

## Coalho cheese with incorporated chitosan and as a coating: effect on the viability of *Staphylococcus aureus* and sensory acceptance

## Queijo de coalho com quitosana incorporada e como revestimento: efeito na viabilidade de *Staphylococcus aureus* e aceitação sensorial

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### Abstract

Coalho cheese is a dairy product typical of the Northeast region of Brazil and widely consumed by the population; however, the poor quality of the raw material used in association with the absence of standardization in the manufacturing process makes it susceptible to microbial contamination, mainly by *Staphylococcus aureus*. The objective of this study was to evaluate the influence of chitosan as a coating and incorporated in coalho cheese on *S. aureus* viability and the sensorial acceptance of the cheese. For this, coalho cheeses were made with chitosan in the coating or added to the curds at 5 mg mL<sup>-1</sup>, 10 mg mL<sup>-1</sup> and 15 mg mL<sup>-1</sup> and 1 mg g<sup>-1</sup>, 2 mg g<sup>-1</sup> and 4 mg g<sup>-1</sup>, respectively. Products without chitosan (C) and 1% acetic acid (CA) were used as controls. The bacterial inhibition of chitosan in the artificially contaminated samples was assessed by comparing the viable cell count of *S. aureus* (ATCC 6538) in each treatment over five time intervals (0, 4, 8, 12 and 16 d storage). Product C was evaluated for moisture and fat content. The sensorial and shelf stability analyses were performed with the control and chitosan treated samples at the most efficient antibacterial concentrations. The sensorial analyses were performed with 100 consumers. Chitosan antimicrobial activity was observed in all treatments, however, the highest concentrations of chitosan added as a coating and to the cheese, 15 mg mL<sup>-1</sup> and 4 mg g<sup>-1</sup>, respectively, inhibited *S. aureus*. The moisture and fat contents met the standards established by the Brazilian legislation. Regarding stability, the samples complied with the regulatory microbiological limits and presented acceptable pH, acidity and water activity values. As far as sensorial acceptance was concerned, the scores corresponded to hedonic concepts between “I liked it slightly” and “I liked it a lot”, with an improvement in the texture of the products when chitosan was incorporated. Acceptability values were higher than 70%, except for the taste the products with chitosan covering them, which was 68.3%. It can be inferred from the results that the use of chitosan as a coating and incorporated in coalho cheese is a favourable alternative for the preservation of this product and has potential commercial applicability.

**Key words:** Conservation. Contamination. Dairy product. Quality control.

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## Resumo

O queijo de coalho é um produto lácteo típico da região Nordeste do Brasil e amplamente consumido pela população, contudo, a qualidade precária da matéria-prima utilizada associada à ausência de padronização no processo de fabricação torna-o susceptível à contaminação microbiana principalmente por *Staphylococcus aureus*. Em vista disso, objetivou-se avaliar a influência da quitosana como revestimento e incorporada em queijo de coalho sob a viabilidade de *S. aureus* e a aceitação sensorial do queijo. Para isso, foram produzidos queijos de coalho com quitosana como revestimento e adicionada à massa nas concentrações 5 mg/mL, 10 mg/mL e 15 mg/mL e 1 mg/g, 2 mg/g e 4 mg/g, respectivamente. Produtos sem quitosana (C) e com ácido acético 1% (CA) foram usados como controles. A capacidade de inibição bacteriana da quitosana nas amostras artificialmente contaminadas foi avaliada por meio da comparação da contagem de células viáveis de *S. aureus* (ATCC 6538) em cada tratamento, no decorrer de cinco intervalos de tempo (0, 4, 8, 12 e 16 dias de armazenamento). O produto C foi avaliado quanto ao teor de umidade e gordura. As análises sensoriais e de estabilidade de prateleira foram realizadas com as amostras controles e tratadas com quitosana nas concentrações antibacterianas mais eficientes. As análises sensoriais foram realizadas com 100 consumidores. Verificou-se atividade antimicrobiana de quitosana em todos os tratamentos, porém, as concentrações mais elevadas de quitosana como revestimento e adicionada à massa, 15 mg/mL e 4 mg/g, respectivamente potencializaram a inibição do *S. aureus*. O teor de umidade e gordura atendeu aos padrões estabelecidos pela legislação brasileira em vigor. Quanto à estabilidade, as amostras cumpriram com os limites microbiológicos regulamentares e apresentaram valores de pH, acidez e atividade de água aceitáveis. No que se refere à aceitação sensorial, as pontuações equivaleram a conceitos hedônicos entre “gostei ligeiramente” e “gostei muitíssimo”, verificando-se melhoria na textura dos produtos com quitosana incorporada. Valores de aceitabilidade foram maiores que 70 %, exceto no quesito sabor dos produtos com quitosana em cobertura, com 68,3 %. Pode-se inferir que a utilização de quitosana como cobertura e incorporada em queijo de coalho é uma alternativa favorável na conservação deste produto evidenciando potencial aplicabilidade comercial.

**Palavras-chave:** Conservação. Contaminação. Controle de qualidade. Produto lácteo.

## Introduction

Coalho cheese is a typical product of the Northeastern region of Brazil. As for the main physico-chemical aspects, it is a cheese with medium to high humidity, with a semi-solid or cooked mass, a semi-hard, elastic consistency and fat content in the total solids varying between 35.0 and 60.0%. Because it is a dairy product, it is an important source of protein, essential amino acids, vitamins, calcium and phosphorus. It is consumed either natural or roasted and is represented as a food cultural symbol and is of socioeconomic relevance for the regional population (BRASIL, 2001a; OPAS, 2009; ALMEIDA et al., 2013; PAQUEREAU et al., 2016; SILVA et al., 2017).

