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Characterization of "Minas" Fresh Cheese Enriched with Calcium Caseinate by Principal Component Analysis

Caracterização de Queijo Minas Frescal Enriquecido com Caseinato de Cálcio pela Análise dos Componentes Principais

AUTORES
AUTHORS

Carmen Cecília TADINI
Laboratory of Food Engineering,
Department of Chemical Engineering,
Escola Politécnica, São Paulo University,
P. O. Box 61548, 05424-970 São Paulo-SP - Brazil
Fax number: 55 11 3021 3020,
E-mail: catadini@usp.br

SUMMARY

The Principal Component Analysis was used to analyze data obtained from "Minas" Fresh cheese, a typical and popular fresh Brazilian cheese, enriched with Ca-caseinate. Data for acidity, pH, total solids, fat, protein, ash, chlorides and yield were obtained. The cheeses were produced in the laboratory according to a 4^2 experimental design with two variables: age of the pure cultures (1 to 4 weeks) and proportion Ca-caseinate (0.0%, 0.5%, 1.3% and 2.0%). From the results obtained, a second factorial experimental design (3^2) was proposed as a combination of the Ca-caseinate proportion and homogenization pressure applied to the milk mixture. Predictive mathematical models showed that the addition of Ca-caseinate decreased the fat content and increased the total protein content and yield. The Principal Component Analysis was performed and three new variables accounted for 97.42 percent of the total variance, the first component (70.61%) basically representing the yield in cheese. The homogenization pressure applied to the milk mixture did not affect cheese yield.

RESUMO

Análise dos Componentes Principais foi aplicada para analisar dados de queijo "Minas Frescal", um típico queijo fresco produzido no Brasil, enriquecido com caseinato de cálcio. Os dados analisados foram: acidez, pH, sólidos totais, lipídios, proteína total, cinzas, cloretos e rendimento. Em laboratório, foram produzidos queijos de acordo com o projeto experimental 4^2, com os fatores: idade das culturas puras (1 a 4 semanas) e proporção de caseinato de cálcio (0,0%, 0,5%, 1,3% e 2,0%). Dos resultados obtidos, um segundo projeto experimental 3^2 foi proposto como uma combinação da proporção de caseinato de cálcio e pressão de homogeneização na mistura leite. Modelos matemáticos indicaram que a adição de caseinato de cálcio diminuiu o conteúdo de lipídios e aumentou o conteúdo de proteína total e rendimento. A Análise dos Componentes Principais foi realizada e três novas variáveis representaram 97,42% da variância total, sendo que o primeiro componente (70,61%) representou basicamente o rendimento dos queijos. A pressão de homogeneização não influenciou o rendimento dos queijos.

PALAVRAS-CHAVE
KEY WORDS

Principal component analysis; Cheese; Control data / Análise dos componentes principais; Queijo; Controle de qualidade.

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1. INTRODUCTION

Milk proteins are highly desirable as ingredients in food. In addition to availability and high nutritive value, their utility is to enhance the bland products they produce. Casein, the major component of cows' milk protein and the caseinates show physicochemical, functional and nutritive properties that make them useful worldwide. These proteins are currently used in a variety of products, including coffee whiteners, cheese analogs and meat products (KONSTANCE; STRANGE, 1991).

Caseinates, according to IFD (1974), are the products formed by drying dispersions prepared by the addition of an appropriate alkali and potable water to edible dried casein or to fresh edible casein, both derived entirely from milk. The alkali(s) and/or alkaline reacting salts (including ammonium and earth alkalis) used should be of food grade and provide the desired ions in the caseinates.

"Minas" fresh cheese, one of the most consumed cheeses in Brazil, accounts for about 13% of the total cheeses produced in Brazil, mainly in the South and Southeast regions (ABIQ, 1992). More recent data indicates that the total production of cheese in Brazil increased from 420 thousand tones in 1998 to 516 thousand tones in 2003 (ANONYMOUS, 1998; BRASIL, 2003). Its calorific value is about 243 Cal/100g of cheese. Each 100g of cheese usually contains: 18g of protein, 19g of fat, 685mg of calcium, 430mg of phosphorus and 0.4mg of iron (FAO, 1974).

