Evaluation of quality of Coalho cheese produced with endogenous *Lactococcus* spp.

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Abstract

Purpose – The objective of this study is to evaluate the quality of Coalho cheese produced using two different strains of *Lactococcus* spp. that were isolated from artisanal Coalho cheese in Bahia, Brazil.

Design/methodology/approach – Three different batches of cheeses were produced using lactic acid *Lactococcus* spp. strains named "LA-02", "RE-02" and "Blend" (LA-02 plus RE-02). To study the quality of these products the paper uses quantitative descriptive analysis (QDA), a test of acceptability and an analysis of physical-chemical parameters.

Findings – For the flavor attribute, the highest acceptabilities were for LA-02 (7.38) and Blend (6.92). The panelists found that 13 attributes were important to describe Coalho cheese. The Blend cheese was characterized by its yellow color, hardness, consistency, chewiness, typical Coalho cheese aroma and flavor. The LA-02 cheese was characterized by its acid flavor, homogeneity and softness. All the samples analyzed showed values of moisture and fat that were in line with Brazilian legislation.

Practical implications – Coalho cheese is a typical Brazilian artisanal cheese that is normally produced with raw milk, even though legislation does not allow this. Pasteurization of the milk used to produce cheeses guarantees the microbiological safety of the product and, in this case, the cheese needs the addition of lactic acid bacteria in order to improve the sensorial quality and to give typical characteristics.

Originality/value – Coalho cheese, as produced in the artisanal form, is widely appreciated for its sensorial characteristics. The Coalho cheese proposed in this research retains the typical sensory characteristics by using different strains of *Lactococcus* spp. that were isolated from artisanal Coalho cheese but according to the specification of good manufacturing practices (GMP).

Keywords Food products, Food additives, Quality, Sensory perception, Animal products, Brazil

Paper type Research paper

Introduction

Coalho-type cheese is widely produced in several Brazilian regions, especially in Northeastern Brazil. It is a typical delicacy of Northeastern cuisine, and it is used in many ways: it can be eaten fresh, grilled or as an ingredient in several regional dishes.

Typical artisanal Brazilian Coalho cheese is normally produced with raw cows' milk (it is produced mainly in small proprieties without the hygienic conduct required for cheese production), even though legislation does not allow this; most of the production is based on the simple coagulation of raw milk and the pressing of the resulting curds without a starter culture. It is considered an artisanal form of production. Cheeses that are made without all the requirements of the legislation do not usually conform to the microbiological specification for the Coalho cheese standard (Santos, 2002; Nascimento et al., 2002; Borges et al., 2003; Teshima et al., 2004; Martins and Silva, 2006).

Owing to the fact that the milk should be pasteurized (a process that eliminates most of the endogenous lactic acid bacteria) for cheese production, the sensorial characteristics of this kind of cheese – such as flavor, aroma, and texture – are changed, and can be improved with the addition of specific lactic acid bacteria such as Streptococcus, Lactobacillus, Leuconostoc (Oliveira et al., 1980; Sbampato et al., 1999), as well as contributing to the safety of the product (Isepon and Oliveira, 1995).

According to the Food and Agriculture Organization (2009), Coalho cheese is a semi-hard, slightly acid, white-colored cheese showing a typical opened texture with mechanical holes and a salty flavor. There are many possibilities of variations during the elaboration of this cheese, including cooking temperatures, salting methods, use or not of lactic cultures, size of the curds after cutting, with consequent changes in its physicochemical composition. It can be found in varying sizes and forms (from long wood sticks with 100 g of cheese to blocks of 8 kg), with either regular and irregular small eyes or without them. The main characteristic of this type of cheese is a well mineralized curd with a high pH; generally, the cheese should present internally a more tender structure recovered by a fine dark-colored pellicle.