Despite its nutritional, social and economic relevance, coalho cheese still is considered a poor quality product in regards to its microbiological

aspects. This is because the production of this food is mainly performed with raw milk and/or Good Manufacturing Practices - GMPs are absent (SILVA et al., 2010; FREITAS et al., 2013). These developmental conditions make it a food susceptible to contamination, directly affecting its quality standard for consumption, and this can consequently cause Foodborne Diseases - FBD (SILVA et al., 2010; TOZZO et al., 2015).

Among the various types of FBD, staphylococcal intoxication, caused by *S. aureus* strains, is considered the most frequent cause of food illness outbreaks in Brazil. The poisoning generated by this bacterium has been attributed mainly to the consumption of cheeses, among which is the coalho cheese; this demonstrates the need to guarantee the microbiological safety of this product due to possible risks to the health of the consumer (BORGES et al., 2008; SANTANA et al., 2008; ANDRADE et

al., 2011; SOUSA et al., 2014; EVANGELISTA-BARRETO et al., 2016).

From this perspective, the development of innovative microbiological control techniques associated with the use of GMPs can improve the microbiological quality and increase the shelf life of coalho cheese. During the previous decades, the use of natural antimicrobial compounds has been spreading due to the potential capacity to guarantee the conservation and safety of food (ALBUQUERQUE et al., 2009; MACHADO et al., 2011).

Chitosan is a natural compound that has been evaluated for its conservation efficiency and microbiological safety in cheeses with medium and high moisture content. As for its applicability, it can be used in food in the form of coating or added directly into the product during the manufacturing process. However, it should be noted that the antimicrobial action of chitosan analysed *in vitro* may not be the same when used in the food; this is attributed to interactions between chitosan and the nutritional and chemical compounds of the food. For this reason, the efficiency of chitosan needs to be evaluated in a specific way for each food (DUTTA et al., 2009; SIRIPATRAWAN; HARTE, 2010; AIDER, 2010; FERNANDEZ-SAIZ et al., 2010). In addition to evaluating the antimicrobial activity of chitosan according to the type of food, it is necessary to perform the analysis of the physical-chemical and sensorial parameters of the product exposed to this agent in order to verify possible influences on quality characteristics (FAI et al., 2008; BENTO et al., 2011). In view of this, the aim of the study was to evaluate the effect of chitosan as a coating and incorporated into coalho cheese in relation to *S. aureus* viability and stability.

## Materials and Methods

### *Preparation of coalho cheese with chitosan*

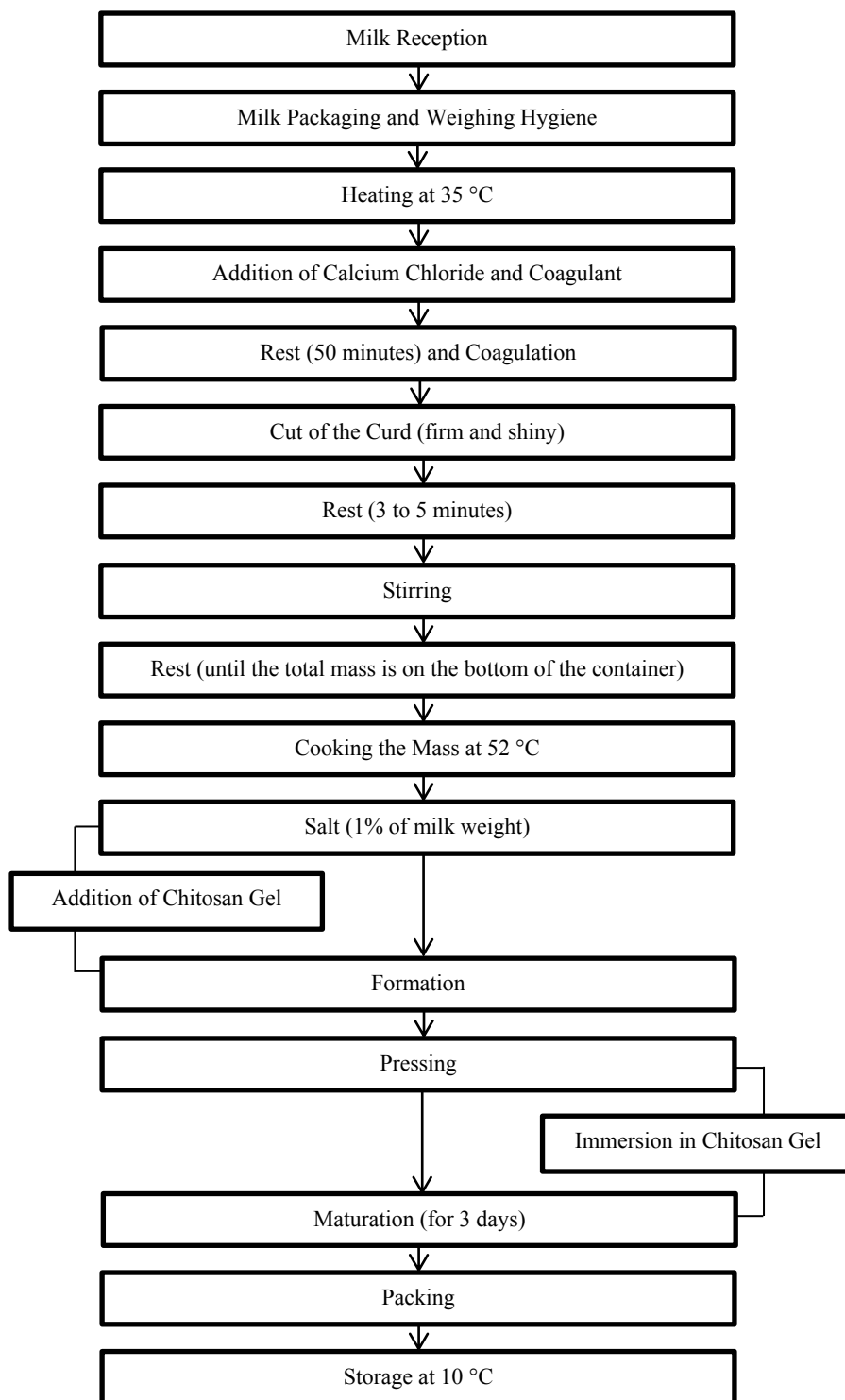
The analyses were conducted in the Laboratories of Technique and Dietetics, Bromatology and Food