Some researchers have studied the production of cheese enriched with protein concentrates to improve quality and yield. FURTADO; COELHO; GOMES (1988) studied the influence of the addition of soy protein concentrate to Mozzarella cheese and the results showed that the yield increased by about 4% in comparison to the traditional process. SOUZA; ANTUNES; SILVA (1992) produced sixteen different formulations of "Minas" Fresh cheese with non-fat soluble soy extract powder and the results showed that the best condition for product firmness was 4% non-fat soy extract powder, 350mg of rennet and 7.5ml of calcium chloride solution per 10 liters of milk. For the process yield the best condition was 16% of non-fat soy extract powder, 400mg of rennet and 6.25ml of calcium chloride solution per 10 liters of milk.

Due to the large number of variables involved in food development and quality control, statistical techniques have been used to identify and measure factors that have significant influence on the results. These statistical techniques have usually been referred to as multivariate statistical methods. Principal component analysis (PCA) has been used by many researchers to analyze multivariate data, since it is successful in investigating relationships among larger numbers of variables. PCA is a useful technique for reducing the number of variables in a data set by finding linear combinations of those variables that explain most of the variability.

Recent articles show Principal Component Analysis as a powerful tool to relate observable variables with variables that cannot be directly measured in food experiments (BAARDSETH; NAES; MIELNIK; SKREDE; HOLLAND, 1992; KARLSSON, 1992; CORDOBA; NAKAI, 1994; BICCHI; BINELLO; PELLEGRINO; VANNI, 1995; INGEMANSSON; KAUFMAN; EKSTRAND, 1995; VILLAR; GARCIA; IGLESIAS; GARCIA; OTERO, 1996; PRIPP; REHMAN; MCSWEENEY; SϕRHAUG; FOX, 2000; PRIPP; STEPANIAK; SϕRHAUG, 2000; THYBO; MARTENS, 2000; FR?ST; DIJKSTERHUIS; MARTENS, 2001).

A Principal Component Analysis is concerned with explaining the variance-covariance structure through a few linear combinations of the original variables. Its general objectives are (1) data reduction and (2) interpretation. This is accomplished by transforming a set of original variables, X_1, X_2, ..., X_p, observed for the samples to a new set, Y_1, Y_2,....., Y_p, the so-called principal components (PCs). Each PC, Y, is a linear combination of the X's:

$$Y_i = a_{i1}X_1 + a_{i2}X_2 + ... + a_{ip}X_p \quad (1)$$

The coefficients, a_{ij}, are called "loadings" and express the contributions of the original variables, X_1, X_2, ..., X_p, to the PC. Algebraically, principal components are particular linear combinations of the p random variables X_1, X_2, ..., $X_{p.}$ Geometrically, these linear combinations represent the selection of a new coordinate system obtained by rotating the original system with X_1, X_2, ..., X_p as the coordinate axes. The new axes represent the directions with maximum variability and provide a simpler and more parsimonious description of the co-varied structure. The algebraic sum for a sample in a PC is called the score for that sample in that PC. The first PC is constructed so that it extracts maximum variance in the data set. Second and further PCs extract maximum remaining variance uncorrelated with previous extracted PCs. Since all PCs are totally uncorrelated, they do not overlap in information content. The first new PCs are usually enough for most of the variance and the rest is mainly error (noise) (JOHNSON; WICHERN, 1992).

VILLAR et al. (1996) studied the relationships between microbial populations in 402 samples of refrigerated raw cows' milk taken from 18 farms, using principal component analysis. The first component was related almost equally to all bacterial counts, and it could represent the bacteriological quality of the bulk farm tank (77.2% of total variance). The second component (11.9%) was defined mainly as thermoduric flora and coliform flora. The predominance of thermodurics over other flora from the milking equipment may be associated with the use of high temperature during cleaning of the equipment. This is quite an interesting example of how PCA can be applied to simplify relationships between different variables related to a process.

The aim of the present study was to analyze physical-chemical data of "Minas" cheese produced with calcium caseinate by Principal Component Analysis (PCA).

