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Effect of the addition of bacterial cellulose on the texture and color properties of sausages obtained from mechanically separated meat from Nile tilapia (*Oreochromis niloticus* L.)

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Abstract

Bacterial cellulose has been considered a potential ingredient in food development. In this study it was used to replace fat in Nile tilapia (*Oreochromis niloticus*) mechanically separated meat sausages. Response surface methodology with complete Central Composite Rotatable Design (CCRD) 2^2 was used, including 4 axial points and 3 center points, with fat ranging from 0 to 8% and bacterial cellulose ranging from 0 to 13.66%. The variables BC and fat had no significant effect ($p>0.05$) on hardness, gumminess and color parameters c and h of sausages. BC had a significant effect ($p<0.05$) on cohesiveness, adhesiveness and luminosity, and tests 1 and 2 provided the best formulations.

Keywords: fat replacement; fish sausage; bacterial cellulose; *Komagataeibacter xylinus*.

Practical Application: Effect of bacterial cellulose as a fat substitute on fish sausage.

1 Introduction

Nile tilapia (*Oreochromis niloticus* L.) is one of the most farmed fish species in the world (Food and Agriculture Organization of United Nations, 2020), with great acceptance in the consumer market, being often marketed as frozen fillet (Uehara et al., 2022; Costa et al., 2019). Tilapia filleting produces a low fillet yield, approximately 30%, and the other 70% is considered waste (Bacelar et al., 2021) which, when used by the fish industry, produce a value-added by-product, mechanically separated meat (MSM). MSM has been used in the development of new products, such as sausage, bologna, restructured products, surimi, fishburgers, among others (Leira et al., 2019; Jerônimo et al., 2021; Tomé et al., 2022). Besides adding value to the by-product, its use reduces the amount of organic waste discarded in the environment (Alexandre et al., 2022; Albergaria et al., 2022).

The aforementioned meat products are the main foods that contribute to the intake of saturated fats, which in turn are related to cardiovascular diseases. Hence these products are unhealthy (Oliveira et al., 2021; Öztürk-Kerimoglu et al., 2022; Ferro et al., 2021). To minimize these negative effects, several studies have been carried out aimed to reduce or replace some components used in the formulation of these products, such as, for example, fat reduction (Ferro et al., 2021), making them healthier (Sariçoban & Unal, 2022).

One of the main challenges for the industry in replacing and reducing fat is maintaining the flavor, texture, juiciness and appearance of emulsified meat products (Ferro et al., 2021; Pietrasik & Soladoye, 2021; Oliveira et al., 2021). An alternative method for improving the technological and functional characteristics in the reformulation of new low-fat products is the use of replacers (Auriema et al., 2021).

Bacterial cellulose (BC), also known as "nata" or "nata-de-coco", is a gelatinous cellulosic film produced by bacteria, and the genus *Komagataeibacter xylinus* is the most efficient. Although BC has the same chemical structure as vegetable cellulose, it has different physicochemical properties, such as high water retention capacity, high tensile strength and greater purity, due to the absence of hemicellulose and lignin (Oliveira et al., 2021; Pereira et al., 2020). BC has been widely studied as a food ingredient and has the potential to be used as a fat replacer (Oliveira et al., 2021).

This study aimed to optimize the addition of BC as a partial fat replacer in fish sausages, to evaluate this effect on the technological properties, texture and color, and to choose the best formulations using the response surface methodology.

2 Materials and methods

2.1 Raw material

Nile tilapia fillet and mechanically separated meat (MSM), soybean oil, bacterial cellulose (BC), maltodextrin, monosodium glutamate, sugar, polyphosphate, sodium chloride, water and spices were used in the sausage preparation. The MSM was acquired from Cooperativa Agroindustrial Consolata (COPACOL), located in Nova Aurora, Paraná, Brazil. BC was acquired from

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BioSmart Nanotechnology, located in Araraquara, SP, Brazil. The other inputs needed to process the sausage were purchased from local shops.

