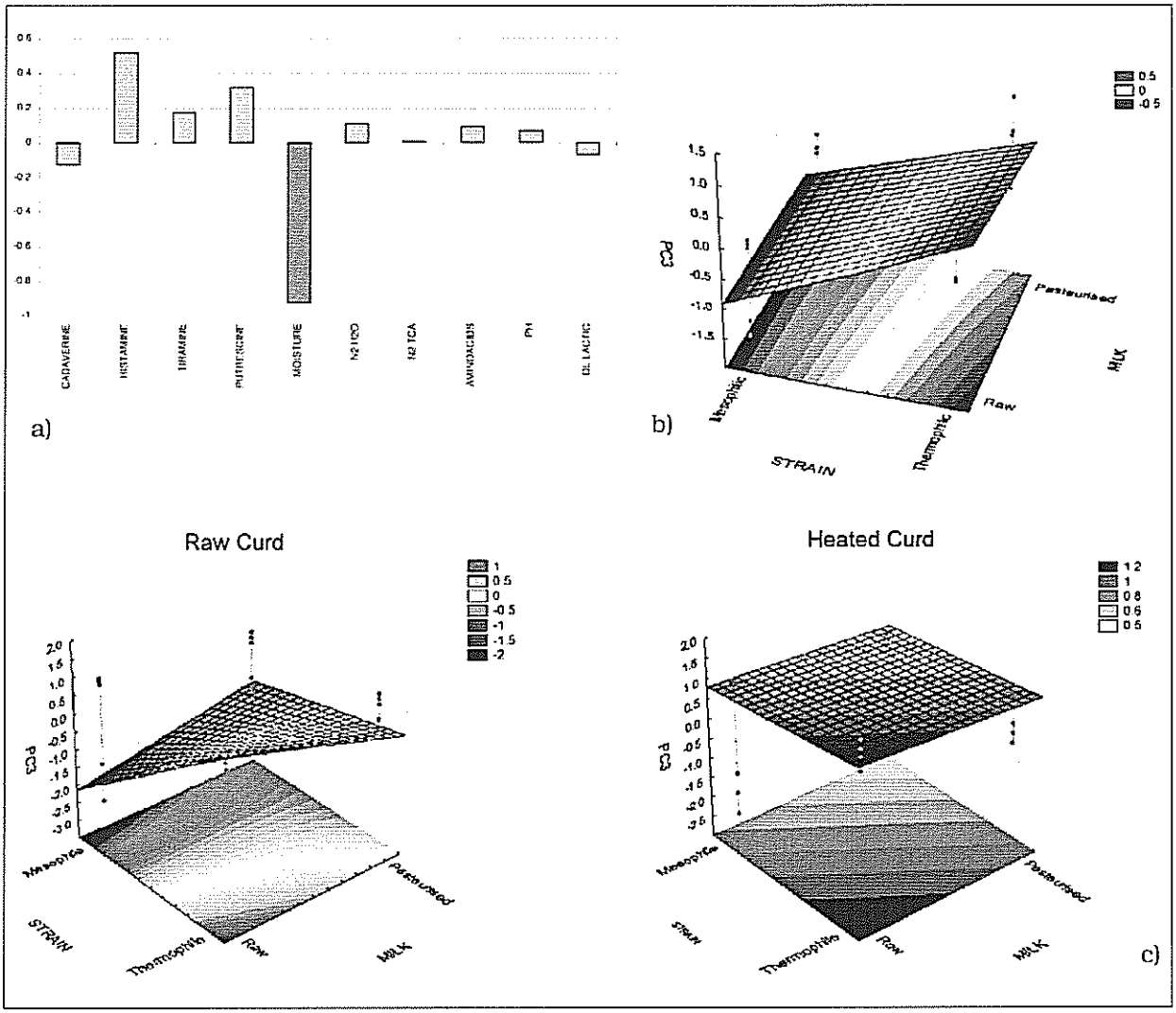

Fig. 3 - Results obtained for PC_3 (Moisture): loading plot (a); surface plots for the two-way MILK*STRAIN interaction for the model after one month ripening (b); surface plots for the three-way interaction for raw and heated curd separately for the model after two months ripening (c).

ophile strains and increase with thermophile strains. Passing from thermophile to mesophile strains, amines increase with raw curd and decrease if the curd is heated. The smallest amounts of amines are detected in correspondence to raw curd/thermophile strain and heated curd/mesophile strain and the largest amount in the other two extreme experimental points.

If the three two-way interactions are considered simultaneously, it appears clear that also after two months ripening the smallest amount of amines is achieved with pasteurised milk, heated curd and mesophile strains. For what regards the achievement of low levels of BAs, potentially dangerous for health, it is better to heat the curd with mesophile strains or using raw curd with thermophile strains: in such cases fermentation is not pushed to extremes. The heating of the curd does not produce always smaller levels of amines: this is true only if pasteurised milk is used.