2. MATERIAL AND METHODS

Commercial milk with 3% fat content (acidity as lactic acid: 0.136% ± 0.013; total solids: 11.65% ± 0.18; fat: 2.96% ± 0.18; total protein: 3.08% ± 0.29; ash: 0.70% ± 0.06 and density: 1.0308g cm^{-3} ± 0.0011), calcium caseinate from CONAPROLE* (acidity as lactic acid: 1.21% ± 0.16; total solids: 92.12% ± 0.55; fat: 1.34% ± 0.05; total protein: 85.26% ± 0.45 and ash: 3.90% ± 0.10), pure cultures of Lactococcus lactis and Lactococcus lactis ssp cremoris from ITAL** (Food Technology Institute), rennet powder from Chr. Hansen***, calcium chloride and sodium chloride, were used to produce cheeses in the laboratory.

The experimental design was a combination of age of the pure cultures of Lactococcus lactis and Lactococcus lactis ssp cremoris and proportion of calcium caseinate added to the milk. Four levels of the factors were set as shown in Table 1, thus resulting in a 4^2 experimental factorial design. For each factor and each level, replicates were conducted, totaling 32 cheeses.

TABLE 1. Runs and limiting values for the factors at the four levels used in the experimental factorial design for "Minas" cheeses produced with calcium caseinate.

Run	Age of pure cultures (weeks)	Calcium Caseinate (% in relation to milk)
1	1 (-1)	0.0 (-1)
2	1 (-1)	0.5 (-0.5)
3	1 (-1)	1.3 (+0.3)
4	1 (-1)	2.0 (+1)
5	2 (-0.33)	0.0 (-1)
6	2 (-0.33)	0.5 (-0.5)
7	2 (-0.33)	1.3 (+0.3)
8	2 (-0.33)	2.0 (+1)
9	3 (+0.33)	0.0 (-1)
10	3 (+0.33)	0.5 (-0.5)
11	3 (+0.33)	1.3 (+0.3)
12	3 (+0.33)	2.0 (+1)
13	4 (+1)	0.0 (-1)
14	4 (+1)	0.5 (-0.5)
15	4 (+1)	1.3 (+0.3)
16	4 (+1)	2.0 (+1)

*CONAPROLE Cooperativa Nacional de Productores de Leche, Uruguay, www.conaprole.com

**ITAL Instituto de Tecnologia de Alimentos, P.O. Box: 139, Zip Code: 13073-001, Campinas, SP, Brazil, www.ital.org.br

***Chr. Hansen Ind. E Com. Ltda. P.O. Box: 371, Zip Code: 13276-970, Valinhos, SP, Brazil, www.chrhansen.com.br

From the results obtained, a second experimental design was proposed as a combination of the calcium caseinate proportional to milk and homogenization pressure of the calcium caseinate-milk mixture, both at three levels, resulting in a 3^2 experimental factorial design (see Table 2).

TABLE 2. Runs and limiting values for the factors used in the second experimental factorial design for "Minas" Fresh cheeses produced with calcium caseinate.

Run	Homogenization pressure (MPa)	Calcium Caseinate (% in relation to milk)
1	0 (-1)	0.0 (-1)
2	0 (-1)	0.4 (0)
3	0 (-1)	0.8 (+1)
4	14 (0)	0.0 (-1)
5	14 (0)	0.4 (0)
6	14 (0)	0.8 (+1)
7	21 (+1)	0.0 (-1)
8	21 (+1)	0.4 (0)
9	21 (+1)	0.8 (+1)

2.1 Procedure

The basic formulation used to produce a "Minas" Fresh cheese was: 1500g of 3% fat content milk; 0.3g of calcium chloride; 45.0g of mixed culture (1:1 proportion of Lactococcus lactis and Lactococcus lactis ssp cremoris); 0.04g of rennet and 2% of sodium chloride with respect to curd mass. The production of cheese enriched with calcium caseinate was similar, adding different proportions of caseinate to the basic formulation according to the experimental design (Table 1), and the mixture was homogenized or not, making it more uniform in consistency (TADINI; 1994; TADINI; CURI; CARDOSO, 1997). The cheeses produced were stored under refrigeration.

