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Effect of the addition of resistant starch in sausage with fat reduction on the physicochemical and sensory properties

Mariana de Souza Leite GARCIA-SANTOS[1], Flaviana Sales CONCEIÇÃO[1], Flávia VILLAS BOAS[1], Bruna Maria SALOTTI DE SOUZA[1], Andrea Carla da Silva BARRETTO[1]*

Abstract

Sausage is one of the meat products most consumed in Brazil, although the incorporation of fat is necessary for its elaboration, influencing its technological and sensory characteristics and its caloric value. The aim of this study was to evaluate the addition of resistant starch as a fat substitute in sausage on physicochemical properties and sensory acceptance. The analyses performed were the centesimal composition, emulsion stability, instrumental colour, texture profile analysis and sensorial acceptance. The resistant starch was evaluated for thermal properties, demonstrating that it required a high temperature for gelatinization, indicating that there was probably no gelatinisation of the starch in the cooking of the sausages. There was a significant difference between the treatments T_1 and T_2 with partial fat reduction and caloric value reduction. The study showed that the partial fat reduction positively influenced the reduction of caloric value, emulsion stability, colour parameters and texture profile analysis. All treatments were well accepted by the consumers. The incorporation of resistant starch in sausages did not influence the centesimal composition, texture profile analysis and sensory analysis, showing it to be a promising ingredient in the making of healthier meat products.

Keywords: sausage; resistant starch; fat substitute.

Practical Application: The use of new technologies in the food industry aims to develop new formulations and healthier products for the consumer. In this way, the partial reduction of fat and the resistant starch addition in the preparation of emulsified meat products presents a viable alternative for the production of sausages, contributing to the reduction of calories, acting positively in the manufacturing process and its sensory acceptance.

1 Introduction

Sausage is a popular meat product in Brazil with a *per capita* consumption of 10 kg/inhabitant (Almeida, 2015) and it is notable for its sensory characteristics, practicality and speed in preparation (Park et al., 2012; Cabral et al., 2014). Sausage is obtained from meat emulsion of one or more animal species with some added ingredients. It can be embedded with natural or artificial wrapping or by extrusion process and subjected to a suitable thermal process, having the maximum fat value of 30% and the minimum protein requirement of 12%, as legally permitted (Brasil, 2000).

In Brazil, the fat concentration in sausages can influence the consumption of this meat product (Weiss et al., 2010) because the consumers are in search of healthier and more functional food (Jiménez-Colmenero et al., 2010; Hygreeva et al., 2014; Kılıç & Özer, 2017). Furthermore, high fat consumption has been associated with cardiovascular diseases, obesity, cancer and hypertension, among other illnesses (Mapiye et al., 2012; Hygreeva et al., 2014).

The development of emulsified products with fat substitution or reduction has been studied as one way of meeting such demand, with the incorporation of ingredients to reduce calories to influence the functional properties of the final product (Yang et al., 2001; Ritzoulis et al., 2010; Schmiele et al., 2015; Zhao et al., 2018; Abbasi et al., 2019). The use of new ingredients as fat substitutes helps with water retention capacity, improves fat functionality, maintains the acceptance of sensory attributes such as appearance, odour, flavour and texture parameters. They also contribute to the challenge of making products that are less caloric, have less fat and have healthier ingredients (Choe et al., 2013; Méndez-Zamora et al., 2015; Zhao et al., 2018; Abbasi et al., 2019).

Several studies have shown the possibility of replacing fat with dietary fibre in meat products with good sensory acceptance (Huang et al., 2011; Grizotto et al., 2012; Schmiele et al., 2015; Talukder, 2015; Barretto et al., 2015; Borrajo et al., 2016; Carvalho et al., 2017; Bis-Souza et al., 2018; Zhao et al., 2018; Abbasi et al., 2019), increasing the intake of this component in the diet. These ingredients can contribute giving technological benefits in meat products in order to improve their nutritional and sensory characteristics (Méndez-Zamora et al., 2015; Paglarini et al., 2018).

