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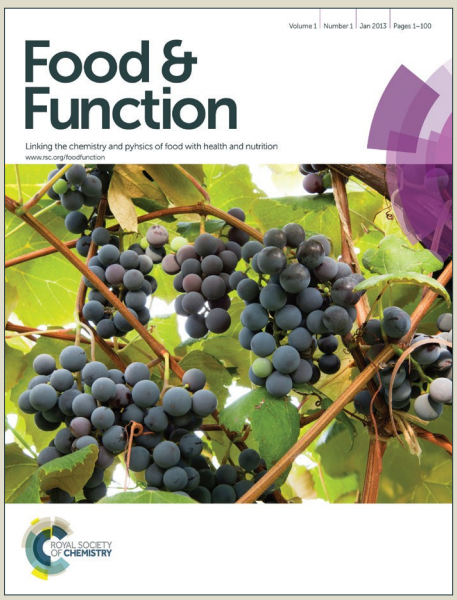

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GOAT SAUSAGES CONTAINING CHITOSAN TOWARDS A HEALTHIER PRODUCT: MICROBIOLOGICAL, PHYSICO-CHEMICAL TEXTURAL EVALUATION

Deborah S. do Amaral[a], Alejandra Cardelle-Cobas[b,c], Bárbara M. S. do Nascimento[a], Marta S. Madruga[a], Maria Manuela E. Pintado[b]

[a]DEA - Department of Food Engineering, Technology Centre, Federal University of Paraiba, 58051-900 João Pessoa, Paraiba, Brazil

[b]CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Rua Arquiteto Lobão Vital, Apartado 2511, 4202-401 Porto, Portugal

[c] Present address: Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Veterinary Medicine, University of Santiago de Compostela, 27002 Lugo, Spain

[*]Corresponding author:

E-mail address: mpintado@porto.ucp.pt

Tel.: +351 225580097

Fax: +351-225090351

Goat meat is extensively known for its interesting nutritional value and for being an important source of protein with high quality. Its food derivatives are, therefore, a good alternative to develop new products addressed to health conscious consumers. In this work, a healthier goat product, namely, a low fat fresh sausage, was produced with the objective of evaluating the effect of inclusion of chitosan on quality, stability and shelf life. Sausages containing 2% chitosan were formulated with different fat levels (5%, 12.5% and 20%, w/ w) and stored at 4 ºC during 15 days. Results indicated the incorporation of 2% (w/w) chitosan was technologically feasible, due to the reduction of microbial growth and lipid oxidation, as well as the enhancement of red color. Additionally, the treated samples improved all characteristics associated to cooking, showing ability to bind water and fat and acquiring a firmer texture compared with control samples. Additionally, the reduction of fat content is technologically feasible without negative influences on the final product.

Keywords: antimicrobial, antioxidant, chitosan, fat reduction, goat meat, shelf life

Introduction

Goats are ruminants widely distributed around the world and have been a source of nutrients for humans since the very beginnings of civilization.[1] The goat meat has a great potential merchandising because of its nutritional value, such as its low fat content, high digestibility, high protein content, iron and unsaturated fatty acids, when compared to other types of red meat.[2] The best cuts (loin, leg, etc) are sold at high prices and are very appreciated by the consumers whereas the remaining cuts such as the shoulder and neck, for example, have low consumer acceptability and no commercial value. In addition to the low appeal for some cuts, consumers reject, in some cases, the

goat meat probably due to texture and strong flavour, particularly present in meat from older animals.[1] In this sense, the industrialization of this kind of meat. focusing, specially, in the elaboration of new products from the less appreciated cuts, could be a good alternative to increase the value of goat meat with low commercial attractiveness[3]

Sausage is a popular processed meat product that traditionally consists of chopped meat, water, binders, fat, and seasonings.[4] Despite its popularity, this kind of product presents some negative perceptions, most of them related to its high fat content since it is well known that the amount and the type of fat consumed is associated with the risk of coronary heart diseases[5] This, combined with the fact that the effect of diet on health is well-known and that the use of foods to improve health and the state of wellbeing is an idea increasingly accepted by society in the last three decades, has caused an increase in the demand for low fat products. Consequently, this change in consumer's mentality could contribute to the loss of market share, especially in this kind of products with high fat content as sausages, when health considerations are important quality criteria.[6]

Another negative aspect usually associated with this kind of products such as sausages, especially when commercialized as a fresh product, is the frequent use of high levels of synthetic additives to avoid damaging lipid oxidation reactions, control the growth of pathogens and food contaminants and thus, increase shelf life.[7] Therefore, a reformulation of meat products based on processing strategies is an important trend to develop products that promote better consumer health. In this sense, the safety of synthetic additives has been questioned in the last few years, increasing the request for natural additives.[8] In this sense, different herb extracts as, for example, rosemary, essential oils as well as concrete compounds (i.e. α-tocopherol) present in fruits and vegetables have been tested as natural additives given rise to interesting results in terms of food preservation.[7] The use of nitrite and nitrate in the manufacturing of meat

products is considered indispensable, since they can promote the red colour of cured products, act as antioxidants by delaying lipid oxidation, prevents or retards microbial growth and give a pleasant flavour.[9] However, nitrite also results in the formation of N-nitrosamines, a group of compounds that are well known for their carcinogenic and mutagenic activities[10] being related to certain types of cancer namely the colon one. These risks have caused a rise in consumer demand for natural products and created a need for developing alternative preservation systems for meat and meat products. [11,12]

In this context, different compounds obtained from natural sources such as grains, oilseeds, spices, fruit and vegetables have been investigated.[8] Among these different natural compounds, chitosan has attracted especial interest from the industry as a potential natural food preservative since it exhibits strong antimicrobial activity against a range of food-borne microorganisms[13] and possess different functional characteristic, such as, antioxidant activity[7] and lipid and water binding capacity[14], that can promote the final quality of food products. At the same time, different studies have reported several health benefits, namely, anti-inflammatory, antitumoral and immune-stimulating.[15] Moreover, many studies report their hypocholesterolemic effects.[15,16]

Chitosan consists of polymeric 1,4-linked 2-amino-2-deoxy-β-D-glucose and it was reported as Generally Recognised as Safe (GRAS) by the US FDA.[17] Previous studies have indicated that chitosan could be effectively used to inhibit microbial spoilage and delaying lipid oxidation in fresh pork sausages at certain concentrations[5,7], as well as, some research can be found on the fat reduction in meat products by the incorporation of chitosan without causing adverse effects in sensory characteristics and textural properties.[5,18] Moreover, a previous study developed by our research team, indicated that incorporation of 2% (w/w) of chitosan in pork sausages to accomplish the EFSA[19] claims on reduction of cholesterol (ingestion of 3 g of chitosan per day (3 sausages), is

technologically feasible, allowing to obtain a product with improved properties.[18] However, the application of chitosan in goat meat products demonstrating the potential of chitosan as a functional ingredient and as technological agent to develop a low fat goat meat product with better quality and stability throughout shelf-life have not been yet established. Additionally, the use of goat meat cuts with low demand could enhance their value and promote the use of this so healthy kind meat. Therefore, the objective of this study was to include chitosan in an adequate concentration to be in accordance with the scientific opinion of EFSA[19] in a fresh low fat goat sausage and establish as this inclusion could affect the quality, stability and shelf life of the product. Thus, the fresh goat sausages were produced with different percentages of fat with required amount of chitosan and stored at 4 ºC during 15 days to evaluate of the microbiological and physico-chemical parameters in order to promote the goat meat with commercial value through the reduction of fat and use of a natural preservative.