Microbiology of the Centro Acadêmico de Vitória, Universidade Federal de Pernambuco - CAV/UFPE. The chitosan used in this study was obtained from Sigma-Aldrich® (Alto de Pinheiros, São Paulo - SP, Brazil) and was described as medium molecular weight (between 120,000 and 150,000 g mol<sup>-1</sup>), deacetylated 75-85 % and from the purification of chitin extracted from the crustacean shell. Chitosan gels were prepared by dissolution in 1% acetic acid and kept under moderate stirring on magnetic stirrer for 24 h at room temperature 25 °C (± 1 °C) until the powder was completely dissolved and a homogenous gel was obtained. The pH was then calibrated to 5.8 using NaOH and HCl as described by Shigemasa and Minami (1996).

The production of coalho cheese was performed according to Nassu et al. (2006), with adaptations (Figure 1). Pasteurised whole milk, standardised with 3 % fat (Bethânia®), HA-LA (Cristian Hansen®) liquid coagulant, calcium chloride and sodium chloride (VETEC®) were used.

The milk containers were sanitised to remove any contaminants, using a neutral detergent solution and 70 % alcohol, followed by weighing and heating the milk at a temperature of 35 °C. Samples with chitosan gel as a coating were treated on the third day of maturation, during which time the cheese mass was firm, that is, in it could be cut without compromising the structure of the product. As for the chitosan samples added to the dough, they were treated immediately after salting. In both treatments, coalho cheeses were cut into cubes of about 25 g in weight and 1.5 to 2 cm in thickness.

In the samples where chitosan was added in the mass, each product received 20 mL of the chitosan gel (m = 25 g) at final concentrations of 1 mg g<sup>-1</sup>, 2 mg g<sup>-1</sup> and 4 mg g<sup>-1</sup> to 75 g of cheese mass. For samples treated with chitosan as a coating, the coalho cheese cubes were individually immersed in 5 mg mL<sup>-1</sup>, 10 mg mL<sup>-1</sup> and 15 mg mL<sup>-1</sup> chitosan gels with the aid of a metal support.

**Figure 1.** Process flow diagram of coalho cheese.

Source: Adapted from Nassu et al. (2006).

To facilitate shelf life evaluation, all samples were aseptically transferred to sterile glass containers with plastic caps and stored under refrigerated conditions at 10 °C.

#### *Chemical characterization of coalho cheese*

The control product was evaluated in relation to the physical-chemical parameters for coalho cheese required by current legislation (BRASIL, 2001a), and the moisture and fat were determined using a butyrometer for milk. The results were expressed as Dry Base Fat (DBF), and were calculated according to the equation following:  $DBF = \% \text{ fat} \times 100 / \text{Total Dry Extract}$  (INSTITUTO ADOLF LUTZ, 2008).

#### *Antibiogram and elaboration of the suspension of cells*

The antimicrobial susceptibility test of the *S. aureus* ATCC 6538 standard strain was performed with paper disks impregnated with 5 mg mL<sup>-1</sup> chloramphenicol and norfloxacin antibiotics. The discs were deposited on the surface of nutrient agar previously inoculated with a pure culture of *S. aureus* ATCC 6538. The plates were incubated for 24 h at 37 °C, and the corresponding inhibition halos were measured for each antibiotic tested for the bacterial strain.

Suspensions of *S. aureus* ATCC 6538, which were employed in the viability tests, were standardised according to the McFarland 0.5 nephelometric scale. Confirmation of the inoculum was performed by spectrophotometry under optical density of 610 nm, which corresponded to approximately  $1 \times 10^8$  CFU mL<sup>-1</sup> (CLSI, 2016).