The application of soluble and insoluble dietary fibres has been studied both individually and in combination with other ingredients in formulations of emulsified meat products for fat reduction (Ktari et al., 2014; Barretto et al., 2015; Méndez-Zamora et al., 2015; Borrajo et al., 2016; Zhao et al., 2018;

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[1] *Departamento de Engenharia e Tecnologia de Alimentos, Universidade Estadual Paulista – UNESP, São José do Rio Preto, SP, Brasil*
*Corresponding author: andreasb@ibilce.unesp.br

Abbasi et al., 2019). The use of resistant starch in emulsified meat products may represent an alternative to help with fat reduction, since it behaves similarly to dietary fibre and is not absorbed in the intestine of healthy individuals (Englyst et al., 1993; Roberfroid, 2007; Viuda-Martos et al., 2010; Peng & Yao, 2017) and does not supply glucose to the body. It can reach the colon and be fermented by intestinal microbiota bacteria, producing short chain fatty acids and other organic acids (Fuentes-Zaragoza et al., 2010; Haenen et al., 2013). A number of studies have shown the positive effects of resistant starch in cases of obesity (Bodinham et al., 2010), cardiovascular disease (Morita et al., 2005), diabetes (Zhou et al., 2014) and colon cancer (Yin & Zhao, 2017; Panebianco et al., 2017; Cray et al., 2017).

The commercially available resistant starch is tasteless, white in colour, it presents high gelatinisation temperatures, has water binding capacity and the ability to replace functional characteristics of fat in meat products (Biswas et al., 2011). Thus, the sensory characteristics of products with resistant starch added can be better when compared to those of traditionally high fibre products (Sajilata et al., 2006) or other carbohydrates.

The present study aimed to evaluate the effect of resistant starch application in sausages with partial replacement of fat on the physicochemical and sensory properties.

2 Materials and methods

2.1 Material

Raw materials

The ingredients were supplied and the sausage treatments produced at the New Max Industrial's pilot plant located in the city of Americana, SP, Brazil. The resistant starch (type 3) incorporated in the treatments was donated by Ingredion, located in Westchester, Illinois, USA.

Sausage processing

The sausages were produced at the New Max Industrial pilot plant. The meat raw materials (beef, bacon and mechanically deboned chicken meat) were refrigerated at -4 °C and ground in a MEW 613 3 mm grinder (Mado, Dornhan Germany). The MDCM was cut with an SL bandsaw (Metalúrgica Siemsen, Brusque, Brazil) and ground in a 6 mm grinder. All ingredients were pre-weighed for preparation of the treatments with the formulae described in Table 1.

Table 1. Different formulations of sausage treatments.

Ingredients	T_0*	T_1*	T_2*
Beef (%)	38.0	38.0	38.0
Mechanically deboned chicken meat (%)	27.3	27.3	27.3
Cure salt (%)	0.2	0.2	0.2
New Mix condiment (%)	1.3	1.3	1.3
Refined salt (%)	1.4	1.4	1.4
Pork back fat (%)	15.0	11.25	7.5
Water and Ice (%)	15.0	15.75	19.5
Cassava starch (%)	2.0	1.0	1.0
Ingredion™ Resistant Starch (%)	0.0	4.0	4.0

*T_0: treatment without resistant starch addition; T_1: treatment with 25% reduction of pork back fat and the addition of 4% resistant starch; T_2: treatment with 50% reduction of pork back fat and the addition of 4% resistant starch.

For all treatments, the following were added: 38% of beef, 27.3% of mechanically deboned poultry meat, 0.2% of curing salt (94% salt, 6% sodium nitrite) and 1.3% of New Max sausage mixed spices and 1.4% of salt.

All the ingredients for the treatments were added to an MTK 662 stainless steel cutter (Mado, Dornhan, Germany) with a capacity of 10 kg until a homogeneous batter was obtained, with a temperature control of 12 °C, in order to maintain the batter at an ideal temperature for the sausage processing.

The batter was transferred to an EM 20 hydraulic sausage filling machine, (Mainca, Barcelona, Spain) and embedded in cellulose gut casing with a 25 mm gauge and twisted into 12 cm long links. After that, the links were placed on skewers to be cooked in a Unimatic 2200 steam oven (Eller, Brazil) until the internal temperature of the product reached 72 °C. The treatments were cooled in a cold water shower for a period of 5 minutes in order to stop cooking inside the product (40 °C). They were then stored in 4 °C refrigeration chambers. The treatments were submitted to manual dewatering for the removal of the cellulose casing and packed in polyethylene nylon vacuum bags at a temperature of 4 ± 1 °C until the physicochemical and sensory analyses which were performed in triplicate at the Department of Engineering and Food Science of the Institute of Biosciences, Languages and Exact Sciences, UNESP.