Comparing the two models (Table 1) the only parameter showing a change of its sign is the intercept (negative value for the first model and positive value for the second model): the average BAs content after one month ripening is low (negative score), while it is larger after two months (positive score), showing that a general increase is recorded during ripening.

In order to maintain low concentrations of BAs, it seems to be sufficient to pasteurise milk with a ripening of one month; for a ripening time of two months it is necessary, in addition, to heat the curd. If the procedure of cheese making requires the use of raw milk, the use of mesophile strains and heated curd assures a low concentration of BAs.

Table 1 - OLS regression models for PC_1 (biogenic amines), PC_2 (proteolysis) and PC_3 (moisture) for the first and second months: regression coefficients, corresponding calculated t-Student and p-level, experimental error (S_{pe}), standard error calculated on the coefficients (Std Error). Coefficients in parenthesis were not included in the final model (p-level > 0.05); R^2, F-Fisher calculated and tabulated for the evaluation of lack-of-fit are indicated for the model excluding not significant coefficients.

PC_1: Biogenic amines	1st Month			2nd Month		
	Coeff.	t calc	p-level	Coeff.	t calc	p-level
Intercept	-0.3086	8.38	0.0000	0.3086	5.69	0.0000
MILK	-0.5061	13.74	0.0000	-0.7110	13.10	0.0000
STRAIN	(0.0332)	0.90	0.3802	-0.2623	4.83	0.0002
CURD	-0.1594	4.33	0.0005	(-0.0398)	0.73	0.4739
MILK*STRAIN	0.0893	2.42	0.0276	0.2463	4.54	0.0003
MILK*CURD	(0.0180)	0.49	0.6317	-0.4177	7.69	0.0000
STRAIN*CURD	-0.1270	3.45	0.0033	-0.7437	13.70	0.0000
MILK*STRAIN*CURD	0.1276	3.47	0.0032	(0.1072)	1.98	0.0657
	S_{pe}	Std Error	R^2	S_{pe}	Std Error	R^2
	0.1804	0.0368	0.9330	0.2660	0.0543	0.9577
	$F_{lof,calc}$	$F_{lof,tab}$		$F_{lof,calc}$	$F_{lof,tab}$	
	0.53	4.69		2.22	4.69	
PC_2: Proteolysis	1st Month			2nd Month		
	Coeff.	t calc	p-level	Coeff.	t calc	p-level
Intercept	-0.7603	18.44	0.0000	0.7603	19.18	0.0000
MILK	0.4863	11.80	0.0000	-0.2800	7.06	0.0000
STRAIN	0.1869	4.53	0.0003	0.2150	5.43	0.0001
CURD	-0.1572	3.81	0.0015	(-0.0093)	0.24	0.8171
MILK*STRAIN	-0.1053	2.56	0.0212	-0.2193	5.53	0.0000
MILK*CURD	-0.0945	2.29	0.0359	0.2553	6.44	0.0000
STRAIN*CURD	(0.0234)	0.57	0.5782	0.2610	6.59	0.0000
MILK*STRAIN*CURD	-0.3209	7.79	0.0000	-0.1548	3.91	0.0013
	S_{pe}	Std Error	R^2	S_{pe}	Std Error	R^2
	0.2019	0.0412	0.9379	0.0377	0.0396	0.9290
	$F_{lof,calc}$	$F_{lof,tab}$		$F_{lof,calc}$	$F_{lof,tab}$	
	0.32	6.11		0.06	6.11	
PC_3: Moisture	1st Month			2nd Month		
	Coeff.	t calc	p-level	Coeff.	t calc	p-level
Intercept	-0.2002	6.42	0.0000	0.2002	3.68	0.0020
MILK	-0.0774	2.48	0.0245	-0.1359	2.49	0.0239
STRAIN	-0.5839	18.73	0.0000	-0.5756	10.57	0.0000
CURD	0.6183	19.83	0.0000	0.6770	12.43	0.0000
MILK*STRAIN	0.0791	2.54	0.0219	0.2269	4.17	0.0007
MILK*CURD	(-0.0446)	1.43	0.1714	(-0.1137)	2.09	0.0532
STRAIN*CURD	(0.0537)	1.72	0.1041	0.3889	7.14	0.0000
MILK*STRAIN*CURD	(-0.0040)	0.13	0.9000	-0.2256	4.14	0.0008
	S_{pe}	Std Error	R^2	S_{pe}	Std Error	R^2
	0.1527	0.0312	0.9729	0.2668	0.0545	0.9462
	$F_{lof,calc}$	$F_{lof,tab}$		$F_{lof,calc}$	$F_{lof,tab}$	
	1.68	4.08		4.36	6.11	

- **PC_2: Proteolysis**

The models built after one and two months ripening show that the three-way interaction is relevant (Table 1); therefore, both models must be discussed considering the surface plots representing the corresponding three-way interactions (Figg. 2b-c).