2.2 Experimental design

Response surface methodology with complete Central Composite Rotatable Design (CCRD) 2^2 was used, including 4 axial points and 3 center points (Table 1), totaling 11 tests (Table 2). The percentages of fat and bacterial cellulose (BC) were used as independent variables, and the response variables were texture profile and instrumental color.

The linear model with interaction that represents the relationship between the variables of the experiment is below (Equation 1). Data were analyzed with Statistica software, version 7.0.

$$\hat{Y} = \hat{\beta}_0 + \hat{\beta}_1 X_1 + \hat{\beta}_2 X_2 + \hat{\beta}_{11} X_1^2 + \hat{\beta}_{22} X_2^2 + \hat{\beta}_{12} X_1 X_2 \quad (1)$$

where: $\hat{Y}$ is the response function; β are the regression coefficients; X_1 and X_2 are the independent variables.

The independent variables evaluated were bacterial cellulose (X_1) and fat (X_2), added to the formulation at five levels of variation (Table 1). The limits of variable X_2 were based on the Brazilian Technical Regulation on Sausage Identity and Quality, which establishes 30% as the upper limit for total fat content in sausage (Brasil, 2000). As the present study aims to reduce the amount of fat, the limits were tested in order to reduce the content of this component. The limits of variable X_1 were established according to Marchetti & Andrés. (2021).

To verify the effects of the independent variables and their interactions on the responses evaluated in the sausages, in data processing and in the Response Surface Methodology, the Statistica software, version 7.0 (StatSoft, 2007) was used. The test results were analyzed using ANOVA and Tukey's test, with p≤0.05.

Table 1. Process variables and levels that were used in experimental design.

Independent variables	Coded values	Levels				
		-1.41	-1	0	+1	+1.41
BC (X_1)	Real values	0	2.34	8	13.66	16
Fat (X_2)		0	1.17	4	6.83	8

Table 2. Matrix of experimental design with coded and real values.

Essay	Coded levels		Real values	
	X_1	X_2	BC (%)	Fat (%)
1	-1	-1	2.34	1.17
2	-1	+1	2.34	6.83
3	+1	-1	13.66	1.17
4	+1	+1	13.66	6.83
5	-1.41	0	0	4
6	+1.41	0	16	4
7	0	-1.41	8	0
8	0	+1.41	8	8
9	0	0	8	4
10	0	0	8	4
11	0	0	8	4

2.3 Sausage processing

Processing was performed at Embrapa Agroindústria de Alimentos, located in Guaratiba-RJ, Brazil, and analyzes were conducted at Federal Fluminense University (UFF), located in Niteroi-RJ, Brazil.

The methodology adapted from Freitas et al. (2022) was used in the preparation of sausages. The formulation analyzed in this study complied with the limits required by Normative Instruction No. 4, March 31, 2000 (Brasil, 2000), according to which sausages maximum content of MSM is 60% and of isolated soy protein is 4%. In addition to MSM, Nile tilapia fillet, water, salt, spices and additives were added.

The ingredients were homogenized in the cutter (Geiger, model UM 12, Brazil) and during this process the temperature of the mass was controlled not to exceed 7 °C. Then, a manual sausage filler (CAF model E8, Brazil) was used, and the sausages were stuffed in synthetic casings of caliber no 24, diameter of 1.5 cm and length of 10 cm. The heat treatment was carried out in a jacketed pan (INCAL, model 99, Brazil) with steam circulation for 1 hour and 20 minutes, and with the aid of a thermocouple, the internal temperature of the sausage was controlled until it reached 72 °C. After cooking, the sausages were cooled in an ice-water bath until the internal temperature reached 40 °C. The manual peeling process was performed after cooling and all samples were packed separately in 11 identified polypropylene packages and stored at 4 °C until the time of analysis.

2.4 Response variables

The analysis of the texture profile response variables (hardness, cohesiveness, adhesiveness and gumminess) and instrumental color (*L**: luminosity, *C**: chromaticity and *h*°: hue angle) were carried out at the Laboratory of Technology for poultry, rabbits, eggs, honey, beeswax and derivatives at UFF, Niteroi, RJ.