After 24 hours, samples were collected and submitted to laboratory analyses according to the Official Methods of Analysis (AOAC, 1990). The acidity, expressed as lactic acid, was determined by titration as described in AOAC (1990). The pH was determined using a pH meter (Metrohm Ltd., model E350 B, Herisau, Switzerland) as described in the Standard Methods for the Examination of Dairy Products (APHA, 1985). The fat content was determined by a gravimetric determination as described in the Official Methods Oil Chemists Society (AOCS, 1987), with prior acid hydrolysis. The total protein content was determined by the micro-Kjeldahl method as described in APHA (1985). Total solids, ash and chloride were determined as described in the Analytical Methods of Adolfo Lutz Institute, Brazil (IAL, 1976). The yield was determined from the ratio between cheese mass and initial total mass of mixture.

The cheeses were stored at refrigerated temperature (4°C) for 8 days and the mass was determined daily to verify if the presence of calcium caseinate influenced syneresis.

3. RESULTS AND DISCUSSION

The ANOVA analysis showed that at the 0.05% level the ash, chloride and total solids contents of the cheeses produced with calcium caseinate, were neither influenced by the age of pure cultures nor by the addition of calcium caseinate in the interval studied.

Table 3 shows the results, indicating that the addition of calcium caseinate influenced the protein and fat contents and yield, whereas the age of the pure cultures influenced the fat content, acidity and pH of the cheeses.

Table 4 shows that the addition of calcium caseinate at a level of up to 0.4% in relation to the milk did not influence the fat content of the cheeses, and that homogenization at 14 MPa decreased the fat content of the cheeses, as confirmed by the total solids contents of the cheeses and the fat contents of the whey.

TABLE 3. Mean values of the results and Tukey's Honestly Significant Difference (HSD) values ($p<0.05$) for the "Minas" Fresh cheeses of the first experimental design.

Cheese quality attributes	Ca–Caseinate proportion (%)					Age of Pure Cultures (weeks)				
	0.0	0.5	1.3	2.0	Tukey HSD (5%)	1	2	3	4	Tukey HSD (5%)
Acidity[1]	0.13^{a}	0.14^{b}	0.13^{a}	0.12^{a}	0.01	0.15^{a}	0.13^{a}	0.12^{b}	0.11^{b}	0.01
Ash[2]	2.26^{a}	2.21^{a}	2.30^{a}	2.30^{a}	0.21	2.30^{a}	2.30^{a}	2.25^{a}	2.22^{a}	0.21
Chloride[3]	1.11^{a}	1.05^{a}	1.11^{a}	1.10^{a}	0.22	1.21^{a}	1.03^{a}	1.13^{a}	1.01^{a}	0.22
Fat[4]	11.23^{a}	10.13^{b}	8.80^{c}	8.82^{c}	0.98	9.66^{ab}	9.30^{a}	9.73^{ab}	10.29^{b}	0.98
pH	5.93^{a}	5.96^{a}	5.90^{a}	5.96^{a}	0.20	5.80^{a}	6.07^{b}	5.98^{ab}	5.91^{ab}	0.20
Protein[5]	10.29^{a}	10.69^{a}	11.02^{a}	12.27^{b}	1.22	11.07^{a}	11.47^{a}	10.66^{a}	11.07^{a}	1.22
Total solids[6]	27.94^{a}	28.61^{a}	27.89^{a}	27.05^{a}	2.15	27.31^{a}	27.95^{a}	28.90^{a}	27.33^{a}	2.15
Yield[7]	23.88^{a}	23.88^{a}	29.65^{b}	27.94^{b}	2.34	26.96^{a}	25.18^{a}	26.42^{a}	26.78^{a}	2.34

[1]Acidity as % lactic acid
[2]Ash as % (g ash/ 100 g cheese)
[3]Chloride as % (g salt/ 100 g cheese)
[4]Fat as % (g fat/ 100 g cheese)
[5]Protein as % (g total protein/ 100 g cheese)
[6]Total solids as % (g total solids/ 100 g cheese)
[7]Yield as % (g cheese mass/ 100 g total mass of mixture)

TABLE 4. Mean values of the results and Tukey's Honestly Significant Difference (HSD) values ($p<0.05$) for the "Minas" Fresh cheeses of the second experimental design.