#### *Viability of *S. aureus* versus chitosan in coalho cheese*

Samples of coalho cheese (25 g) containing chitosan and control samples (without chitosan or with only acetic acid) were inoculated with 2.5

mL of the *S. aureus* ATCC 6538 ( $1 \times 10^8$  CFU mL<sup>-1</sup>) suspension and kept under refrigeration (6-8 °C). The time intervals (0, 4, 8, 12 and 16) for the evaluation of the samples were established based on the marketing period of the artisanal coalho cheese, which normally occurs within 10 days after the manufacturing (NASSU et al., 2006). The 0 day interval corresponds to the moment of chitosan application and artificial inoculation of the microorganism (*S. aureus* ATCC 6538) in the samples. At the 0, 4, 8, 12 and 16 day post-incubation intervals, the product was homogenised in 0.1% peptone water (25 g sample to 225 mL peptone water) and then serially diluted (1:9 v/v) in sterile distilled water ( $10^{-1}$ - $10^{-6}$ ). Then, 100 µL aliquots of the dilutions were seeded in Petri dishes containing Baird-Parker (BP) agar medium enriched with egg yolk emulsion and 1% potassium tellurite, evenly distributed with Drigalski loop and incubated at 35 °C for 48 h. After counting the number of viable cells, the results were expressed as log CFU g<sup>-1</sup> (SAGDIÇ, 2003).

#### *Stability of coalho cheese with and without chitosan*

In the evaluation of stability, the samples of coalho cheese controls and with chitosan as cover or added to the mass (in the concentrations that obtained greater antimicrobial efficiency), were analysed for physicochemical and microbiological parameters at 0, 10 and 20 d of storage. The following physical-chemical parameters were determined: pH, acidity in lactic acid according to the norms of Instituto Adolfo Lutz (2008) and water activity (*A<sub>w</sub>*) according to the descriptions of the equipment manual.

The analysis times were established based on the marketing period of artisanal coalho cheese, which normally occurs within 10 d after manufacture. The time of 30 d was also used, however there were visible changes in relation to the sensorial characteristics of the product (mainly odour and colour).

Microbiological analyses were performed according to RDC No. 12, dated January 2, 2001 (BRASIL, 2001b), which recommends counts of coliforms at 45 °C and staphylococci coagulase positive and noting the presence or absence of *Salmonella* spp. and *Listeria monocytogenes*. Microbiological analyses were performed according to Normative Instruction No. 62 for food control (BRASIL, 2003).

### *Sensory analysis*

Samples of coalho cheese and more efficient antimicrobial treatments incorporated into the mass and as edible covers against *S. aureus* were offered to a panel of 100 untrained tasters (teachers, students and technicians from the Centro Acadêmico de Vitória, CAV/UFPE), chosen according to availability and inclusion criteria (liking curd cheese and being over 18 years old).

Sensory analysis was performed in individual booths with artificial white light. The samples were supplied to the tasters in a fractionated manner, in cubes of about 25 g in weight and 1.5-2.0 cm in thickness under refrigeration at 10 °C. These samples were in dishes coded with random three digit numbers accompanied with a glass of water to rinse the mouth during the test between samples. Three samples were presented in the same period along with a sensory assessment sheet containing evaluation parameters regarding colour, odour, taste, texture and appearance.

The acceptance test was carried out with a structured hedonic scale of 9 points, where 9 represented “I liked it a lot” and 1 “I greatly disliked”. From the results obtained, an acceptability index (AI) was calculated according to Teixeira et al. (1987). Participants signed the Written Informed Consent Form (WICF) in accordance with the

research project that had been submitted and approved by the Ethics and Research Committee of the Universidade Federal de Pernambuco-UFPE (CAAE 56538416.7.0000.5208), in accordance with Resolution 196/96 of the National Health Council.

### *Statistical analysis*

The experimental data from triplicate analyses were evaluated using descriptive statistics (means and standard deviations) and inferential statistics (Duncan test) to determine significant statistical differences ( $p < 0.05$ ) between the control samples and the different treatments applied where the analysis of variance (ANOVA) was performed. The effect of storage time on the treated and control samples was evaluated according to the physical-chemical and microbiological parameters described in the section: *Stability of coalho cheese with and without chitosan*.

## **Results and Discussion**

### *Viability of S. aureus versus chitosan in coalho cheese*

The cultures of *S. aureus* ATCC 6538 evaluated against the antibiotics chloramphenicol and norfloxacin were classified as sensitive, with inhibition halos varying from 20 to 29 mm, as recommended by CLSI (2016). It is assumed with this result that the bacterial strains revealed normal conditions of activity and having an active metabolism, that is, the species showed normal characteristics of behaviour.

The results of viable cell counts of *S. aureus* versus chitosan as a coating and added to the mass of coalho cheeses under different concentrations are shown in Table 1 and Table 2.

**Table 1.** Survival curve of *S. aureus* in coalho cheese with chitosan added the mass of the product in different concentrations and stored for 16 days under refrigeration.

Days	C	CA	T1	T2	T3
0	6.52 ± 0.01 Aa	7.58 ± 0.06 Cd	6.60 ± 0.02 Ba	6.50 ± 0.05 Aa	6.49 ± 0.09 Ab
4	8.37 ± 0.10 Dd	7.09 ± 0.15 Cc	7.18 ± 0.06 Cc	6.85 ± 0.06 Bb	6.67 ± 0.10 Ac
8	7.90 ± 0.01 Dc	9.26 ± 0.01 Ee	7.08 ± 0.01 Cb	6.91 ± 0.02 Bb	6.40 ± 0.02 Ab
12	10.01 ± 0.01 De	6.80 ± 0.01 Ab	7.09 ± 0.02 Bb	7.12 ± 0.01 Cd	6.79 ± 0.01 Ad
16	7.49 ± 0.15 Db	6.67 ± 0.04 Ba	7.48 ± 0.04 Dd	7.01 ± 0.02 Cc	6.07 ± 0.04 Aa

<sup>ABC</sup>Models followed by uppercase letters do not differ significantly ( $p > 0.05$ ) by the Duncan test. <sup>abc</sup>Means followed by lowercase letters do not differ significantly ( $p > 0.05$ ) by the Duncan test. C: Control; T1: incorporated (1 mg g<sup>-1</sup> Chitosan); T2: incorporated (2 mg g<sup>-1</sup> chitosan); T3: incorporated (4 mg mL<sup>-1</sup> chitosan) and CA: Control with acetic acid.