Results and discussion

Fresh samples analysis

Microbiological results. Results on microbiological counts of total mesophilic and psychrotrophic bacteria, *Enterobactereaceae* and moulds and yeasts in fresh goat sausages during refrigerated storage are shown in Fig. 1. In general, the enumerations for all determined microbiological groups were significantly ($p < 0.05$) affected by storage time, addition of chitosan and variation of fat. In the beginning (day 0) mesophilic bacteria (Fig. 1a) was between 5.03 ± 0.07 and 5.24 ± 0.06 for the sausages control and 3.86 ± 0.06 to 4.83 ± 0.07 for the sausages with chitosan, which denotes an efficient and rapid antimicrobial effect of chitosan. These values are in agreement with previously published results[20] for fresh goat meat (5.68 log CFU/g) and goat meat salted

for 0.6 day of salting/kg (4.17 and 3.30 log CFU/g for samples without and with olive oil and paprika, respectively). During storage the counts increased gradually in all samples as expected due to the initial microbial load, however the growth was always higher in control sausage and on the day 15 the counts for control sausages (8.42 ± 0.03 to 7.37 ± 0.02) were very high exceeding the recommended limit of 10^7 cfu/mL[12], while in the treated samples with chitosan the initial reduction of ca. 2 log cycles was maintained assuring that the value is in the recommended range (6.14 ± 0.06 to 6.97 ± 0.06). Similar behavior was reported by Soultos et al. [21] which reported that after storage at 4 °C of Greek style fresh pork sausages for 14 days the total counts in the samples without chitosan (7.82 ± 0.59) had already exceeded the maximum levels acceptable (10^7 CFU/g) of mesophilic bacteria, while samples with chitosan reached 21 days (6.82 ± 0.48). In our study, considering the counts mesophilic bacteria, the shelf life of control sample could be limited at 10 days, while the chitosan sausages could prolong at least in 5 days the shelf-life of the treated samples.

Regarding the variation of fat, the bacterial counts decreased with the increase in fat content, being always lower in all sausages containing chitosan where the differences were between 0.5 - 2 log cycles in relation to the control sample, varying with the level of fat, but with no relation established. In general, the increase of fat led to a reduction on microbial growth explained by the reduction of moisture and the negative effect of fat. Additionally, the inhibition of mesophilic bacteria by chitosan is also in agreement with results reported by other research groups for pork meat products. Other authors[7] observed a decrease between 1 and 2 log cycles in pork sausage added 1% chitosan after 20 days of storage, while Roller et al. [22] and Sayas-Barberá et al. [12] reported a reduction of 2 log units in fresh pork sausage with 1% chitosan after 24 days and in pork model burgers after 7 days, respectively.

The psychrophilic bacteria counts (Fig. 1b) in the early day of storage were between 6.24 ± 0.02 and 7.27 ± 0.03 log CFU/g. During storage, the counts increased up to values of 8.35 ± 0.02 to 8.58 ± 0.01 log CFU/g after 15 days in control samples (without chitosan) and values of 7.32 ± 0.04 to 7.79 ± 0.06 log CFU/g for samples with chitosan. Similar counts were found in goat meat under aerobic storage (9 log cfu/g after 28 days) and vacuum storage (7 log CFU/g after 40 days).[23] Regarding the percentage of fat, the psychrophilic bacterial counts showed a slight decrease, at 10 and 15 days of storage, with the increase in fat content. Psychrophilic bacterial counts were always lower for the samples containing chitosan and along all the period of storage, showing a reduction in the range of ca. 0.8 – 1.0 log CFU/g in comparison with samples without chitosan. Petrou et al.[24] reported that chitosan produced significantly lower psychrotrophic counts as compared to the control samples (day-12). Similarly Bostan et al.[25] reported that during storage the increase in the number of psychrotrophic bacteria in sausage treated with 1% chitosan was significantly lower than the control sausage.

The values of *Enterobacteriaceae* (Fig. 1c) found on day 0 for the sausages with and without chitosan were between 3.54 ± 0.02 and 3.87 ± 0.02, respectively. These values increased during storage at 4 °C reaching values of ca. 4.72 ± 0.04 and 6.53 ± 0.02 for the sausages with and without chitosan, respectively. Babji et al[23] reported similar counts in goat meat under aerobic storage (6 log CFU/g) and vacuum storage (4 log CFU/g) after 28 days. Regarding to the fat content, the samples containing higher fat level (F20 and F20C), also showed significant lower values of *Enterobacteriaceae*. The addition of chitosan caused a reduction of 1 log CFU/g at 5, 10 and 15 days as compared to the control samples. The antimicrobial effect of chitosan upon *Enterobacteriaceae* observed in this study is in accordance with Petrou et al.[24], which reported that in chicken meat the *Enterobacteriaceae* reached final counts of ca. 6 log

cycles on day-12, while the treated samples with chitosan showed counts of ca. 3-4 log cycles. Georgantelis et al.[7] studied the effect of rosemary extract addition, with chitosan and α- tocopherol in fresh pork sausages and reported a decreasing trend on *Enterobacteriaceae* counts in samples containing chitosan (3.9 log CFU/g) and a reduction during storage for 20 days of ca. 1 - 1.5 log CFU/g compared to the control sample.

Finally, with regard to moulds and yeasts (Fig. 1d) fresh goat sausages counts at initial time were between 6.62 ± 0.01 and 7.24 ± 0.02 log CFU/g, respectively for sausages with and without chitosan. These values increased during the storage up to 7.25 ± 0.02 and 8.21 ± 0.03 log CFU/g, respectively after 15 days storages at 4 °C. The lowest levels were always found lower in samples with chitosan (F5C, F12.5C and F20C), in comparison with those with no inclusion of chitosan. The presence of chitosan promoted a reduction in moulds and yeast counts of ca. 0.5 - 0.8 log CFU/g during storage as compared to the control samples. Soultos et al.[21] reported similar results for yeast and molds. They determined that the counts in fresh pork sausage reduced approximately 0.8 log CFU/g at the end of 15 storage days at 4 °C when in presence of 1% of chitosan. Sagoo et al.[11] in chilled pork products found a reduction of ca. 2 log CFU/g at the end of 18 days storage by the presence of 1% chitosan. Petrou et al.[24] also reported that chitosan could reduce the growth of these species as compared to untreated samples. Therefore, corroborating the results found in our study.