- *First month ripening* (Fig. 2b). With raw curd, the largest proteolysis (largest positive score) is observed with mesophile strains and pasteurised milk. If raw milk is used, proteolysis is always low. With raw curd, the strain plays an effect only if pasteurised milk is used. Milk pasteurisation always plays the same effect: it increases proteolysis, but the effect is more relevant (steeper curve) if mesophile strains are used. When the curd is heated, instead, proteolysis is always low (negative scores), particularly with thermophile strains and raw milk. With heated curd, the effect of the type of strain is the most relevant (steepest curve) with raw milk (opposite to the behaviour with

raw curd). The effect of the type of milk is relevant only with thermophile strains (less negative scores).

- *Second month ripening* (Fig. 2c). One of the most evident differences between 1st and 2nd month models (Table 1) is the sign of the intercept, changing from negative (first model) to positive (second model): after one month ripening the average proteolysis is low, while it increases on the average after two months ripening. In general, we can state that the effects played by the factors remain almost unvaried with heated curd even if proteolysis in general increases after the second month: the smallest proteolysis is always obtained with raw milk and thermophile strains. However, proteolysis is large above all with raw milk and mesophile strains. With heated curd the effect of milk is similar to the first month but in this case it is relevant even with mesophile strains (opposite to that recorded for thermophile strains). With raw curd, the behaviour after two months ripening is quite different from one month ripening: proteolysis is large with raw milk and low with pasteurised milk, while the strain does not play a relevant effect. With raw curd, the type of milk plays the same effect with both strains but the effect is opposite to that recorded after one month ripening (proteolysis increases with raw milk). The type of strain plays no relevant effect nor with raw or pasteurised milk. The effect of the use of raw milk, when using raw curd, becomes visible only at the second month, increasing proteolysis, but shows no effect on proteolysis if the ripening is stopped at the first month.

- **PC_3: Moisture**

In the 1st month model only the three principal factors and the interaction *MILK - STRAIN* are relevant (Table 1). The regression coefficient of *CURD* shows that passing from raw to heated curd, moisture of the final product decreases, since draining increases. The effect of *MILK* and *STRAIN* instead must be discussed on the basis of the two-way interaction (Fig. 3b). With both pasteurised and raw milk, passing from mesophile to thermophile strains decreases moisture (more positive scores). In particular with mesophile strains, pasteurising milk does not produce relevant effects (moisture is always large), while it reduces moisture with thermophile strains.

For what regards the 2nd month model, the effects must be discussed on the basis of the three-way response surface (Table 1), reported in Fig. 3(c). With raw curd, the interaction between MILK and STRAIN seems similar to the first month: the lowest moisture is recorded with raw milk and thermophile strains. On the contrary, the largest values are recorded with mesophile strains for both types of milk. In general, the effect of the type of milk and strain on the final moisture is more significant with raw curd.

With heated curd, moisture is always low (positive scores) but the lowest possible values are recorded when raw milk and thermophile strains are adopted. Heating of the curd therefore levels moisture after two months ripening.

Again, if 1st and 2nd month models are compared, only the intercept changes its sign: a negative value for the first model corresponds to an average large value of moisture after one month, while the positive value for the second model corresponds to a general decrease of the moisture along ripening, as expected.

In moisture control, the most significant parameter is the heating of curd, as expected; if cheese has to be produced from raw curd, the use of thermophile strains, in association with both pasteurised and raw milk, assures its minimum value.

CONCLUSIONS

The conclusions that can be drawn from the joint analysis of the regression models built for each PC independently, can lead to the establishment of optimal cheese making practices aimed to obtain products characterised by high quality features and high food safety standards.

The approach here presented can be used as a guideline for product optimization: the optimization step has to take into account the technological features of the product and food safety issues but also receipt constraints. In facts, the optimal cheese-making conditions will not probably correspond to the absolute optimal conditions but will be the best conditions considering all receipt constraints, e.g. the use of raw milk, the use of raw curd etc.

Since the use of raw or heated curd leads to fundamentally different final products, final considerations are drawn according to a preliminary choice of a desirable ripening time (one or two months) and of the procedure used for curd preparation. For example, if the making conditions state a maximum ripening of one month and the use of raw curd (the worst conditions for BAs production), it emerges that pasteurised milk has to be preferred since the final product shows anyway a small BAs amount and very advanced proteolysis (especially when mesophile strains are used); the use of mesophile strains leads to a very moist product, but if thermophile strains are adopted, intermediate moisture levels can be obtained.

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