**Table 2.** Survival curve of *S. aureus* in coalho cheese added chitosan as edible cover at different concentrations and stored for 16 days under refrigeration.

Days	C	CA	T1	T2	T3
0	5.54 ± 0.03 Ea	4.95 ± 0.04 Ca	5.17 ± 0.03 Da	4.85 ± 0.02 Ba	4.62 ± 0.02 Aa
4	7.38 ± 0.05 Dc	6.18 ± 0.04 Bb	6.49 ± 0.03 Cb	5.28 ± 0.03 Ac	5.22 ± 0.02 Ab
8	8.54 ± 0.02 De	7.29 ± 0.00 Ce	7.28 ± 0.00 Cd	5.54 ± 0.03 Bd	5.50 ± 0.01 Ad
12	7.66 ± 0.01 Dd	6.43 ± 0.01 Cc	6.46 ± 0.01 Cb	5.55 ± 0.02 Bd	5.37 ± 0.04 Ac
16	7.26 ± 0.08 Db	6.84 ± 0.12 Cd	6.89 ± 0.08 Cc	5.26 ± 0.07 Bb	4.59 ± 0.15 Aa

<sup>ABC</sup>Models followed by uppercase letters do not differ significantly ( $p > 0.05$ ) by the Duncan test. <sup>abc</sup>Means followed by lowercase letters do not differ significantly ( $p > 0.05$ ) by the Duncan test. C: Control; T1: coating (5mg mL<sup>-1</sup> Chitosan); T2: coating (10 mg mL<sup>-1</sup> Chitosan); T3: Coating (15 mg mL<sup>-1</sup> Chitosan) and CA: Control with acetic acid.

The antimicrobial action of chitosan added to the dough and as an edible cover on the viability of *S. aureus* in coalho cheese revealed either reduction or maintenance of the microbial load of the product (depending on the concentration used and the period of exposure) when comparing the rates of microorganism growth in control and treatment samples (Table 1 and Table 2).

The survival curve of *S. aureus* in coalho cheese with chitosan added to the mass confirmed the antimicrobial effectiveness of the biopolymer against the product (Table 1). It was verified that T3 (4mg g<sup>-1</sup> chitosan) showed greater activity against *S. aureus*. The 4th and 12th storage days of the T3 sample were the time intervals in which there was the most reduction of the logarithmic microbial load, with values of 1.70 and 3.22 respectively (Table 1). When comparing T3 to other treatments and control samples, the growth rate of *S. aureus* during all storage days exhibited a linear function (Table 1).

The results indicated that, during the 16 d of storage of the product T3 (4 mg g<sup>-1</sup> chitosan), the samples remained stable for the growth of the microorganism (*S. aureus* ATCC 6538) in relation to the control sample (Table 1).

Corroborating with data found in this study, Juneja et al. (2006) found that the addition of 3 mg g<sup>-1</sup> chitosan to perishable foods (cooked beef and turkey) was efficient in significantly reducing the potential risk of production of *Clostridium perfringens* spores during the cooling stage (54.4 to 7.2 °C) for up to 18 h.

The use of chitosan with antimicrobial efficiency in food products is commonly observed in the form of coatings, with a small number of studies that use it as a direct ingredient in the food matrix (ASSIS; BRITTO, 2014; GOL et al., 2015; ARAÚJO; SHIRAI, 2016; CHEVALIER et al., 2016; RICARDO-RODRIGUES et al., 2017).

The survival curve of *S. aureus* in coalho cheese with chitosan as a coating (Table 2), showed that treatments T2 (10 mg mL<sup>-1</sup> chitosan) and T3 (15 mg mL<sup>-1</sup> chitosan) had similar results with logarithmic microbial reductions ranging from 0.69 to 3.00 and 0.92 to 3.04, respectively, in relation to the control, considering all the time intervals evaluated (Table 2). Treatment T3 (15 mg mL<sup>-1</sup> chitosan) showed greater efficacy on the 8th and 16th d of storage when compared to sample C with microbial log reductions of 3.04 and 2.67, respectively (Table 2).

MEI et al. (2013) when evaluating a protective film composed of chitosan at a concentration of 13 mg mL<sup>-1</sup> for Mongolian cheese storage, observed reductions in total bacterial counts at all time intervals (0, 15 and 30 d) under 8 °C, strengthening the support for the use of this coating for inhibition of microbial growth.

Di Pierro et al. (2011), when using 8 mg mL<sup>-1</sup> chitosan in ricotta cheese in a modified atmosphere at 4 °C, verified a logarithmic reduction of mesophilic and psychrotrophic microorganisms in the cheese when compared to the control samples, demonstrating that this coating provided an effective action in inhibiting the growth of microbial contaminants and reinforcing the possible use of the biopolymer in other dairy products.

The ability to protect food because of its action against film formation has been attributed to chitosan. In addition, chitosan is composed of indigestible components, so it has no energy value, which makes it more appealing to the food industry, which looks for natural components with preservative functions (DUTTA et al., 2009).