Physico-chemical analysis

Proximate composition and pH. Proximate composition of fresh goat sausages added of chitosan and prepared with different fat levels is shown in Table 1. On day 0 the moisture, protein and fat content were similar to those reported by Leite et al.[26] in goat meat sausages manufactured with different pork fat levels (0, 10 and 30%). They found

values of 59.46-69.53, 18.92-14.29 and 5.33-21.81, respectively for moisture, protein and fat. In our study, the moisture content decrease during the storage period at 4 °C ($p < 0.05$) due to the loss of water since the covering material is not high barrier. Regarding the percentage of fat and the addition of chitosan the values obtained were statistically different ($p < 0.05$). Thus, the moisture content decreased with the increase of fat content, as well as, by adding of chitosan when compared with their control samples, without chitosan and equivalent level of fat. With the exception of samples F20 and F20C (20% fat, without and with chitosan, respectively), which, although lower values of moisture in sample treated with chitosan than the control was observed, the difference was not significant on day 0 and 15 of storage. The reduction in moisture content is due to the chitosan ability to absorb water by hydrogen bonding through its hydroxyl and amine groups. Sayas-Barberá et al.[12] in pork model burgers and Amaral et al.[18] in fresh pork sausage also reported that the addition of chitosan caused a decrease in the moisture content.

The addition of chitosan reduced total fat content of sausages compared to control ones. This effect is due to the ability of chitosan to bind fat.[14] During storage, the fat content increased for all samples, which can be explained by the occurrence of concentration due to the reduction of moisture. This increase during storage was also observed on the ash content, although not showing statistically difference ($p > 0.05$) by addition of chitosan and variation of fat.

Regarding the results obtained for the protein content, higher values were found in sausages with lower fat content as expected. Protein levels decreased as a function of storage time in all samples, being statistically different ($p < 0.05$) only for samples F5C, F12.5 and F12.5C. Estevez et al.[27] reported that the proteins can also be affected by

oxidative reactions, so it could reduce the protein content. There was no statistically difference ($p < 0.05$) due to the addition of chitosan.

The results of pH (data not shown) on the day 0 for all batches ranged between 5.37 and 6.38. These values were similar to those reported in previous studies[26,28] for goat sausages. There was a gradual increase of pH in all samples (5.97 – 6.73) during storage ($p < 0.05$), which can be attributed to microbial proteolysis, which cause protein and amino acid degradation resulting in the accumulation of basic compounds such as ammonia.[29] For samples with added chitosan, the samples showed higher values (5.86 ± 0.03 to 6.33 ± 0.06) than control samples (5.37 ± 0.08 to 5.50 ± 0.02). This increase of pH in meat products has also been reported by others studies[7,18,21] and can be attributed to the basic nature of chitosan[7,12], promoted by the amino groups present.

Lipid oxidation. Together with microbial spoilage, chemical deterioration especially lipid oxidation is a main factor limiting the shelf-life of meat foods.[30] Lipid oxidation is a rather complex process, whereby unsaturated fatty acids react with molecular oxygen via a free radical chain mechanism. This reaction constrain nutritional and sensory properties of foods and promotes toxicity since it involves the loss of essential fatty acids and vitamins and the generation of toxic compounds as thiobarbituric acid reactive substances (TBARS) as for example the malondialdehyde (MDA), as well as, affects sensory traits of meat product, causing flavor, color and texture deterioration.[27]

The results of TBARS expressed as mg of MDA per kg (see supplementary material) content in fresh goat sausages with and without chitosan prepared with different fat levels during the 15 d of storage increased proportionally with increasing fat and storage period, being lipid oxidation more intense the control samples than in the samples with added chitosan. The reduction in the TBARS values was greater in samples with 12.5 and 20% of fat treated with chitosan (F12.5C and F20C), resulting in

250 decreases of ca. 44% and 50% on day 0 and 25% and 20% after 15 days, respectively,
251 compared to control sample with same fat content. Our results confirm the fact that
252 chitosan may retard oxidative rancidity in muscle foods, by acting as a chelator on
253 transition metal ions, such as ferrous ions.[31] The effectiveness of chitosan on the
254 oxidative stability of meat and meat products has already been demonstrated[7,18,21]. Thus,
255 the addition of chitosan can result in better quality and longer shelf life concerning lipid
256 oxidation profile either in pork or goat meat products.

257 **Color measurement.** Changes in color parameters L*, a* and b* are shown in table 2.
258 The results obtained for samples on day 0 were similar to those reported by Guerra et
259 al.[32] for goat mortadella prepared with different levels of fat (10%, 20% and 30%). The
260 addition of chitosan resulted in the lowest L* values, while control samples (without
261 chitosan) had significantly higher values ($p < 0.05$). Sayas-Barberá et al.[12] reported
262 similar results in pork model burgers, indicating that the increase in L* during the first
263 day could be related to oxidation increasing metmyoglobin concentration. During
264 storage, the lightness (L*) of fresh goat sausages decreased significantly ($p < 0.05$),
265 remaining lower in samples with added chitosan when compared with the corresponding
266 samples used as a control (no chitosan addition and same level of fat). This decrease in
267 samples F5C, F12.5C and F20C could be due to the water binding ability of chitosan,
268 being in accordance with Fernández-López et al.[33] which indicated that the increase in
269 water holding capacity (WHC) reduces L*. Regarding fat content, the lightness
270 increased proportionally with addition of chitosan ($p < 0.05$), so the higher L* values
271 were found in samples with 20% fat. Similar behavior was reported by Guerra et al.[32] in
272 goat mortadella prepared with different levels of fat, indicating that higher added fat
273 provides greater clarity to the sample.

The redness (a* values) of fresh goat sausage decreased during refrigerated storage ($p < 0.05$) and the decrease was more intense in the samples without chitosan. This effect may be explained by the fact that chitosan presents antioxidant properties which may contribute to maintain redness in muscle foods, due to its ability to act as a chelator on transition metal ions which catalyse oxidative reactions (i.e. oxidation of myoglobin).[31] According to Georgantelis et al.[7], chitosan could be chelating iron ions of meat hemoproteins during heat processing or storage. Similar results were also obtained for pork meat products with chitosan.[12,18] Nitrate and nitrite are added to meat products due to their important role on color and flavor development and their antioxidant activity.[9] However, N -nitrosamines (NA) may be formed increasing demand of additive-free products by consumers. In this aspect, we highlight the absence of chemical additives in our study, as well as, confirm the protective role of the chitosan showing a more stable and improved red color during refrigerated storage than the control samples.