Cheese quality attributes	Ca–Caseinate proportion (%)				Homogenization Pressure (MPa)			
	0.0	0.4	0.8	Tukey HSD (5%)	0	14	21	Tukey HSD (5%)
Fat[1]	12.19^{a}	11.28^{a}	10.16^{b}	0.98	11.90^{a}	10.58^{b}	11.15^{b}	0.71
Whey Fat[2]	0.41^{b}	0.25^{a}	0.49^{a}	0.08	0.32^{a}	0.45^{c}	0.39^{b}	0.06
Total solids[3]	30.53^{a}	27.88^{a}	28.75^{a}	2.94	31.14^{a}	27.65^{b}	28.36^{b}	2.55
Yield[4]	21.70^{a}	22.37^{ab}	25.49^{b}	3.40	23.42^{a}	23.58^{a}	22.56^{a}	3.04

[1] Fat as % (g fat/ 100 g cheese)
[2] Fat as % (g fat/ 100 g whey)
[3]Total solids as % (g total solids/ 100 g cheese)
[4]Yield as % (g cheese mass/ 100 g mixture total mass)

Predictive mathematical models obtained by multiple regression analysis showed that the addition of calcium caseinate decreased the fat content and increased the total protein content, that is, the curd retained the calcium caseinate captured within its structure.

Figure 1 illustrates how the calcium caseinate influenced the variation in weight during storage time. For cheeses produced without caseinate, it varied from 24% to 19% whereas for the cheese produced with more than 1.3% of Ca-caseinate, it varied from 28.5% to 25%, decreasing the syneresis effect and denoting the property of caseinate to retain water (in this case, whey) in the protein structure.

The fitted model to predict the weight variation of the cheeses is presented as equation 2:

$$\hat{Y}_1 = (29.52 + 3.35X_2 - 1.62X_3 + 0.49X_1^2 + 0.90X_1X_2 - 1.61X_2^2 + 0.09X_3^2) \pm 1.61\% \quad (2)$$

$\hat{Y}_1$ is the predicted weight variation (%) of the cheese
X_1 is the age of the cultures, from 1 to 4 weeks
X_2 is the calcium caseinate proportion, from 0.0% to 2.0%
X_3 is the storage time, from 1 to 8 days

The fitted model indicated that the addition of calcium caseinate influenced the weight variation of the cheeses more than the storage time, which influenced it more than the age of the pure cultures (see Figure 2).

To get another point of view, the age of the pure cultures was considered as constant and equal to 2.5 weeks (mean value), obtaining a second fitted model as equation 3, which indicated that the addition of calcium caseinate in the interval studied, increased the variation in weight, whereas the storage time decreased it, caused by whey syneresis, as can be seen in the response surface model illustrated in Figure 3.

$$\hat{Y}_2 = 24.46 + 5.60X_2 - 1.62X_3 - 1.61X_2^2 + 0.09X_3^2 \quad (3)$$

The PCA analysis was then applied to establish the relationships amongst the physicochemical characteristics of the cheeses produced (acidity, total solids, fat, protein, pH, ash, chloride and weight retention). Table 5 shows that the relationships between three new components accounted for 97.42% of the total variability and the original variables, as well as the amount of information being explained by each one. From the data shown in Table 5, it is possible to interpret the meaning of the new variables. The first component (PC_1) is strongly related to the weight retention of the cheeses. The second one (PC_2) is defined by the total solids (positively) and by the fat content (negatively), while the third component (PC_3) is defined by the protein content (negatively) and is also defined by the total solids and fat contents, with both of them influencing in the same direction (positively).

Table 5 shows the component "loadings" for each variable, illustrating that the first PC is basically constituted of the weight retention of the cheeses. A weight close to 0.0 indicates that the original variable contributes little to the component, such as acidity as related to the 1st PC.