The results obtained in the study show that the use of chitosan extracted from the shells of crustaceans was effective in inhibiting *S. aureus* strain ATCC 6538 in samples of coalho cheeses, since the addition of the antimicrobial resulted in smaller microbial counts with better performances for samples with chitosan added to the mass than as a coating at the concentrations 4 mg g<sup>-1</sup> and 15 mg mL<sup>-1</sup>, respectively.

The antimicrobial action of chitosan in cheese samples was more evident when added to the mass than it was as a cover, due to the interaction of the biopolymer with the microorganism. In a high protein and humid food (BORGES et al., 2008; FREITAS FILHO et al., 2009; FORSYTHE, 2013), facultative anaerobic *S. aureus* bacteria (FORSYTHE, 2013) grows homogeneously throughout the product, without limiting its development to the external region. Thus, incorporating chitosan in the mass caused a higher antimicrobial response, that is, the greater the contact of chitosan with the microorganism, the greater its antibacterial efficiency.

The antibacterial action of chitosan has not yet been completely elucidated (GOY et al., 2009), however, it is known that its action is influenced by intrinsic factors such as the degree of deacetylation and extrinsic factors such as nutrients, chemical substrates and the conditions of the medium environment (COSTA SILVA et al., 2006). Other evidence indicates that the antimicrobial activity of chitosan derives from the polycationic nature of the molecule, which interacts with the surface of the bacterial membrane (KONG et al., 2010). Electronic micrographs performed by Lifeng et al. (2004) of *S. aureus* (gram-positive bacteria) have demonstrated that, in the presence of chitosan, the bacterial cell membrane was fragmented. Zheng and Zhu (2003), in turn, found that the antimicrobial activity against gram-positive bacteria increased with higher molecular polymer masses. The hypothesis was that chitosan of greater molecular mass forms films around the bacterial cell that end up inhibiting the absorption of nutrients, which supports the choice of the medium molecular weight chitosan for the accomplishment of the experimental tests.

#### *Physicochemical analysis*

The moisture contents (46.0-54.9%) and fat on dry basis (35.0-60.0%) of the coalho cheese met the standard established by the current legislation, with values of  $54.30 \pm 0.37$  and  $36.40 \pm 0.51$ , respectively (BRASIL, 2001a).



Considering the results of this study and according to other scientific findings, the average percentage of moisture and fat for coalho cheese is relatively varied. Sousa et al. (2014), when investigating the moisture content of the artisanal and industrial manufacturing coalho cheese under state and federal inspection, reported moisture variations between 14.38 and 29.38%. Santos et al. (2011), had moisture values for coalho cheeses between 49.07 and 62.33%. Samples of the same dairy, from the Sertão Alagoano, showed average humidity values between 45.5 and 51.5% (SILVA et al., 2010). Ferreira and Freitas Filho (2008), when evaluating the moisture content of coalho cheeses sold in Barreiros-PE, obtained values between 31.11 and 40.62%, and Cavalcante et al. (2007), when evaluating coalho cheese produced with endogenous lactic acid culture found moisture values from 31.50 to 42.73%.

As for the dry matter fat content in coalho cheese, Santos et al. (2011) verified values from 16.83 to 24.00%, Silva et al. (2010) obtained values ranging from 36.59 to 48.16%. Freitas Filho et al. (2009), reported average values of 18.99-31.88% in artisanal coalho cheese produced in the municipality of Jucati-PE, and Cavalcante et al.

(2007) reported percentage values between 28.00 and 34.50%. These changes in the moisture and fat content of coalho cheeses can be attributed to the lack of standardization in the production process, reflecting variation in the characteristics of the product composition (SILVA et al., 2010).

#### Stability analysis

The results of the parameters A (pH), B (acidity) and C ( $A_w$ ) of the coalho cheeses are described in Table 3. At the time intervals of 0, 10 and 20 d of storage, the samples of C, T1 (15 mg mL<sup>-1</sup> chitosan) and T2 (incorporated as 4mg g<sup>-1</sup> chitosan) had values with significant differences ( $p < 0.05$ ) in relation to the CA sample (control with acetic acid). It should be noted that the CA sample had pH values between 4.97 and 5.1 due to the acidity of the acetic acid that influenced the pH reduction.

The physico-chemical factors of pH, acidity and water activity ( $A_w$ ) of coalho cheese are not discussed by specific legislation, however, these parameters are fundamental to establish the type of microbial degradation, which strengthens the importance of obtaining these data for the food industry (SOUSA et al., 2014).

**Table 3.** Physical and chemical analyses of coalho cheese with chitosan added in the mass and as a cover in different concentrations and stored for 20 days under refrigeration.

<b>A (pH)</b>				
Days	C	CA	T1	T2
<b>0</b>	6.57 ± 0.21 Ba	5.00 ± 0.10 Aa	6.53 ± 0.06 Ba	6.73 ± 0.06 Bb
<b>10</b>	6.60 ± 0.15 Aa	4.97 ± 0.06 Aa	6.50 ± 0.10 Ba	6.67 ± 0.20 Ba
<b>20</b>	6.23 ± 0.29 Bb	5.10 ± 0.17 Aa	6.50 ± 0.10 Ba	6.77 ± 0.06 Bb
<b>B (acidity %)</b>				
<b>0</b>	0.12 ± 0.02 Aa	0.35 ± 0.01 Db	0.27 ± 0.01 Ca	0.22 ± 0.01 Bb
<b>10</b>	0.11 ± 0.01 Aa	0.31 ± 0.01 Da	0.26 ± 0.01 Ba	0.15 ± 0.01 Ca
<b>20</b>	0.29 ± 0.03 Bb	0.34 ± 0.01 Cb	0.30 ± 0.02 Bb	0.22 ± 0.02 Ab
<b>C (<math>A_w</math>)</b>				
<b>0</b>	0.96 ± 0.01 Ab	0.98 ± 0.01 Aa	0.97 ± 0.01 Ac	0.96 ± 0.01 Ab
<b>10</b>	0.95 ± 0.01 Ab	0.96 ± 0.01 Aa	0.95 ± 0.01 Ab	0.95 ± 0.01 Ab
<b>20</b>	0.92 ± 0.01 Ba	0.97 ± 0.01 Aa	0.93 ± 0.01 Ba	0.90 ± 0.01 Ba