In sensorial analysis there are several descriptive methods that provide qualitative information beyond the quantitative data of the products. The flavor profile developed by Little (1940) and cited in Meilgaard et al. (2007) is considered qualitative and semi-quantitative, and only evaluates the aroma and flavor. The texture profile created by Szczesniak (1963), although considered qualitative and quantitative, uses descriptive terms that are definite (i.e. established according to products that were analyzed previously) and only evaluates the texture. In this case, the vocabulary of the panelist becomes limited and this forces the panelist to evaluate terms that can be unknown or only slightly understandable. Stone et al. (1974) developed a quantitative and qualitative method called "quantitative descriptive analysis" (QDA) that evaluates all the sensorial aspects present in products. The QDA values generally use a nine-point non-structured hedonic scale. In this method the descriptive terms are created by the panelists forming the evaluation team for the product.

Andrade et al. (2006) employed descriptive analysis to describe the characteristics of Coalho cheese; however quantification was not fulfilled. Consumer testing has already been employed to estimate the quality of Coalho cheese (Benevides et al., 2000; Mangueira et al., 2002. Recently, Cavalcante et al. (2007), used an acceptance test to evaluate four different types of Coalho cheese produced with endogenous lactic acid bacteria, but the samples were not measured for a set of common attributes of the

vocabulary (such aroma, flavor, texture, appearance). Instead they were measured in a global form (general), but the hedonic values of these attributes that could contribute to improving of the quality of product were not measured. Thus, a quantitative score of these attributes could add new information about the sensorial characteristics of Coalho cheese.

The objective of this study was to evaluate by using the quantitative descriptive analysis (QDA®) method, acceptance testing and physico-chemical parameters the quality of Coalho cheese produced using two different strains of endogen lactic acid bacteria (*Lactococcus* spp. and a blend of the two strains).

Material and methods
Source and maintenance of starter culture
Lactococcus spp. from artisanal Coalho cheese produced in Bahia, Brazil, were isolated and characterized in a previous study (Assis *et al.*, 2006) in Food Biotechnology Laboratory of Stated University of Feira de Santana. Three types of starter culture with two different strains of *Lactococcus* spp were used:

(1) LA-02 (an acidifying *Lactococcus* spp.);

(2) RE-02 (an aromatic *Lactococcus* spp.); and

(3) a blend of both bacteria in the proportion 1:1.

These cultures were isolated in MRS broth and then cultivated in restored skimmed milk at 10 percent during all identification steps. The isolates were measured for morphology, Gram stain and catalysis test according to methodology described by Holdeman and Moore (1987) and Teuber *et al.* (1991). The culture thus isolated presented a morphology of bacillus or coccus, was Gram-positive and negative for catalase and was identified at the grade of genre through a growth test at a specific temperature (10, 15, 22 and 45℃) and tolerance to sodium chloride 6.5 percent (v/v). The results were analyzed according to the characteristics described by Carr *et al.* (2002).

These cultures were maintained as frozen stocks at -24℃ in sterile 12 percent (w/v) reconstituted non-fat dry milk (NFDM) with 10 percent (v/v) of glycerol. Before use, each strain was sub-cultured three times in sterile 10 percent (w/v) NFDM using 5 percent (v/v) inoculums followed by incubation for 24 h at 30℃. Theses cultures were selected between other cultures tested for characteristics of acetoin and lactic acid production in large concentrations. These characteristics were observed in the study of Assis *et al.* (2006).

Processing of Coalho cheese
The production of Coalho cheese followed the methodology proposed by Munck (2004) and was conducted at the Dairy Processing Laboratory of the State University of Feira de Santana, Bahia, Brazil. To pasteurized cows' milk (purchased from a local market in Feira de Santana, Bahia, Brazil) was added rennet (CHR Hansen of Brazil at 0.1 percent v/v) and calcium chloride (0.05 percent v/v), which is necessary due to the low solubilization of part of the calcium during the pasteurization. Each batch of cheese was produced using 15 l of milk. The cheese production was repeated twice and the results presented in this paper are the mean.