Parameter b* (yellowness) was not affected by the variation of fat level on day 0 and was always higher in the control samples than in samples added of chitosan ($p < 0.05$). This behavior may be due the antioxidant properties of chitosan. However, generally the b* values increase during storage by intensity of the oxidation process that tend to increase yellowness of samples by rancidity.[33] However, our results showed slight difference on yellowness (b*) throughout storage time of fresh sausages, probably because samples were stored for short time and at refrigerated temperature.

Cooked samples analysis

Water retention capacity. The water retention capacity (Table 1) of the goat sausages after cooking was significantly affected by the variation of fat, time of storage and the addition of the chitosan ($p < 0.05$). Samples with higher fat level (F20 and F20C) showed higher values of water retention capacity than the other samples with lower fat

content (F5, F5C, F12.5 and F12.5C). According to Cavestany et al.[34], higher the percentage of fat, the more concentrated and dense will be the emulsion's continuous phase, favoring, thus, the formation of the structure with greater water-holding ability. During storage, water retention capacity increases for both samples (with and without chitosan). This increase is probably due to the slight water loss during storage. Regarding the influence of chitosan, the control samples (F5, F12.5 and F20) showed a lower percentage of water retention capacity compared with the sausages with added chitosan (F5C, F12.5C and F20C), which also had the lowest moisture content. Claus et al.[35] reported that the lower moisture content provides a medium of greater ionic strength, which will lead to greater extraction of proteins and thus improves the binding properties. Similar behavior was reported by Sayas-Barberá et al.[12] in pork model burgers, which justified the highest cooking yield of samples with chitosan due to its water binding ability.

Texture profile analysis. Textural parameters are crucial to monitor the impact of chitosan and fat on final sausages texture and consequently predict the impact on sensory quality. Among them the hardness is one of more relevant markers and represents the maximum force required to compress the sample. Results obtained from fresh goat sausages texture (data not shown) showed that the addition of chitosan increased the hardness values (5.99 ± 0.07 to 7.27 ± 0.08) when compared with their control samples, without chitosan and same level of fat (5.66 ± 0.25 to 5.81 ± 0.49). The other textural parameters in general were not affected by the addition of the chitosan. Regarding cooked goat sausage (Table 3), the sausages with chitosan showed higher hardness values than the control samples. The increase in hardness by adding of chitosan has been reported by García et al.[38] in pork sausage and Lin and and Chao[5] in reduced-fat Chinese-style sausage, Amaral et al.[18] in cooked pork sausage. This effect

can be due to the fact that chitosan have ability to act as binder, thus favoring the formation of a stronger gel promoting a more stable structure.

After 15 days of refrigeration, the hardness of cooked goat sausage increased significantly ($p < 0.05$) in all groups, with this increase being significantly higher in the products with chitosan than in the control sample. This behavior can be explained not only by the slight drying of the product during storage Ganhao et al.[39], but also due to the stabilizations of chitosan linkages with matrix components at refrigerated temperature. Furthermore, this parameter showed a tendency to increase proportionally with the reduction of fat content. This result is consistent with those obtained by Cavestany et al.[36] that have assessed the effects of sardine surimi in Bologna sausage containing different fat levels, which reported that fat may act as a lubricant to allow myofilaments to slide past one another more easily, thus increasing tenderness and resulting in lower shear-force values. In our study, the lower hardness values were presented by the samples with higher fat content (F20 and F20C), which also showed the highest water retention capacity, as described before.

Regarding the other parameters of texture profile, chitosan addition increased slightly the values of springiness, cohesiveness, gumminess, chewiness and resilience of cooked sausages compared with control sausages, though this tendency was not always significant. García et al.[36] in pork sausage reported that chitosan addition did not affect significantly the results of the texture profile analysis. The gumminess is the force necessary to disintegrate a semi-solid state sample until swallowing and chewiness is defined as the product of hardness and cohesiveness. These two parameters have their results dependent on the hardness, thus showed similar behavior, increasing with the addition of chitosan and time storage, as well as reducing with the fat. The resilience was not affected by the addition of chitosan, reducing after 15 days of storage.

Principal component analysis (PCA) and Pearson correlation analysis

For a global view of the results presented in this work, a PCA (Fig. 2) and Pearson correlation analysis (Table 4) was performed on microbial counts and physicochemical parameters, such as texture (by the TPA test) and color (L*, a* and b*). On PCA, the resulting grouping of the variables analyzed in relation to the formulations (with and without chitosan; PC1) and storage time (PC2) showed 89% of confidence. The microbiological parameters were all grouped in the right and lower part of the graph indicating the highest level of microorganisms for the sausages formulated without chitosan at 15 days (F5-15, F12.5-15 and F20-15). Since, for all the groups of microorganisms analyzed the increase on storage time led to increase of microbial levels the significant correlation it was expected. Therefore, data was not shown in the Pearson correlation table. The texture parameters (Hardness, Gumminess and Chewiness) were all grouped in the right and upper part of the graph, showed highest values in all the sausages with chitosan after15 days of storage (F5C-15, F12.5C-15 and F20C-15). This was due to the chitosan incorporation and the time of storage that increased the compressive forces and, therefore, resulting in an improvement of the texture. The correlation between hardness, gumminess and chewiness it is explained because these two last parameters have their results dependent on the hardness, as previously mentioned. Finally, the color parameters showed a trend of reduction with storage time. The L* and b* showed a correlation and were grouped in the left and lower part of the graph, possibly because they have similar behavior, such as, a decrease over the time and with the addition of chitosan. Already the a* located on left and upper part of the graph was affected by chitosan, thus improving red color of goat sausage, resulting in positive effects on appearance.

Experimental

Sausage ingredients

Sausages were formulated according to previous studies.[18] Thus, ingredients such as salt, fresh garlic and powder white pepper and dried oregano were obtained in local markets of the city of Porto (Portugal). Goat meat was removed of leg, shoulder, rib, neck and loin cuts of male animals, without defined breed, slaughtered between 8 and 10 months old and artificial casings were also bought in traditional local markets.

Chitosan was provided by Sigma-Aldrich (Steinheim, Germany) and previously characterized in the laboratory.[38]

Sausages manufacture

Fresh sausages manufacture and chitosan addition were carried out under the same procedure as indicated in a previous work.[18] In brief, three different formulations were made with 2% (w/w) of chitosan and different fat concentrations: 5% (w/w) (Formulation F5C), 12.5% (w/w) (Formulation F12.5C) and 20% (w/w) (Formulation F20C). A sample without chitosan was used as a control for each formulation (F5, F12.5 and F20). Each fresh sausage weighed 50 g and had 3 cm of diameter. They were packed in plastic bags without vacuum and stored under refrigeration at 4 °C for 15 days. One lot of 1000 g of fresh goat sausage of each formulation was prepared and divided into two replicates for consequent analysis.