<sup>ABC</sup>Models followed by uppercase letters do not differ significantly ( $p > 0.05$ ) by the Duncan test. <sup>abc</sup>Means followed by lowercase letters do not differ significantly ( $p > 0.05$ ) by the Duncan test. C: Control; CA (Control with Acetic Acid); T1: Coating (15 mg mL<sup>-1</sup> Chitosan) and T2: incorporated (4 mg mL<sup>-1</sup> Chitosan).

The analysed samples revealed similar values for all parameters evaluated when compared to those described in the literature (Table 3). In all storage periods (0, 10 and 20 d), sample T2 had a more acidic pH than sample C; however, the data obtained were not considered statistically significant. Although it was not significant, it is worth noting that pH difference can be justified by the presence of the acetic acid used for the dissolution of the powder and the pH adjustment of the chitosan in order to reach amine groups with a high degree of protonation (HERNÁNDEZ-MUÑOZ et al., 2008). Sousa et al. (2014), consider pH a parameter of fundamental importance in determining a cheese profile, given its effect upon texture, maturation and microbial action.

The percentage of lactic acid in the samples ranged from 0.11 to 0.35% (Table 3) for control samples and treatments. The higher acidity of chitosan-treated cheese samples (T1 and T2) occurred because chitosan is dissolved in an acidic medium to activate it (VARMA; DESHPANDE; KENNEDY, 2004; MOURA et al., 2005). The values obtained resembled data from the literature, although there have been several values reported for the acidity parameter. Mamede et al. (2010) reported values ranging from 0.53% to 0.91% acidity, and Sousa et al. (2014) found values of 0.12-1.01% for the coalho cheeses analysed.

The  $A_w$  values varied between 0.90 and 0.98 for all samples of cheese analysed, with no significant differences between samples ( $p > 0.05$ ) at 0, 10 and 20 d post-storage (Table 3). Thus showing that the samples treated with chitosan did not influence changes in this parameter. The control sample and chitosan-treated products showed a reduction in  $A_w$  during the stability evaluation period, however, these values were not considered significant. Other studies corroborate the results found, Sousa et al. (2014) verified values between 0.89 and 0.96 for coalho cheeses sold in the Northeast. When evaluating the  $A_w$  of curd cheese produced in the

state of Paraíba, Freitas et al. (2013) found values between 0.97 and 0.98.

As for the microbiological profile, the samples of coalho cheese C, T1 (coating of 15 mg mL<sup>-1</sup> chitosan) and T2 (incorporated at 4 mg g<sup>-1</sup> chitosan) at all time intervals (0, 10 and 20 d of storage) with the limits recommended by the legislation (BRASIL, 2001b). Coagulase positive staphylococcal counts in the coalho cheese samples were < 10 CFU g<sup>-1</sup>. For thermotolerant coliforms, counts were < 5 × 10<sup>2</sup> CFU g<sup>-1</sup>. Not isolated *Salmonella* spp. and *L. monocytogenes* (in 25 g of each sample analysed).

Thus, it is understood that in laboratory conditions, the samples of coalho cheese analysed did not show differences in microbiological growth, strengthening the importance of food production in adequate hygienic-sanitary conditions.

The literature in turn reveals a high level of cheese contamination, especially for coalho cheeses that are commonly contaminated by pathogens in an industrial production line. This situation has been attributed mainly to the absence of quality criteria for the selection of raw material, standardization in the production process and application of GMP procedures (BORGES et al., 2008; SILVA et al., 2010; ANDRADE et al., 2011).

Santana et al. (2008) analysed 60 samples of coalho cheeses sold in the central market in Aracajú - SE reported contamination in 46.7% of samples by coagulase positive staphylococci, revealing the unviability of the product for human consumption.

Borges et al. (2008) through the monitoring of the hygienic conditions used for the processing of coalho cheese, observed contamination by *S. aureus* in a production line of this product in the metropolitan region of Fortaleza-CE. In their case, 100% (25/25) of the samples of raw milk showed the presence of coagulase positive staphylococci, and staphylococcal enterotoxin was detected in 20% of raw milk samples and consequently in pasteurised milk, curds and cheese (final product).

When evaluating the microbiological aspects of coalho cheeses sold in establishments in the states of Pernambuco, Piauí, Ceará, Rio Grande do Norte, Sergipe and Paraíba, which either did or did not have the Federal Inspection Seal (SIF), Sousa et al. (2014) verified that, of the 104 samples collected, 100 were above the accepted limits for coagulase positive staphylococci.

### Sensory analysis

From the grades obtained in the acceptance test (Table 4), the three samples of coalho cheese were successful, since the averages obtained ranged from 6.83 to 8.14, representing in the hedonic terms “I liked it slightly” and “I liked it a lot”. In addition, it was observed that the samples did not differ significantly ( $p > 0.05$ ), except for the texture attribute, which differed ( $p > 0.05$ ) from sample C.