The endogenous starter culture is an important ingredient in the production of cheeses, and is used to provide acidity development, and the formation of eyes, flavor and aroma. *Lactococcus* RE-02, *Lactococcus* LA-02 and the blend culture of *Lactococcus* were used to inoculate, individually, a batch of 15 l of milk at 35℃, in a proportion of 3 percent (v/v), and the mixture was stirred for 2 min.

Coagulation of the milk was at 35℃, over 50 min, and when the curd was firm with a bright aspect, it was cut into blocks of 2×2 cm. After cutting, the curds were rest for 5 min to acquire the required firmness. Then the curd was agitated, which was initially performed with gentle movements, gradually increasing the speed. This step took 30 min, and part of the whey was removed. Heating was applied indirectly, via the injection of steam into the jacket of the preparation vat.

Heating proceeded slowly up to 45℃. The final point of the curd occurred after 60 min, when the whey was completely drained and the refined salt was directly incorporated into the curd at a rate of 2 percent in relation to the initial volume of the milk. In order to remove the whey, the mass was set into plastic casts and they were coated with fine cloths and subjected to a pressing process. The first pressing was made with 10 kg weights for 1 h and the second pressing was made after turning the cheeses in the moulds and inverting for an additional period of 2 h at the same pressure. The cheeses were vacuum packaged and stored in a cold room at 7℃ for ripening for ten days before the final analysis.

Sample preparation, standardization, coding, and order of presentation
For sensory analyses the samples were cut into pieces of $2 \times 3 \times 1.5$ cm and grilled (in an Electric Grill Baby, Cotherm Eletrotérmica LTDA) for 3 min at 170℃. The samples were identified by three-digit codes and served on plastic plates. The order of presentation of the samples was balanced (complete balanced block) and the tests were conducted in an individual cabinet with white light.

Acceptance test
Acceptation was evaluated by 50 consumers (students of the Federal University of Bahia) who normally eat Coalho cheese. It was used nine-point hedonic nominal scales ("Like extremely" to "Dislike extremely") for the attributes of appearance, aroma, flavor and texture. To evaluate purchasing intention a nominal five point scale was used (5 = "Definitely would not buy" and 1 = "Definitely would buy"; Meilgaard *et al.*, 2007).

Descriptive Quantitative Analysis (DQA®)
Descriptive Quantitative Analysis (DQA®) was conducted according to Stone and Sidel (2004). The tests were undertaken at the Sensory Analysis Laboratory of Pharmacy Faculty of the Federal University of Bahia (UFBA).

First we recruited consumers of Coalho cheese (students from Federal University of Bahia) according to their availability, whether they were consumers of Coalho cheese, and how often they consumed it (i.e. at least once per week).

The selection of panelists was executed according to the power of discrimination of each one using a triangle test and analysis of the results was based on the table (critical number of correct responses in a triangle test) described by Meilgaard *et al.* (2007), with $p < 0.05$. In this way balanced complete blocks were made. Panelists who hit two of

three combinations presented (AB, AC and BC) were selected to search the descriptive terminology. The descriptive terminology was developed by the repertory grid method (Moskowitz, 1983).

Then, the individual terminologies were evaluated in an open discussion about the vocabulary, with reference material to choose the most important attributes to describe the cheese. Definitions were reached in consensus with the group, and this discussion was undertaken with the help of a leader. After the discussion, in a consensus the panelists wrote down the definition for all the attributes that were considered important and references for the extremes of the scale (see Table I). In the final step the panelists evaluated the three samples of Coalho cheese with three repetitions.