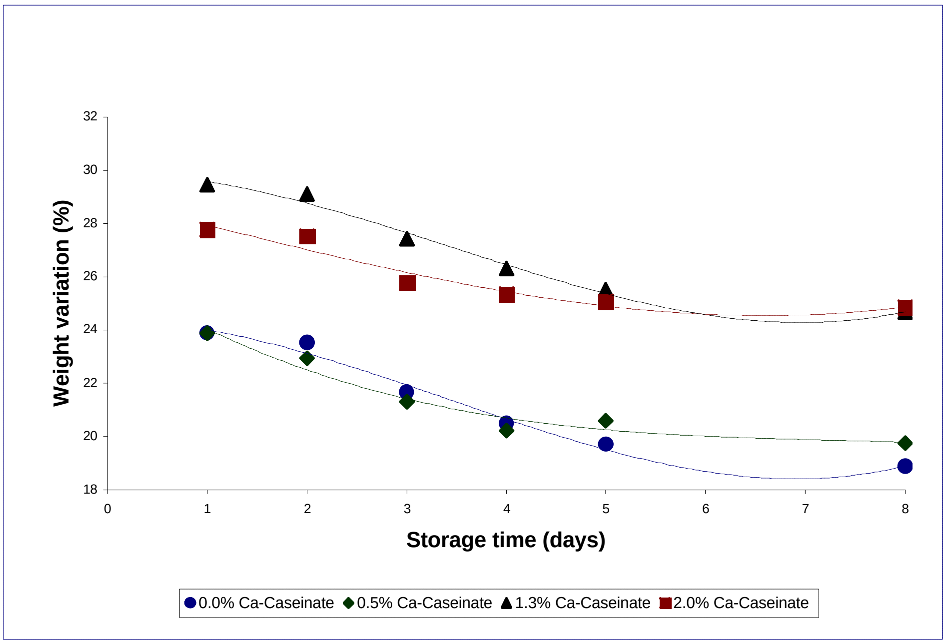

FIGURE 1. Weight variation of the "Minas" Fresh cheeses produced with calcium caseinate during refrigerated storage time.

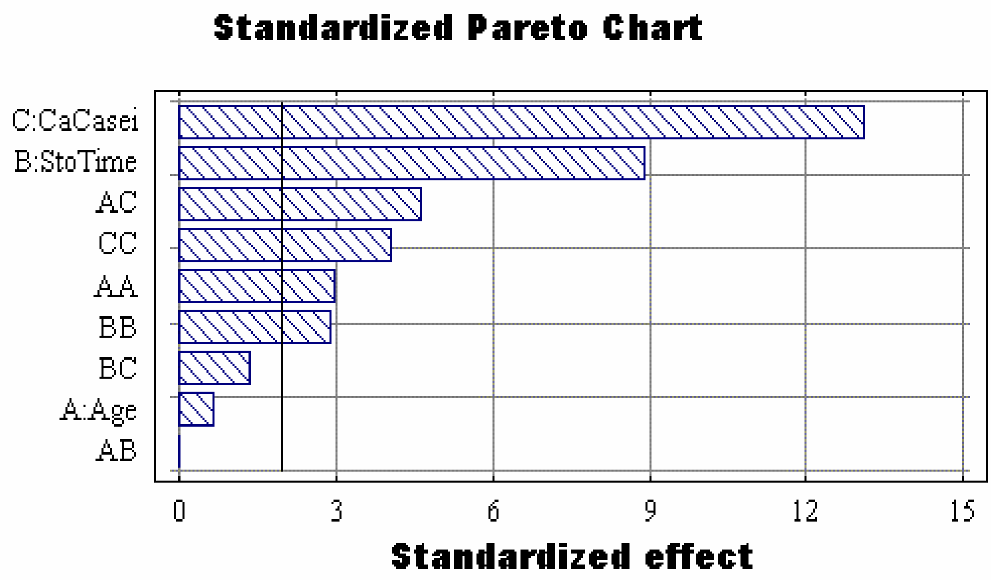

FIGURE 2. Pareto Chart for the weight variation of the "Minas" Fresh cheeses produced with calcium caseinate, showing each effect divided by its standard error.