Microbiological analysis

Mesophilic and psychrophilic bacteria, *Enterobacteriacea*e and yeast and molds were determined along the storage period at 4 °C of the samples on days 0, 5, 10 and 15. For this, 8 g of sample were placed in plastic bags and homogenized for 2 min in a stomacher (Lab Blender 400, London, UK) with 80 ml of sterile 0.1% peptone water. The homogenate was serially diluted using the peptone water as diluent and plated in duplicate using the drop method (20 µl of each dilution) to enumerate viable counts of

mesophilic and psychrophilic bacteria (PCA-Plate Count Agar, Biokar diagnostics) incubated at 30 ºC for 48 h and 7 ºC for 7 d, respectively and yeasts and molds (PDA-Potato Dextrose Agar, Biokar diagnostics) incubated at 25 ºC for 5 d. Enterobacteriaceae (VRBGA- Violet Red Bile Glucose Agar, Lab) were evaluated by pour plate technique[39] incubated at 37 ºC for 24 h. After incubation, the colonies were enumerated and colony forming units (CFU/mL) were calculated. Each replicated sample of fresh goat sausages were analysed in duplicate.

Physicochemical analyses

Fresh samples

Proximate analysis, lipid oxidation and color analysis

Proximate analysis was determined in the samples at 0, 5, 10 and 15 days of storage at 4 ºC. Moisture, ash, protein, and fat content were determined by the official AOAC methods of analysis 24.003, 24.009, 24.027, and 24.005, respectively.[40] Results were expressed in all cases as g /100 g of sample.

The pH values of samples were also measured by an AOAC method of analysis. Specifically, it was analyzed by the 943.02 method.[41] A combined pH glass electrode connected to a pH-meter MicropH 2001 Crison potentiometer (MicropH 2001, Barcelona, Spain) was used. Each replicated sample of fresh goat sausages were analysed in duplicate.

Lipid oxidation

Lipid oxidation was assessed by measuring the thiobarbituric acid reactive substances (TBARS). 2 g of sample were homogenized by vortexing in 10 mL of 10% of tricloroacetic acid (TCA biochemical/Applichem) and 5 ml of 0.02 M 2-thiobarbituric acid (TBA, Merck). Then, it was centrifuged at 5000 rpm for 20 min in a UNiversal 320R centrifugue (Zentrifugem, HETTICH). The supernatant was collected and filtered,

424 heated in boiling water for 35 min at 100 °C and chilled in iced water for 10 min.
425 Finally, absorbance at 532 nm was measured in a spectrophotometer UV mini 1240
426 (Shimadzu, Tokyo, Japan). 1,1,3,3 tetraethoxypropane (Sigma Aldrich) was used as
427 standard in the range $1x10^{-6}$ – $14x10^{-6}$ mol/L. TBARS concentration was expressed as
428 mg malondialdehyde per Kg of sample. Each replicated sample of fresh goat sausages
429 were analysed in duplicate.

430 **Color analysis**

431 Color was determined as previously reported[18], using a digital Minolta colorimeter
432 (Model CR-300, Minolta, Osaka, Japan). The parameters lightness (L*),
433 redness/greenness (a*) and yellowness/blueness (b*) were determined according to the
434 specifications of the Commision Internationale de L'éclairage (CIE, 1986)., being,
435 illuminant D65, 8 ° viewing angle, standard observer angle of 10 ° specular included.
436 The determinations in each replicated sample of fresh goat sausages were performed in
437 triplicate.

438 **Cooked samples**

439 Two fresh goat sausages of each formulation were subjected to cooking after 0, 5, 10
440 and 15 days of storage at 4 ºC. For this, the sausages were cooked in hot water until
441 reaching 72 ° C at the geometric center, controlled by a thermocouple, then were taken
442 and maintained at room temperature until cooling. Since chitosan impacts especially in
443 the moisture and fat retention, as well as some textural parameters, those parameters
444 were also analyzed in cooked samples.

445 **Water retention after cooking**

446 The water holding capacity in cooked samples was determined according to the
447 methodology previously described by other authors.[12] The estimation the amount of
448 moisture retained in the samples was calculates according the following equation:

449 % Water retention = 100 × cooked weight (g) × % moisture in cooked sample

450 raw weight (g) × % moisture in raw sample

451 **Texture profile analysis**

452 Fresh and cooked samples were submitted to textural analysis. Thus, two sausages of
453 each formulation at 0, 5, 10 and 15 days of storage at 4 ºC (fresh and cooked) were cut
454 into three pieces of 3 cm high and compressed twice using a texture analyzer TA-XT2
455 (Stable Micro Systems, Haslemere, England). Textural parameters were measured by
456 compressing with a cylinder probe of 2 cm of diameter. Force-time curves were
457 recorded at a crosshead speed of 5 mm/s at a distance of 35 mm. Hardness (peak force
458 of first compression cycle, N), chewiness (hardness × cohesiveness × springiness, N x
459 mm), cohesiveness (ratio of positive areas of second cycle to area of first cycle,
460 dimensionless), gumminess (hardness × cohesiveness, N), springiness (distance of the
461 detected height of the product on the second compression divided by the original
462 compression distance, mm/mm) and resilience (area during the withdrawal of the first
463 compression divided by the area of the first compression) were the textural parameters

464 **Statistical analysis**

465 The statistical package used was SAS version 9.4 (2013) to explore the statistical
466 significance of results. The analysis of variance (ANOVA) using the Tukey test was
467 applied to the results of physicochemical and microbiological analysis to determine the
468 statistically significant differences between formulations during storage. Data from
469 microbiological, texture and color analysis were evaluated by principal component
470 analysis (PCA) and Pearson correlation analysis. A confidence interval of 95% ($p <$
471 0.05) was considered in all cases.

472

473 ## Conclusions

The use of chitosan in the manufacture of fresh goat sausages has been studied for the first time. A better preservation of quality and extension of the product shelf life was observed through a significative reduction of microbial growth and lipid oxidation in chitosan added sausages, when compared with the controls. Moreover, an enhancement of the physical stability of sausages with chitosan was also obtained with an improvement in the red color and also in a firmer texture through the increase of hardness. Although it is necessary to conduct further studies to show the beneficial properties of this product, the results included in the present study indicate that incorporation of 2% (w/w) chitosan in meat goat sausage is technologically feasible to formulate a product with a reduced fat content and that, at the same time, accomplish the requirements of the EFSA (ingestion 3g chitosan/day; 3 sausages) to cause a decrease in serum cholesterol

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TABLES

Table 1. Proximate composition obtained, during the storage period, for fresh goat sausages and water retention capacity (WRC) calculated for cooked goat prepared with 2 % of chitosan and without chitosan (chitosan and control samples, respectively) and different amounts of fat 5% (F5), 12.5%(F12.5) and 20% (F20). Samples were stored at 4 °C for 15 days.