**Table 4.** Mean of the grades assigned in the acceptance test for coalho cheeses added by chitosan in the dough, as a cover and without chitosan.

Products	Colour	Smell	Flavour	Texture	Appearance
C	7.95 ± 1.30 a	7.47 ± 1.27 a	7.29 ± 1.52 a	7.05 ± 1.78 a	7.5 ± 1.66 a
T1	7.79 ± 1.30 a	7.25 ± 1.44 a	6.83 ± 1.80 a	6.93 ± 1.60 a	7.1 ± 1.55 a
T2	8.14 ± 0.98 a	7.65 ± 1.23 a	7.67 ± 1.36 a	7.73 ± 1.28 a	7.82 ± 1.32 a

Means followed by lowercase letters do not differ significantly ( $p > 0.05$ ) by the Duncan test. C: Control (without addition of chitosan); T1: coating (15mg mL<sup>-1</sup> Chitosan); T2: incorporated (4 mg g<sup>-1</sup> Chitosan).

As for the colour parameter, the averages obtained for the cheese ranged from 7.79 to 8.14, which represented in the hedonic terms “I liked it moderately” and “I liked it a lot” (Table 4). It was observed that the samples did not differ significantly ( $p > 0.05$ ), however the coalho cheese T2 had the highest score in the evaluation of the colour attribute. El-Diasty et al. (2012) reported similar results for chitosan-treated Kariesh cheese at concentrations of 5 mg mL<sup>-1</sup> and 10 mg mL<sup>-1</sup>, where sensory evaluators did not identify significant differences in colour between cheeses with and without addition of chitosan.

The odour attribute of the evaluated samples obtained average scores between 7.25 and 7.65, which denotes in hedonic terms “I liked it moderately”. The cheeses analysed showed no significant difference ( $p > 0.05$ ). The study showed that the flavour parameter had the lowest mean (6.83), among all attributes evaluated, only for cheese treated with chitosan (coating 15 mg / mL), but within the hedonic terms, this evaluation is considered satisfactory, equivalent to the concept “I liked it slightly” (Table 4). The other samples

(C and T2) had averages greater than 7.0. As for the texture, the averages obtained were distributed between the hedonic terms “I liked it slightly” and “I liked moderately”, with averages of 6.93 and 7.73, respectively. In addition, the sample treated with the incorporated antimicrobial had a higher score (7.73) according to the averages assigned in the acceptability test when compared to the other cheeses evaluated, showing a significant difference ( $p < 0.05$ ). The use of chitosan as an agent that controls and extends the texture in foods (SHAHIDI; ARACHCHI; JEON, 1999; NAIR et al., 2009) can be inferred from the fact that its homogeneous incorporation into the product improved this aspect.

Garcia et al. (2008), when evaluating the odour, taste and texture of commercial coalho cheeses (obtained from buffalo milk, goat milk and cow milk) obtained in the hedonic concepts notes between 6.55 and 7.40 (odour), 6.23 and 7.65 (flavour), 7.32 and 7.68 (texture). For the appearance attribute, the evaluators assigned the grade  $7 \pm 1$  for all the samples, which reflects in the hedonic terms the concepts “I liked it slightly” and “I liked it a lot”.

As to the Acceptability Index (AI) of sample C (without addition of chitosan) and treatments, T1 (coating of 15 mg mL<sup>-1</sup> chitosan) and T2 (incorporated 4 mg g<sup>-1</sup> chitosan), results were higher than 70.0% for the different parameters, except in

the taste aspect of the sample treated with chitosan as edible cover (Table 5). According to Dutcosky (1996), indices of acceptance  $\geq 70.0\%$  reflect a positive repercussion of the product with promising potential in the market sector.

**Table 5.** Acceptability of coalho cheese added with chitosan in the mass, as a cover and without chitosan, per evaluated parameter.

Products	Colour	Smell	Flavour	Texture	Appearance
C	88.33%	83.00%	81.00%	78.33%	83.33%
T1	86.60%	80.55%	68.30%	77.00%	78.88%
T2	90.44%	85.00%	85.22%	85.88%	86.88%

C: Control (without addition of chitosan); T1: coating (15mg mL<sup>-1</sup> Chitosan); T2: incorporated (4 mg g<sup>-1</sup> Chitosan).

## Conclusions

The antimicrobial efficiency of chitosan incorporated and used as a coating in coalho cheese at all concentrations used was proven. The treatments with chitosan at concentrations of 4 mg g<sup>-1</sup> (incorporated into the mass) and 15 mg mL<sup>-1</sup> (edible coating) were the most efficient in inhibiting the growth of *S. aureus* ATCC 6538 during 16 days of coalho cheese storage. The physico-chemical characteristics of the products obtained during the experiment are in accordance with the regulatory standards for coalho cheese and showed stability as to pH, acidity and Aw. In addition, the evaluated samples met the microbiological limits advocated by the specific legislation, evidencing that chitosan associated with GMPs can assure the conservation and quality of the food. As for the sensorial acceptance, the addition of chitosan did not influence the parameters of quality except for improving the texture when incorporated into the mass. On the acceptability index, there was satisfactory acceptance for the chitosan treated samples for the parameters evaluated, except flavour when chitosan was added as a cover. In view of this, the application of chitosan as a coating and incorporated into coalho cheese can be considered a profitable and promising alternative for the preservation of this food.

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