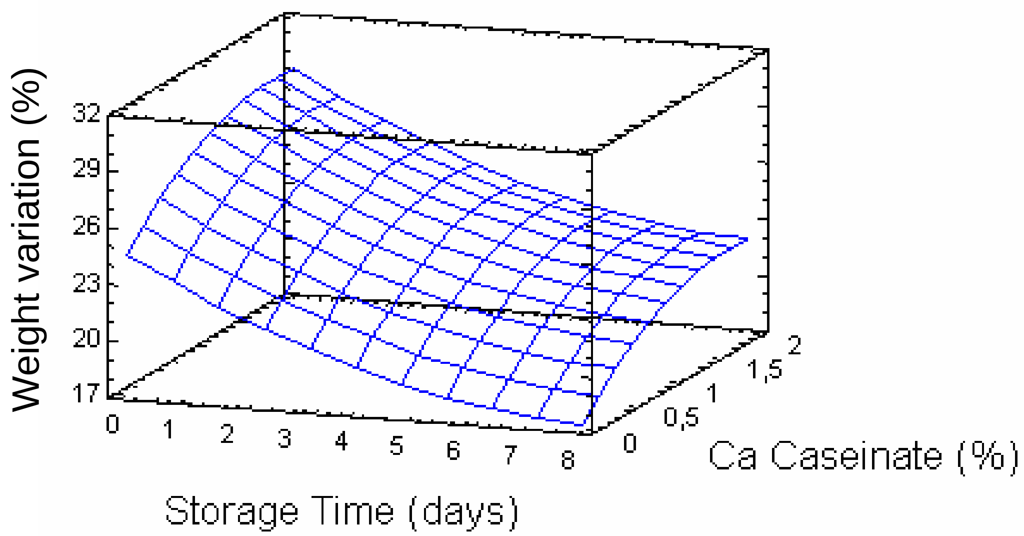

FIGURE 3. Surface Plot for an estimated response surface to predict the weight variation of the "Minas" Fresh cheeses produced with different proportions of calcium caseinate, according to the storage time under refrigeration.

TABLE 5. Principal component analysis for "Minas" Fresh cheeses produced with a different proportion of calcium caseinate: table of the three component weights, which account for 97.42 percent of the variability in the original data and proportion of variance explained.

Original data	Component 1	Component 2	Component 3
Acidity	-0.001618	0.001841	-0.000331
Ash	0.006107	0.023189	0.025434
Chloride	0.016709	-0.026082	0.044639
Fat	-0.239859	-0.496980	0.333963
pH	-0.009998	0.028246	-0.027677
Protein	0.049961	0.388891	-0.710714
Total solids	-0.260622	0.771492	0.550966
Weight retention	0.933610	0.067494	0.276376
Explained variance	70.61%	16.78%	10.03%

Figure 4 shows the distribution of the cheeses produced with different quantities of calcium caseinate on a plot of PC_1 x PC_2 and PC_2 x PC_3. The position of the different cheeses in relation to each defined axis characterizes the fact that cheeses with higher calcium caseinate contents present higher weight retention values (quadrant I, positive values for PC_1) and lower fat contents (quadrants II and III on a plot of PC_2 x PC_3). Furthermore, Figure 5 shows that the homogenization pressure applied to the milk mixture did not influence the weight retention of the cheese, as indicated by ANOVA.