Attributes	Definitions	Reference minimum	Reference maximum
Yellowness	Intensity of yellow color in the cheese mass	Ricota cheese brand: Veneza	Cheddar cheese brand: Sadia
Holes	Amount of holes in cutting surface on average	None	Artesanal Coalho cheese
Homogeneity	Absence of particles in the cheese mass	Minas Frescal cheese brand: Natville	Mussarela cheese brand: Cotochés
Hardness	Hardness of cheese mass appearance	Requeijão cheese brand: Polenguinho	Parmesan cheese brand: Cristina
Buttery aroma	Aroma characteristic of butter	None	Prato cheese brand: Natville
Typical of Coalho cheese	Aroma characteristic of Coalho cheese	None	Artesanal Coalho cheese
Salty	Intensity of salt flavor	Minas Frescal cheese brand: Veneza	Parmesan cheese brand: Cristina
Fat flavor	Intensity of fat flavor	Queijo ricota brand: Veneza	Reino type cheese brand: Tina
Typical of Coalho cheese	Similar to Artesanal Coalho cheese	None	Artesanal Coalho cheese
Sour taste	Intensity of sour taste	Skimmed yogurt brand: Batavo	Fermented milk brand: Vigor
Elasticity	Drag felt while bending a piece of cheese. Elastic cheese does not break	Minas Frescal cheese brand: Natville	Mussarela cheese brand: Cotochés
Chewiness	How many times you have to chew to disintegrate the sample, like rubber?	Minas Frescal cheese brand: Natville	Bala de Gelatina brand: Dori
Softness	Softness of cheese mass is determined by chewing or cutting the cheese	Parmesão brand: cheese Cristina	Minas Frescal cheese brand: Natville

Table I.
Definitions and references of descriptive terms for Coalho cheese

The reproducibility ($F_{\text{repetition}} \geq 0.05$) and the discriminatory power ($F_{\text{sample}} < 0.5$) of the team was measured according to the methodology described by Damásio and Costell (1991).

Physico-chemical analysis
Physico-chemical analyses were undertaken to characterize the three types of cheese produced and to verify whether the new product was in accordance with the regulations for Coalho-type cheese. The samples were evaluated in terms of the moisture, fat, sodium chloride and lactic acid contents (percent) and pH according to the methodology of the Association of Official Analytical Chemists (1990). The content of ash was evaluated according to Cecchi (2003).

Statistical analyses
Statistical analyses were conducted using the procedure of SAS (SAS Institute, Cary, NC) to analyze the data. We used analysis of variance (ANOVA), the mean test of Tukey (at 5 percent significance) and principal components analysis (PCA), while to produce the graphics we used Minitab V15 software (15.12.0).

Results and discussion
Acceptance test
The cheeses produced with the blend (LA-02 plus RE-02) did not present a difference to the samples RE-02; however, the blend samples showed the highest acceptance due to appearance (Table II).

It was hoped that the cheese produced with the RE-02 culture would present a greater degree of acceptance for aroma and that the difference would be highly significant in relation to the other samples because this culture showed the potential to produce the characteristic aroma of the cheese, but the results achieved were not as expected. The average values for aroma showed that none of cultures of the study were demonstrated to improve the aroma. Nevertheless, a mean value of above 6.0 reveals that the acceptance of this attribute in relation to samples is included in the concept "like slightly". This result is good from the point of view that acceptance of this attribute was greater than "neither like or dislike".

Sample LA-02 had the highest acceptance for texture and also for the flavor attribute, which did not differ statistically from the blend samples ($p \leq 0.05$).

| Sample | Mean of acceptance | | | |
	Appearance	Aroma	Texture	Flavor
LA-02	6.38[b]	6.86[a]	7.16[a]	7.38[a]
RE-02	7.06[a,b]	6.6[a]	6.08[b]	6.62[b]
Blend	7.34[a]	6.38[a]	6.32[b]	6.92[b,a]

Notes: LA-02, *Lactococcus* spp. (acidifying); RE-02, *Lactococcus* spp. (aromatic); Blend, a mixture of LA-02 and RE-02 in the proportion 1:1. Means followed by the same letter within a column are not significantly different according to a Tukey test ($p < 0.05$)

Table II.
Mean of acceptance of
Coalho cheese

The samples were evaluated in relation to purchasing intention (Figure 1), and the 24 percent and 18 percent of panelists answered that they "definitely would buy" the LA-02 and blend samples, respectively.

Descriptive Quantitative Analysis (DQA®)

Descriptive Quantitative Analysis (DQA®) was used to evaluate the three samples of Coalho cheese produced with LA-02, RE-02 and a blend of the two. The panel was composed of 11 panelists (male and female, ages between 20 and 35 years old).