Variables	Treatments (% of Fat)	Storage period (Days)*							
		0		5		10		15	
		Control	Chitosan	Control	Chitosan	Control	Chitosan	Control	Chitosan
Moisture (g/100)	F5	69.96 ± 0.22^{aA}	67.27 ± 0.22^{aB}	69.24 ± 0.07^{abA}	66.48 ± 0.30^{abB}	69.59 ± 0.68^{aA}	66.00 ± 0.89^{bB}	68.66 ± 0.23^{bA}	65.80 ± 0.11^{bB}
	F12.5	65.52 ± 0.57^{aC}	62.90 ± 0.09^{aD}	64.69 ± 0.08^{abC}	62.91 ± 0.41^{aD}	64.55 ± 0.09^{bC}	62.77 ± 0.11^{aD}	64.05 ± 0.61^{bC}	61.81 ± 0.26^{bD}
	F20	60.36 ± 0.75^{abE}	59.95 ± 0.30^{aE}	60.79 ± 0.01^{aE}	59.56 ± 0.05^{aF}	59.58 ± 0.52^{bcE}	58.54 ± 0.28^{bF}	58.76 ± 0.24^{cE}	58.37 ± 0.24^{bE}
Fat (g/100)	F5	9.55 ± 0.79^{bD}	8.06 ± 0.36^{bD}	10.72 ± 0.48^{abE}	8.61 ± 0.60^{bF}	11.24 ± 0.35^{aE}	9.36 ± 0.48^{abF}	11.37 ± 0.32^{aD}	10.19 ± 0.61^{aD}
	F12.5	13.93 ± 0.80^{bC}	12.64 ± 0.65^{cC}	14.94 ± 0.31^{abC}	13.37 ± 0.41^{bcD}	16.12 ± 0.48^{aC}	14.43 ± 0.29^{abD}	16.31 ± 0.45^{aC}	15.15 ± 0.67^{aC}
	F20	21.27 ± 0.01^{aA}	17.19 ± 0.41^{bB}	21.72 ± 0.30^{aA}	18.40 ± 0.08^{bB}	21.18 ± 32^{aA}	18.13 ± 0.78^{bB}	22.49 ± 0.60^{aA}	20.34 ± 0.51^{aB}
Ash (g/100)	F5	2.13 ± 0.04^{bA}	2.25 ± 0.18^{aA}	2.29 ± 0.03^{abA}	2.21 ± 0.05^{aAB}	2.46 ± 0.15^{aA}	2.33 ± 0.08^{aAB}	2.50 ± 0.04^{aA}	2.31 ± 0.21^{aAB}
	F12.5	2.07 ± 0.02^{aA}	1.80 ± 0.00^{bB}	2.10 ± 0.04^{aABC}	2.03 ± 0.03^{aBC}	2.21 ± 0.13^{aBC}	2.17 ± 0.08^{aBC}	2.20 ± 0.01^{aBC}	2.05 ± 0.07^{aC}
	F20	1.62 ± 0.21^{bBC}	1.55 ± 0.19^{bC}	2.06 ± 0.02^{aABC}	1.87 ± 0.01^{aC}	1.97 ± 0.04^{aCD}	1.91 ± 0.8^{aD}	2.04 ± 0.09^{aC}	2.01 ± 0.05^{aC}
WRC (%)	F5	87.49 ± 0.20^{cE}	98.00 ± 0.51^{bC}	91.65 ± 0.18^{bE}	101.91 ± 0.35^{aC}	90.79 ± 0.53^{bF}	101.14 ± 0.31^{aD}	94.03 ± 0.24^{aE}	102.48 ± 0.22^{aD}
	F12.5	94.46 ± 0.02^{cD}	102.72 ± 0.25^{bB}	95.54 ± 0.02^{bcD}	100.48 ± 0.33^{cC}	96.90 ± 0.73^{bE}	104.12 ± 0.12^{bC}	101.14 ± 0.11^{aD}	106.21 ± 0.25^{aC}
	F20	104.47 ± 0.50^{cB}	107.58 ± 0.22^{cA}	104.19 ± 0.28^{cB}	108.54 ± 0.26^{cA}	106.24 ± 0.44^{bB}	112.83 ± 0.27^{bA}	108.30 ± 0.20^{aB}	114.99 ± 0.64^{aA}

* Different letters (a–b) in the same row differ significantly ($p < 0.05$) in time. Different letters (A–B) in the same column differ significantly ($p < 0.05$) in samples.

Table 2. Color analysis during the storage period, for fresh goat sausages prepared with 2 % of chitosan and without chitosan (chitosan and control samples, respectively) and different amounts of fat 5% (F5), 12.5%(F12.5) and 20% (F20). Samples were stored at 4 °C for 15 days.

Variables	Treatments (% of Fat)	Storage period (Days)*							
		0		5		10		15	
		Control	Chitosan	Control	Chitosan	Control	Chitosan	Control	Chitosan
L*	F5	55.21 ± 1.13^{aD}	52.27 ± 1.46^{aE}	54.46 ± 0.65^{aC}	51.93 ± 0.88^{aD}	52.79 ± 0.97^{bB}	46.2 ± 1.99^{bC}	47.91 ± 1.02^{cC}	45.51 ± 0.82^{bD}
	F12.5	57.83 ± 0.76^{aAB}	55.52 ± 0.52^{acD}	56.91 ± 0.24^{abA}	54.96 ± 0.61^{aBC}	55.93 ± 0.43^{bA}	52.86 ± 1.10^{bB}	52.46 ± 1.15^{cAB}	51.68 ± 0.86^{bB}
	F20	59.02 ± 1.03^{aA}	57.04 ± 0.43^{aBC}	58.07 ± 1.39^{aA}	56.33 ± 1.11^{aAB}	56.01 ± 1.91^{bA}	53.01 ± 1.60^{bB}	54.12 ± 0.77^{cA}	51.83 ± 0.83^{bB}
*a**	F5	8.51 ± 1.30^{aC}	12.49 ± 0.74^{aA}	4.48 ± 0.89^{bC}	10.39 ± 0.52^{bA}	2.48 ± 0.82^{cC}	9.54 ± 0.60^{bcA}	2.56 ± 0.20^{cC}	8.59 ± 0.36^{cA}
	F12.5	10.39 ± 0.29^{aB}	10.56 ± 0.22^{aB}	4.36 ± 0.51^{bC}	9.72 ± 0.63^{aAB}	2.82 ± 0.37^{cC}	8.08 ± 1.01^{bB}	3.33 ± 0.59^{bcC}	7.62 ± 0.68^{bAB}
	F20	7.60 ± 1.73^{aC}	10.14 ± 0.38^{aB}	3.81 ± 0.61^{bC}	9.03 ± 0.63^{abB}	2.41 ± 0.39^{bcC}	8.03 ± 0.21^{bcB}	2.62 ± 0.28^{cC}	7.29 ± 1.20^{cB}
*b**	F5	12.09 ± 0.66^{aA}	10.51 ± 0.29^{aB}	10.87 ± 1.87^{bBC}	9.68 ± 0.50^{bB}	10.20 ± 0.82^{bB}	9.67 ± 0.49^{bB}	10.07 ± 0.55^{bB}	8.81 ± 0.40^{bC}
	F12.5	12.15 ± 0.65^{aA}	10.43 ± 0.81^{aB}	11.91 ± 0.28^{aAB}	10.50 ± 0.41^{aCD}	12.27 ± 0.45^{aA}	9.75 ± 0.39^{aB}	12.12 ± 0.68^{aA}	9.85 ± 0.41^{aBC}
	F20	12.90 ± 0.58^{aA}	10.71 ± 0.30^{aB}	12.30 ± 0.77^{aBA}	10.57 ± 0.75^{abCD}	11.75 ± 0.97^{bA}	9.85 ± 0.64^{abB}	11.31 ± 0.81^{bA}	9.65 ± 0.57^{bBC}

* Different letters (a–b) in the same row differ significantly ($p < 0.05$) in time. Different letters (A–B) in the same column differ significantly ($p < 0.05$) in samples.