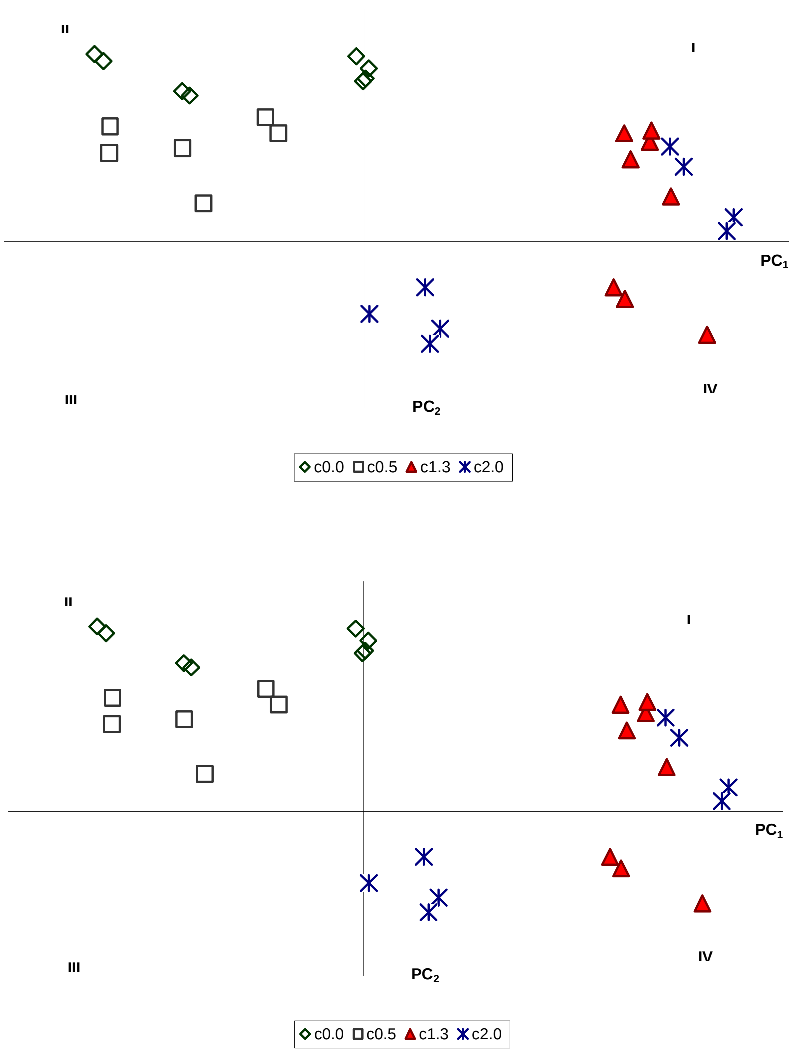

FIGURE 4. Distribution of cheeses produced with different quantities of calcium caseinate on a plot of components PC1 (weight retention) and PC2 (weight retention and fat content) and on a plot of components PC2 and PC3 (protein and total solids contents and fat content).

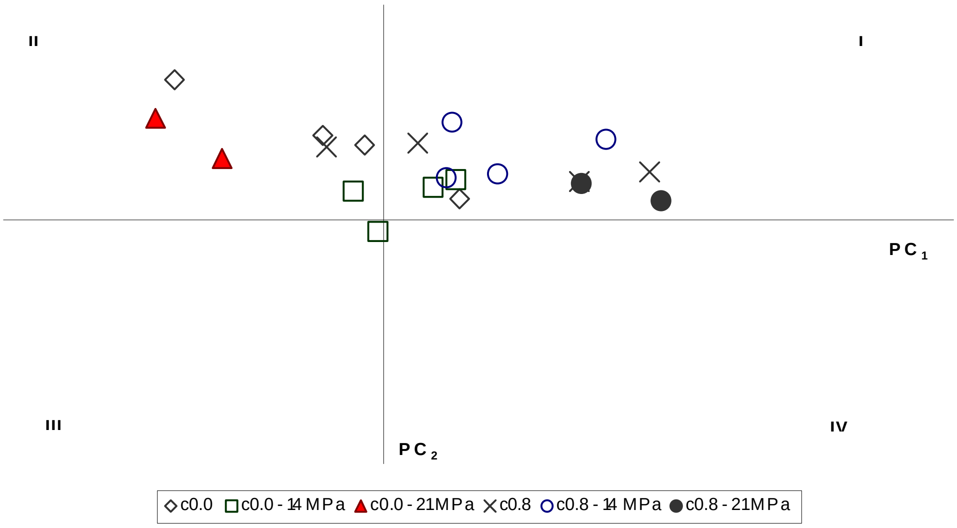

FIGURE 5. Distribution of cheeses produced with different quantities of calcium caseinate and submitted to homogenization on a plot of components PC1 (weight retention) and PC2 (weight retention and fat content).

4. CONCLUSIONS

The Principal Component Analysis applied to the physicochemical data of "Minas" Fresh enriched with calcium caseinate, reduced the original set of variables (acidity, total solids, fat, protein, pH, ash, chloride and weight retention) to three new PC, that account for 97.42 percent of the total variance.

The results obtained by PCA confirmed the same tendencies obtained by Multiple Regression Analysis, that is, that cheeses produced with added calcium caseinate presented higher weight retention (yield), total protein contents and lower fat contents.

The first principal component accounted for 70.61% of the variability in the data and it is basically constituted of the weight retention or yield of the cheese. Homogenization pressures of up to 21 MPa applied to the milk mixture, did not affect the weight retention of the cheeses.

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