The panelists analyzed and quantified 13 attributes that were important for the description of Coalho cheese (the definitions and references can be seen in Table I). Some attributes – such elasticity, hardness, holes and salt – have already described as important in other types of cheese according to Ritvanen *et al.* (2005); this could mean that these attributes are important for, and characteristic of, different types of cheeses.

Some attributes such as yellow color, holes, buttery flavor, salty taste and chewiness were described both in this work and in Andrade *et al.* (2006), highlighting their importance in the description of the sensorial characteristics of Coalho cheese. Andrade *et al.* (2006) studied the sensorial characteristics of artisanal Coalho cheese using descriptive analysis of terminologies generated using a Kelly repertory grid method with 23 panelists pre-selected by a triangular test; however the authors did not make a quantification of the intensity of the attributes used which endangers the characterization and correlation of product quality.

According to Table III, only two attributes did not differ across the samples ($p \leq 0.05$), i.e. homogeneity and fat flavor. The yellowish color and elasticity were the most important attributes to characterize the samples and there were differences across all samples ($p \leq 0.05$).

The yellowish color, holes, hardness and attributes related to the appearance of the product demonstrated a higher intensity in the blend sample. The intensity of these attributes is reflected in the acceptance test because the blend samples had a higher acceptance for appearance (Table II).
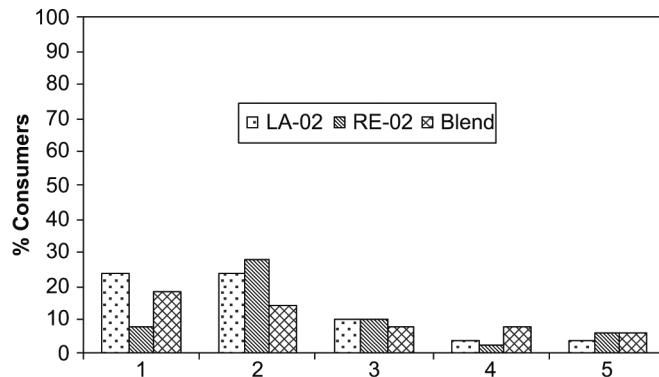

Figure 1.
Consumer purchasing intention of the samples LA-02, RE-02 and Blend

Notes: (1) Definitely would buy; (2) Probably would buy;
(3) Maybe/maybe would buy; (4) Probably would not buy;
(5) Definitely I would not buy

Attribute	Sample	Means
Yellow color	LA-02	2.82[c]
	RE-02	3.63[b]
	Blend	4.47[a]
Holes	LA-02	1.61[b]
	RE-02	2.30[a,b]
	Blend	2.58[a]
Homogeneity	LA-02	5.70[a]
	RE-02	5.40[a]
	Blend	5.25[a]
Hardness	LA-02	4.26[b]
	RE-02	5.36[a]
	Blend	6.23[a]
Buttery aroma	LA-02	4.09[b]
	RE-02	5.20[a]
	Blend	4.10[b]
Typical aroma of Coalho cheese	LA-02	3.15[b]
	RE-02	3.61[b]
	Blend	5.18[a]
Salty flavor	LA-02	2.96[a,b]
	RE-02	3.30[a]
	Blend	2.42[b]
Fat flavor	LA-02	3.70[a]
	RE-02	4.22[a]
	Blend	4.45[a]
Sour taste	LA-02	3.72[a]
	RE-02	2.48[b]
	Blend	2.25[b]
Typical flavor of Coalho cheese	LA-02	3.30[b]
	RE-02	3.95[a,b]
	Blend	4.32[a]
Elasticity	LA-02	4.32[b]
	RE-02	3.30[c]
	Blend	5.71[a]
Softness	LA-02	5.89[a]
	RE-02	5.21[a,b]
	Blend	4.57[b]
Chewiness	LA-02	4.80[b]
	RE-02	4.35[b]
	Blend	6.62[a]