Table 3. Texture profile analysis obtained for cooking goat sausages prepared with 2 % of chitosan and without chitosan (chitosan and control samples, respectively) and different amounts of fat 5% (F5), 12.5% (F12.5) and 20% (F20). Samples were stored at 4 ℃ for 15 days

Variables	Treatments (% of Fat)	Storage period (Days)*							
		0		5		10		15	
		Control	Chitosan	Control	Chitosan	Control	Chitosan	Control	Chitosan
Hardness (N)	F5	13.56 ± 1.60^{aAB}	14.01 ± 0.82^{bAB}	14.04 ± 0.60^{aAB}	16.20 ± 1.02^{abA}	14.33 ± 1.31^{aB}	17.76 ± 0.63^{aA}	15.12 ± 0.67^{aAB}	18.03 ± 2.07^{aA}
	F12.5	11.71 ± 3.36^{abABC}	14.57 ± 0.68^{bA}	11.77 ± 1.55^{abB}	14.31 ± 3.87^{bAB}	10.65 ± 1.00^{bC}	15.62 ± 0.63^{abAB}	14.31 ± 2.82^{aBC}	17.79 ± 0.51^{aA}
	F20	9.54 ± 1.24^{aC}	10.91 ± 3.21^{bBC}	11.42 ± 1.57^{aB}	13.29 ± 1.44^{abAB}	9.18 ± 1.28^{aC}	13.9 ± 0.90^{aB}	11.83 ± 1.23^{aC}	16.12 ± 2.23^{aAB}
Springiness (mm)	F5	0.93 ± 0.02^{aA}	0.95 ± 0.03^{bA}	0.92 ± 0.04^{aB}	0.95 ± 0.06^{bB}	0.89 ± 0.02^{aA}	0.98 ± 0.03^{bA}	0.93 ± 0.02^{aB}	1.33 ± 0.65^{aA}
	F12.5	0.96 ± 0.05^{aA}	0.96 ± 0.08^{bA}	0.87 ± 0.02^{aB}	1.32 ± 0.43^{aA}	0.94 ± 0.00^{aA}	0.98 ± 0.04^{bA}	0.81 ± 0.32^{aB}	0.96 ± 0.03^{bB}
	F20	0.90 ± 0.05^{aA}	0.95 ± 0.04^{aA}	0.91 ± 0.07^{aB}	0.95 ± 0.04^{aB}	0.88 ± 0.02^{aA}	0.96 ± 0.05^{aA}	0.89 ± 0.03^{aB}	1.00 ± 0.14^{aAB}
Cohesiveness	F5	0.33 ± 0.11^{bB}	0.38 ± 0.06^{aB}	0.41 ± 0.04^{abB}	0.43 ± 0.09^{aB}	0.45 ± 0.12^{aBC}	0.40 ± 0.02^{aC}	0.45 ± 0.05^{abC}	0.44 ± 0.03^{aC}
	F12.5	0.37 ± 0.07^{bB}	0.33 ± 0.05^{bB}	0.50 ± 0.11^{aAB}	0.56 ± 0.00^{aA}	0.42 ± 0.02^{abC}	0.47 ± 0.07^{aABC}	0.45 ± 0.04^{abC}	0.46 ± 0.02^{aAB}
	F20	0.50 ± 0.04^{abA}	0.43 ± 0.02^{bAB}	0.41 ± 0.07^{bB}	0.43 ± 0.05^{bB}	0.55 ± 0.01^{aAB}	0.57 ± 0.09^{aA}	0.57 ± 0.03^{aAB}	0.56 ± 0.07^{aA}
Gumminess (N)	F5	5.10 ± 0.84^{aA}	5.22 ± 0.80^{cA}	5.78 ± 0.68^{aAB}	6.41 ± 1.53^{bcA}	6.57 ± 1.86^{aABC}	7.16 ± 0.04^{abAB}	6.72 ± 1.44^{aAB}	8.38 ± 0.08^{aA}
	F12.5	4.21 ± 0.94^{aA}	4.85 ± 0.92^{cA}	5.74 ± 0.53^{aAB}	5.76 ± 1.03^{bcAB}	5.20 ± 0.64^{aBC}	7.32 ± 0.90^{abA}	5.71 ± 0.72^{aB}	8.27 ± 0.12^{aA}
	F20	5.40 ± 1.21^{aA}	5.86 ± 1.70^{abA}	5.10 ± 0.43^{aAB}	4.23 ± 1.23^{bB}	4.95 ± 0.33^{aC}	6.35 ± 1.35^{aAC}	5.61 ± 1.08^{aB}	6.82 ± 1.02^{aAB}
Chewiness (N mm)	F5	4.73 ± 0.87^{aAB}	5.27 ± 0.43^{bAB}	5.33 ± 0.81^{aAB}	6.73 ± 1.40^{bA}	5.21 ± 2.05^{aABC}	7.02 ± 0.21^{abA}	5.37 ± 0.63^{aB}	8.80 ± 0.52^{aA}
	F12.5	3.66 ± 0.49^{bB}	4.61 ± 0.64^{bAB}	4.68 ± 1.07^{abAB}	6.52 ± 0.97^{aAB}	3.82 ± 0.73^{abC}	7.14 ± 0.59^{aA}	5.57 ± 1.34^{aB}	8.05 ± 0.31^{aA}
	F20	4.13 ± 0.20^{aAB}	5.77 ± 1.39^{abA}	4.64 ± 0.51^{aB}	4.46 ± 0.38^{bB}	4.65 ± 0.74^{aBC}	6.56 ± 1.65^{aAB}	5.73 ± 0.54^{aB}	5.91 ± 2.01^{abB}
Resilience	F5	0.11 ± 0.08^{abB}	0.10 ± 0.04^{aB}	0.11 ± 0.05^{abAB}	0.10 ± 0.01^{aB}	0.16 ± 0.08^{aAB}	0.10 ± 0.03^{aBC}	0.05 ± 0.01^{bB}	0.10 ± 0.03^{aB}
	F12.5	0.11 ± 0.05^{abB}	0.10 ± 0.04^{abB}	0.18 ± 0.07^{aA}	0.19 ± 0.04^{aA}	0.21 ± 0.00^{bC}	0.09 ± 0.04^{bBC}	0.05 ± 0.08^{abB}	0.07 ± 0.01^{bB}
	F20	0.23 ± 0.07^{aA}	0.25 ± 0.03^{aA}	0.10 ± 0.06^{bAB}	0.14 ± 0.05^{bAB}	0.21 ± 0.03^{abA}	0.05 ± 0.10^{bABC}	0.22 ± 0.03^{abA}	0.06 ± 0.02^{bB}

* Different letters (a–b) in the same row differ significantly ($p < 0.05$) in time. Different letters (A–B) in the same column differ significantly ($p < 0.05$) in samples.