2.5 Texture profile

Texture profile analysis (TPA) of the tilapia sausages was performed using a texture analyzer (TAXT2 Plus, Stable Micro System, England) with a P036 metallic probe. The parameters used to analyze the mechanical properties of sausages were defined by Surasani et al. (2020) and Wongpattananukul et al. (2022) hardness, cohesiveness, adhesiveness and gumminess. The samples were cut in longitudinal sections. For each sample, three replicates were analyzed.

2.6 Instrumental color (L, C*, h°)*

For the instrumental color analysis of the sausages, Minolta CR-410 colorimeter (Konica Minolta Sensing Inc., Japan) was used. The values of *L**(luminosity), *C**(chromaticity) and *h*° (hue angle) were calculated (Simsek, 2022). Color measurements were performed on the sausage in longitudinal sections, taking three different reading points per sample.

3 Results and discussion

The texture properties of meat products vary depending on the amount of some components such as protein, fat and water (Ghafouri-Oskuei et al., 2022). When reformulating low-fat meat products, it is important to consider the texture of the product to avoid changes that could make them less acceptable to consumers (Marchetti & Andrés, 2021). The results obtained for the parameters of hardness, cohesiveness, adhesiveness and gumminess were evaluated. The general results of the tests of all analyzes are described in Table 3.

The independent variables BC and fat had no significant effect ($p>0.05$) on sausage hardness. Nonetheless, as it can be seen in Table 3, the control sample, without the addition of BC, obtained a higher hardness value compared to the other tests. Akoglu et al. (2015) obtained a similar result in their study with Turkish fermented sausage with BC as a fat replacer. According to these authors, the referred effect is explained by the water retention capacity of BC, which provided higher humidity, and consequently a lower hardness value. The same behavior was observed by Cui et al. (2022) who evaluated an emulsion gel based on soybean protein isolate/curdlan (SPI/CL), as an analogue to pork backfat (PBF) used in meat products. The SPI/CL emulsion gel did not show significant differences compared to PBF ($p>0.05$) in hardness, and it can be used as an analogue to PBF, being an alternative ingredient for the development of low-fat meat products. In the analysis of variance (ANOVA) for CCRD for the concentrations of BC and fat in the hardness parameter of the sausages, it was found that the calculated F value (3.5646) was smaller than the table F value (4.5337). Hence the proposed model was not validated ($p >0.05$).

The influence of CB and fat concentrations on sausage cohesiveness resulted in a regression coefficient (R^2) of 0,72, indicating that mathematical model can explain 72% of the experiment variance. The BC variable had a significant effect ($p<0.05$) on sausage cohesiveness. However, the percentage of fat had no significant effect ($p>0.05$). This result was similar to that found by Akoglu et al. (2015) who reported that the increase in BC in Turkish fermented sausages had a significant effect ($p<0.05$) on the cohesiveness value. According to Younis et al. (2022), reduction of fat promotes a stronger fusion between the sausage ingredients, favoring cohesion. Moreover, other studies reported the addition of fat replacers that significantly ($p<0.05$) affected the cohesiveness of meat products (Auriema et al., 2022; Fan et al., 2020). In the CCRD analysis for BC and fat concentrations in the cohesiveness parameter, the calculated F value (6.5993) was greater than the table F value (4.3468), which allowed the validation of the proposed model (<0.05). Figure 1 shows the influence of the independent variables fat and BC on the cohesiveness response. To obtain higher cohesiveness values of sausage, fat concentration should be 4%. For the variable BC, under the conditions of the study, the best condition is 2.34%. However, the parameter is not optimized for this condition, suggesting that better results can be found in smaller ranges than the studied range. Thus, test 2 was the one that presented the best results, closer to the conditions of the study.