Notes: LA-02, *Lactococcus* spp. (acidifying); RE-02, *Lactococcus* spp. (aromatic); Blend, a mixture of LA-02 and RE-02 in the proportion 1:1. Means followed by the same letter within a column are not significantly different according to a Tukey test ($p < 0.05$)

Table III.
Results of the means of
the attributes (Tukey test)

The typical flavor of Coalho cheese, elasticity and hardness were higher in the blend sample than the LA-02 sample, which displayed a more soft character. The buttery aroma was more intense in the RE-02 sample (5.20) and the typical Coalho cheese aroma was higher in the blend sample (5.18).

The salty flavor was higher in sample RE-02 than the other samples and was more intense (3.30), which were not expected due to the fact that the amount of salt added to the three samples of Coalho cheese did not differ. The difference could be related to the drainage stage.

The sour taste was more intense in the LA-02 sample due to the fact that this strain of *Lactococcus* spp. produces more lactic acid during its metabolism. The fat flavor and the typical flavor of Coalho cheese were observed in higher intensity in the blend sample. Comparing the results of Table II and Table III it is possible perceive correlations; for example the flavor acceptance (Table II) of sample LA-02 obtained a higher score for this attribute class, but did not differ statistically in relation to the blend sample. As such, we can suggest that the intensity of lactic acid noticed favored the acceptance of the flavor of sample LA-02 and furthermore the intensity of the fat flavor and typical flavor of Coalho cheese noticed in QDA in the blend sample could likewise have contributed to the blend sample's flavor acceptance (Table II).

Elasticity, softness and chewiness were attributes related to the texture class. The results in Table III show that the sample LA-02 had the highest score for texture acceptance (Table II) and presented significant differences ($p \leq 0.05$) to the blend and RE-02 samples. In this case, we can highlight that softness was the attribute that contributed more to texture acceptance, followed by the attributes described as elasticity and chewiness. The blend sample had the highest score for elasticity and chewiness, and this result could have been the reason for the second higher score for texture acceptance (Table II).

Principal components analysis (PCA)
Analysis of the principal components explains 77.6 percent of the variability of the difference between the samples. Principal component I explains 25.1 percent and principal component II explains 52.6 percent (Figure 2).

In Figure 2 we can see that the samples were well discriminated, with each one in a different quadrant. The blend sample was characterized by the vectors C_1, C_2, C_4, C_6, C_8, C_{10}, C_{11} and C_{13}. Vector C_4 is hardness and vector C_8 is fat flavor; they are the smallest and therefore the least important for the characterization of this sample.

Attribute homogeneity did not show any significant difference ($p \leq 0.05$) between all samples (Table III), but it was not a short vector compared with the others, which confirms its importance in the characterization of sample LA-02.

Vectors C_9 and C_{12} pointed to sample LA-02, showing their importance for the characterization of this sample. Sample RE-02 is near the vectors of salty flavor and buttery aroma, and therefore these are relevant attributes for the characterization of this sample. Data from the PCA analysis are in accord with the results from Table III.