Table 4. Pearson correlation between textural parameter (cooked sample) and color analysis (fresh sample) of goat sausages prepared with different amounts of fat. 5% (F5), 12.5% (F12.5) and 20% (F20) and with 2% of chitosan and without chitosan stored at 4 °C for 15 days.

	Hardness	Springiness	Cohesiveness	Gumminess	Chewiness	L*	a*	b*
Hardness		-0.241^{ns}	-0.443^{ns}	0.894**	0.791*	-0.898**	-0.025^{ns}	-0.766*
Springiness			0.710^{ns}	-0.071^{ns}	0.188^{ns}	0.482^{ns}	0.403^{ns}	0.085^{ns}
Cohesiveness				-0.079^{ns}	0.100^{ns}	0.608*	0.471^{ns}	0.097^{ns}
Gumminess					0.902**	-0.764*	0.141^{ns}	-0.823*
Chewiness						-0.572^{ns}	0.285^{ns}	-0.663*
L*							0.315^{ns}	0.774*
a*								-0.090^{ns}
b*								

ns - not significant
* Significant at p <0.05% probability
** Significant at p <0.01% probability

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LEGENDS OF FIGURES

Figure 1 Evolution of mesophilic (a) and psychrotrophic (b) bacteria, *enterobactereaceae* (c) and moulds and yeasts (d) in fresh goat sausages prepared with different amounts of fat, 5%, 12.5% and 20% and with 2% of chitosan (samples F5C, F12.5C and F20C) and without chitosan (samples F5, F12.5 and F20) stored at 4 °C for 15 days. Different letters (a–b) differ significantly ($p < 0.05$) in time. Different letters (A–B) differ significantly ($p < 0.05$) in samples. A confidence interval of 95% ($p < 0.05$) was considered in all cases.

Figure 2 Principal component analysis (PCA) for textural parameter (cooked sample), color and microbiological analysis (fresh sample) of goat sausages prepared with different amounts of fat, 5%, 12.5% and 20% and with 2% of chitosan (samples F5C, F12.5C and F20C) and without chitosan (samples F5, F12.5 and F20) on 0 and 15 days of stored at 4 °C. A confidence interval of 95% ($p < 0.05$) was considered in all cases.

FIGURES

Figure 1

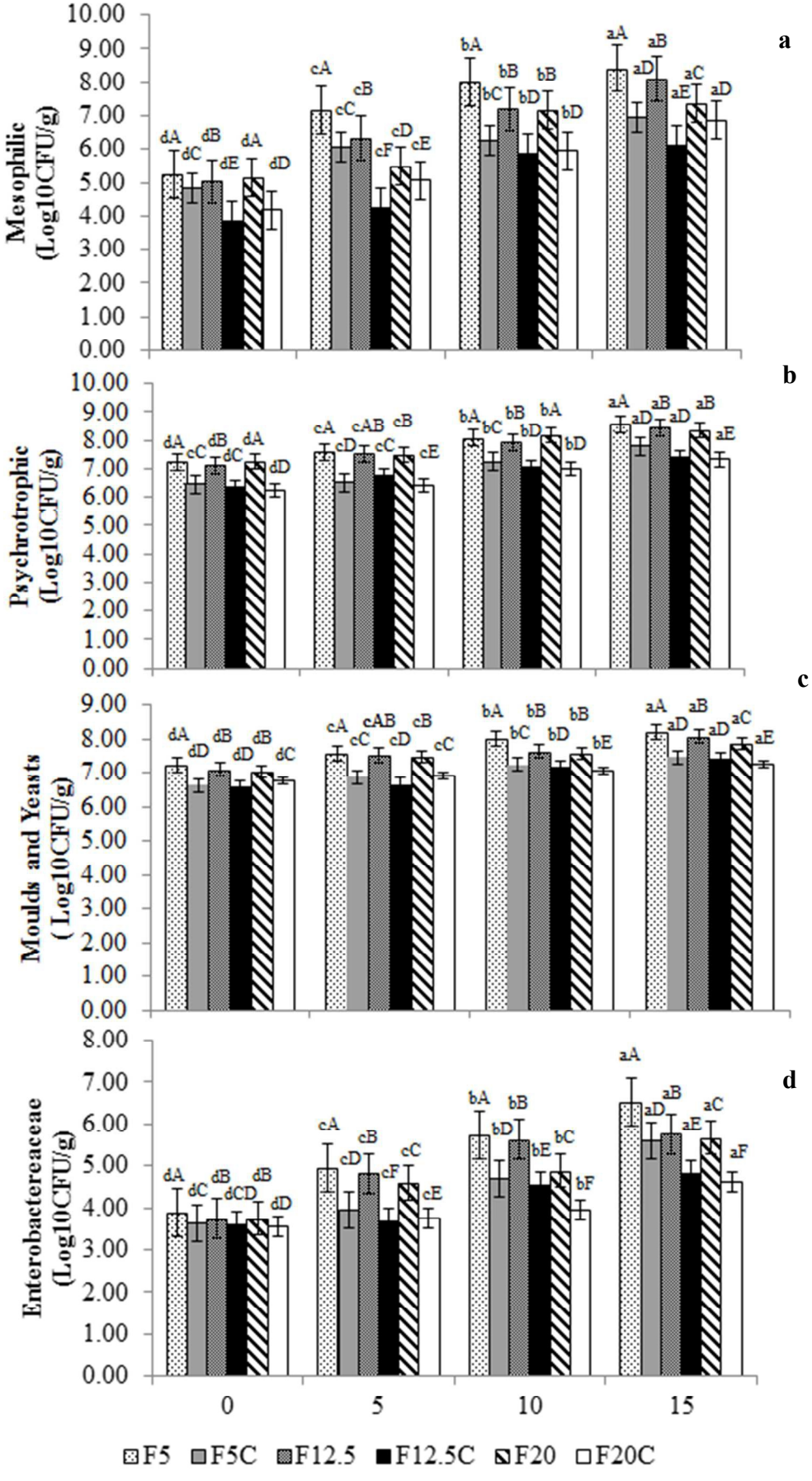

Figure 2