The influence of CB and fat concentrations on sausage adhesiveness resulted in a regression coefficient (R^2) of 0,75, indicating that mathematical model can explain 75% of the experiment variance. The variable BC and fat had a significant effect ($p<0.05$) on sausage adhesiveness. Glisic et al. (2019) also observed a significant effect ($p<0.05$) of the use of inulin gel as

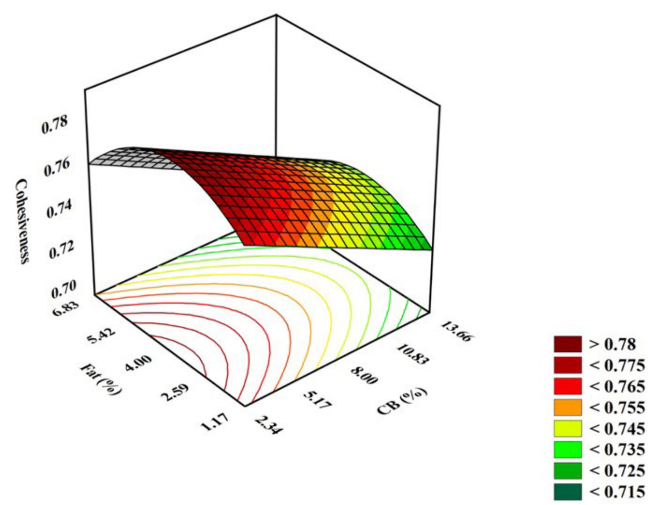

Figure 1. Response surface for cohesiveness as a function of BC and fat.

Table 3. Values obtained in the experimental tests for the variable response of the texture profile investigated in relation to BC and fat.

Essay	Independent variables		Dependents variables			
	BC (%)	Fat (%)	Hardness (g)	Cohesiveness	Adhesiveness (g.seg)	Gumminess (g)
1	2.34	1.17	1994.02 ± 192.89	0.78 ± 0.01	-15.83 ± 5.51	1555.72 ± 127.35
2	2.34	6.83	2165.30 ± 122.43	0.77 ± 0.01	-16.18 ± 5.21	1665.47 ± 79.66
3	13.66	1.17	2611.63 ± 86.04	0.75 ± 0.02	-22.03 ± 4.22	1950.01 ± 79.10
4	13.66	6.83	1756.32 ± 132.24	0.72 ± 0.02	-21.23 ± 4.76	1264.93 ± 88.51
5	0	4	2662.29 ± 139.65	0.78 ± 0.01	-7.08 ± 4.88	2098.03 ± 132.33
6	16	4	1567.75 ± 22.59	0.71 ± 0.02	-33.56 ± 5.46	1127.40 ± 12.89
7	8	0	1861.87 ± 103.75	0.70 ± 0.04	-19.77 ± 6.88	1306.38 ± 57.90
8	8	8	1231.49 ± 28.69	0.70 ± 0.02	-24.16 ± 7.63	846.99 ± 18.85
9	8	4	1673.34 ± 240.86	0.75 ± 0.01	-18.01 ± 3.05	1258.46 ± 168.12
10	8	4	1585.07 ± 99.65	0.76 ± 0.01	-18.61 ± 4.30	1229.28 ± 89.13
11	8	4	1469.56 ± 99.21	0.77 ± 0.01	-18.23 ± 1.62	1131.85 ± 74.15

a replacer for pork back fat on the adhesiveness of sausages. In CCRD analysis for the concentrations of BC and fat in the adhesiveness parameter, the calculated F value (6.9358) was greater than the table F value (4.3468). Therefore, validation of the proposed model ($p<0.05$) and the construction of the response surface of the independent variables for the hardness response variable shown in Figure 2 were possible. In this case, it was found that, regardless of the fat concentration, the best results for adhesiveness are obtained with 2.34% BC, which represents tests 1 and 2. For Oliveira et al. (2021), and according to Leonard et al. (2019), the use of dietary fiber can influence the adhesiveness of the analyzed product. A study by Younis et al. (2022) also highlighted this fact when using fructooligosaccharides (FOS), a dietary fiber, in meat products.