Physico-chemical analysis (means of the samples)
The samples of the Coalho cheese had high moisture; according to the specifications of Brazilian legislation (Diário Oficial da Republica Federativa do Brasil, 1996), they are classified as high humidity if they have moisture between 46.0 percent and 54.9
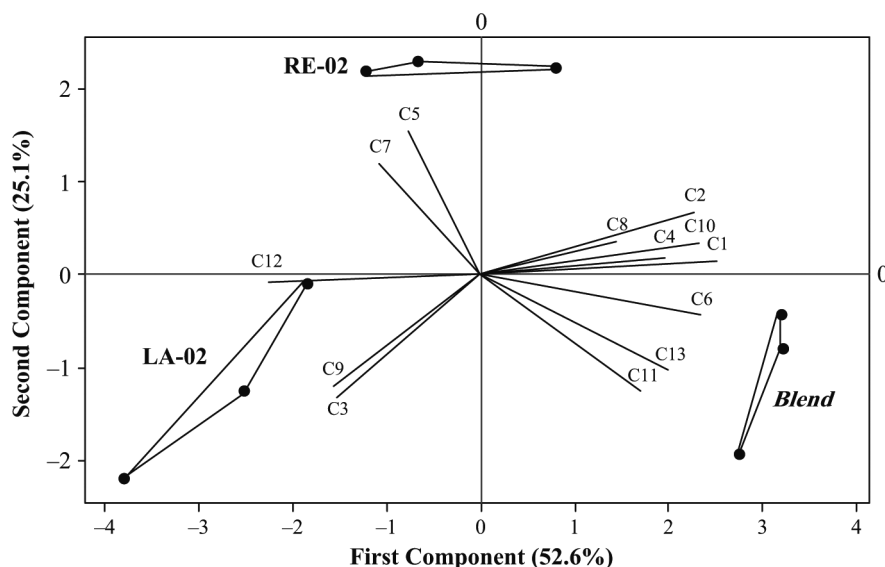
Notes: LA-02: *Lactococcus* spp (acidifying); RE-02: *Lactococcus* spp (aromatic) and Blend:
it is a mixture of the LA-02 and RE-02 proportion 1:1. C1 = YELLOW COLOR;
C2 = HOLES; C3 = HOMOGENEITY; C4 = HARDNESS; C5 = BUTTER AROMA;
C6 = TYPICAL AROMA OF COALHO CHEESE; C7 = SALTY; C8 = FAT TASTE;
C9 = SOUR TASTE; C10 = TYPICAL FLAVOR OF COALHO CHEESE;
C11 = ELASTICITY; C12 = SOFTNESS; C13 = CHEWING

percent. According to the results in Table IV, all samples have moisture percentages
within the limits set by legislation.

The content of fat "in dry base" in Coalho cheese should be between 35.0 and 60 per
cent (Diário Oficial da União, 2001). All the samples were within this range, and can be
classified as a fat cheese (i.e. with fat between 45.0 percent and 59.9 percent).

Sample LA-02 had a higher lactic acid content (variable acidity) than the other
samples, and this complemented the results of the QDA (Table III), where the result
shows that the LA-02 sample has the highest intensity of sour taste over all three
sample.

There is no specification for the amount of salt, but according to the results of this
study, we can suggest that it should be between 0.83 percent and 1.04 percent
(Table IV), because a concentration of 1.14 percent was detected in the sample RE-02
and this had the lowest average of acceptance for flavor of all the samples (Table II).

Conclusion
According to all the results, Coalho cheese produced with any one of the two cultures
isolated showed potential for the production of Coalho cheese, but the samples of
cheese produced with the LA-02 (acidifier) and blend (LA-02 plus RE-02) cultures had a
higher mean acceptance for aroma, texture, flavor and appearance.

In QDA, the samples were described by 13 attributes generated; and the yellowish
color was the most important attribute in the discrimination of the three samples. The

Sample	Moisture (percent)	Ash (percent)	Fat "in dry base" (percent)	pH	Protein (percent)	Acidity (percentage of lactic acid)	Salt (percentage NaCl)
LA-02	51.93[a]	2.85[a]	54.77[a]	6.20[a]	17.53[b]	1.58[a]	0.83[b]
RE-02	51.65[a]	3.13[b]	50.18[b]	6.07[b]	17.80[b]	1.22[c]	1.14[a]
Blend	49.89[b]	3.32[c]	50.21[c]	6.08[b]	19.77[a]	1.30[b]	1.04[a]

Notes: LA-02, *Lactococcus* spp. (acidifying); RE-02, *Lactococcus* spp. (aromatic); Blend, mixture of LA-02 and RE-02 in the proportion 1:1. Means followed by the same letter within a column are not significantly different according to a Tukey test ($p < 0.05$)

Table IV.
Results of
physico-chemical means

blend sample presented a higher intensity of holes, typical aroma of Coalho cheese and typical flavor of Coalho cheese; therefore, this sample was more similar to commercial samples.

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