According to Wongkaew et al. (2020), gumminess is the product of hardness and cohesion, which is a characteristic of semi-solid foods with a low degree of hardness and a high degree of cohesion. The independent variables BC and fat had no significant effect ($p>0.05$) on sausage gumminess. In the study conducted by Akoglu et al. (2015) in Turkish fermented

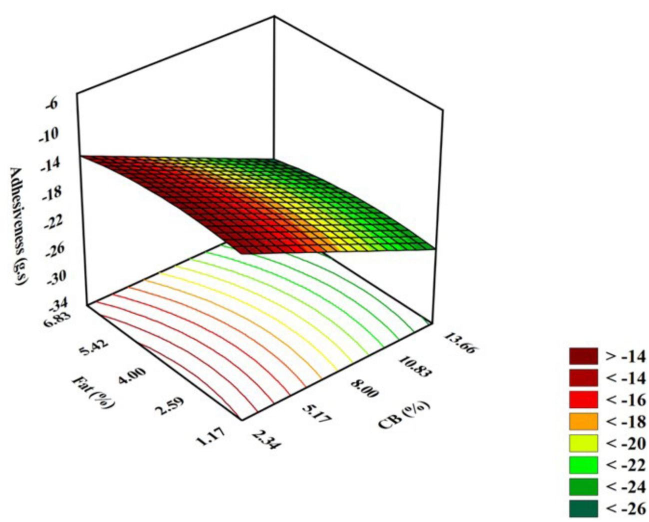

Figure 2. Response surface of adhesiveness as a function of BC and fat.

sausages, addition of BC did not cause a significant difference, and this result is desirable in terms of structure quality. Similar results were obtained by Ferjančič et al. (2021) who evaluated the use of inulin, oat fiber and psyllium in chicken sausages, and which also did not change gumminess. Wongkaew et al. (2020) observed that higher amounts of pectin from mango peel in Chinese sausages resulted in lower values of gumminess. However, these values were not significant ($p>0.05$). In ANOVA for CCRD for the concentrations of BC and fat in the gumminess parameter of sausages, the calculated F value (4.0378) was lower than the table F value (4.5337), and thus the proposed model was not validated ($p >0.05$).

Color is an important factor for the quality of meat and meat products, being decisive in the purchase of these products by consumers (Xue et al., 2022; Gao et al., 2022). Table 4 shows the values obtained in the experimental tests for the variable color.

The influence of CB and fat concentrations on sausage luminosity resulted in a regression coefficient (R^2) of 0,96, indicating that mathematical model can explain 96% of the experiment variance. The independent variables BC and fat had a significant impact ($p<0.05$) on the luminosity (L^*) of the sausages. In their study, Yu & Lin (2014) found that the addition of nata to Frankfurt-type sausages resulted in a slight increase in L^* value. Kumar (2019) reported that replacing animal fat with vegetable oils results in meat products with higher L^*, as vegetable oil globules have greater reflectance than animal fat globules. In ANOVA for CCRD for the concentrations of BC and fat in the L^* parameter of the sausages, the calculated F value (31.9929) was lower than the table F value (4.5337), so the proposed model ($p >0.05$) and the construction of the graph could be validated. Figure 3 shows that higher L^* values tend to be obtained with 4% fat and 8% BC, and these results are represented by tests of the central points (9, 10 and 11). However, Lago et al. (2017) performed acceptability test for tilapia MSM sausages and found a lower acceptance in lighter colored sausages. Furthermore, in their study, Yu & Lin (2014) found that the sausage with the highest percentage of BC had the lowest score in the sensory evaluation of color. Therefore, from a sensory point of view, it would be interesting to choose the test with the lowest luminosity value, that is, close to the points of 2.34% BC and 1.17% fat (Figure 3), which represents

Table 4. Values obtained in the experimental tests for the variable color response investigated in relation to BC and fat.

Essay	Independents variables		Dependents variables		
	BC (%)	Fat (%)	L*	C*	h°
1	2.34	1.17	69.72 ± 0.29	14.88 ± 0.38	83.03 ± 0.25
2	2.34	6.83	70.36 ± 0.14	14.83 ± 1.01	87.74 ± 0.12
3	13.66	1.17	71.58 ± 0.26	15.28 ± 1.29	82.67 ± 0.65
4	13.66	6.83	72.20 ± 0.37	15.53 ± 0.63	86.78 ± 0.85
5	0	4	69.30 ± 0.30	14.56 ± 1.71	88.55 ± 0.10
6	16	4	71.30 ± 0.62	14.96 ± 0.67	87.40 ± 0.64
7	8	0	68.83 ± 0.88	15.09 ± 3.15	85.00 ± 0.51
8	8	8	74.22 ± 0.23	13.42 ± 0.67	75.37 ± 0.36
9	8	4	75.16 ± 0.32	14.20 ± 2.83	85.06 ± 0.87
10	8	4	75.39 ± 1.01	14.20 ± 2.21	84.70 ± 0.79
11	8	4	75.34 ± 0.33	13.83 ± 0.17	84.83 ± 0.21

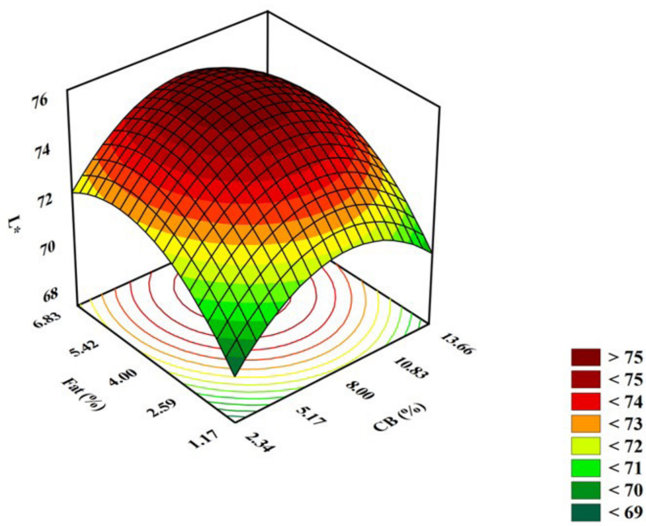

Figure 3. Response surface of luminosity (L*) as a function of BC and fat.

test 1. This result corroborates the studies of Auriema et al. (2022) who explain that low luminosity is expected in meat products with reduced fat content, since light reflectance occurs through fat globules.

According to Signore et al. (2021), the parameter C*, defined as Chroma, is considered the quantitative attribute of color, representing color intensity. The higher the Chroma values, the greater the color intensity of samples perceived by humans. The independent variables had no significant effect ($p>0.05$) on the C* parameter. In ANOVA for CCRD, the calculated F value (1.6599) was smaller than the table F value (4.4590), and therefore the proposed model was not validated ($p>0.05$).

As in the C* parameter, there was no significant effect ($p>0.05$) for the investigated range of the independent variable BC and fat. Therefore, in the present study, the parameters were not affected by variations in concentrations in the investigated range of starch and FRA, and hence a mathematical model cannot be established.

Although the study presents satisfactory results, it is suggested that complementary sensory studies be carried out in order to corroborate the results found (Vidal et al., 2020; Paglarini et al., 2020).

4 Conclusion

The concentration of the variables fat and bacterial cellulose significantly influenced ($p<0.05$) the parameters of cohesiveness, adhesiveness and the parameter of luminosity (L*) of tilapia MSM sausage. To obtain better values of cohesiveness, test 2 was chosen. Regarding the adhesiveness parameter, tests 1 and 2 showed the best behavior. For luminosity, although the central point tests represented the best results of the response surface graph, they are not interesting from the sensory point of view. Therefore, the test chosen in this parameter was also test 2. Therefore, the formulations that showed the best characteristics were tests 1 and 2.

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