In order to verify the repeatability of the process, triplicates of the processing were performed for each treatment.

2.2 Methods

Resistant starch: thermal properties

The gelatinisation temperatures and enthalpy changes of the resistant starch were determined using a Pyris1 differential scanning calorimeter (DSC) (Perkin Elmer, USA) as described by Franco et al. (2002).

Physicochemical characteristics of sausages

The moisture, protein, lipid and ash contents of the three sausage treatments were determined in triplicate at room temperature (25 °C) following the Association of Official Analytical Chemists' methodology (Association of Official Analytical Chemists, 2005). The moisture was determined in direct oven drying at 105 °C. For the protein content, the Kjeldahl method (N x 6.25) was used. The lipid content was determined according to Bligh & Dyer (1959). For the ash content, the samples were incinerated and the carbohydrate content was calculated by the difference. The results of all analysis were expressed as percentages. The caloric value of the sausage treatments was determined using Chemin & Mura's (2008) calculation, where the content of the protein nutrient, carbohydrates and lipids corresponds to 4 kcal, 4 kcal and 9 kcal respectively.

The pH was evaluated using a PG1800 pH meter (Gehaka, São Paulo, Brazil) with a drill electrode inserted directly into the sample at room temperature (25 °C).

Instrumental colour analysis

The instrumental colour analysis was determined using a Color Flex45/0 spectrophotometer *(*Hunterlab, USA*)*, the universal software version 4.10 with the D65 illuminating and 10° observer configurations. The absolute values of the rectangular coordinates L* (Luminosity), a* (Intensity of red) and b* (Intensity of yellow) allowed the calculation of the cylindrical coordinates. Twelve sausages cut in lengthways format at room temperature (25 °C) were analyzed for each treatment.

Emulsion stability

The emulsion stability was determined according to the method described by Jiménez-Colmenero et al. (1995) with some modifications where approximately 50 g of batter were placed in sealed tubes and centrifuged for 5 minutes at 2 °C, after which they were submitted to heat at 40 °C for 15 minutes and after that, the heat was increased to 70 °C for 20 minutes. The exudate liquid was measured for the "emulsion break" evaluation and the results were expressed as a percentage (%).

Texture profile analysis

The texture profile analysis (TPA) of the sausages was carried out in a TA.XT.Plus/50 texturometer (Stable Micro Systems, Godalming, UK) previously calibrated with a standard weight of 5 kg. The sausages were pre-cut in 20 mm lengths in order to be inserted into the equipment. A 25 mm aluminium probe with a speed of 5 mm/s and a 13 mm platform distance was used, which compressed 50% of the sample axially in two consecutive cycles, a total of totalling 11 samples for each treatment were tested, at a temperature of 25 °C, according to Bourne et al. (1978). The data collection and the construction of the TPA curves were performed by the Texture Exponent 32 program (Stable Micro Systems, Godalming, UK). The parameters determined were hardness, cohesiveness, springiness and chewiness. The hardness was defined by the peak force during the first compression cycle. Cohesiveness was calculated as the ratio between the second and first peak areas (A2/A1). The springiness was defined between the peak and the time from the beginning of the first area to the first peak (b/a). The chewiness was obtained by multiplying hardness × springiness × cohesiveness.

2.3 Sensory analysis

The sensory acceptance test of the sausage treatments was carried out at the Sensory Analysis Laboratory of the Department of Engineering and Food Science at the Institute of Biosciences, Languages and Exact Sciences, UNESP. The study was approved by the Ethics Committee in Research from the same institution with Opinion nº 864.959.

Seventy potential sausage consumers were recruited to evaluate the treatments. The sensory analysis was performed the day after the manufacturing process of the sausage treatments. The samples were prepared according to the recommendation of the manufacturers of commercial sausages and were served in circular format with a 3 cm thickness and presented to consumers in white plastic cups, coded with random three digit numbers, presented in monadic form and in individual cabins illuminated with white light.

The treatments were evaluated as the sensory attributes of appearance, colour, odour, taste and overall acceptance using a structured hedonic scale of nine points (1=I highly disliked it, 9=I liked it a lot) and, for purchase intention, a 5 point scale was used 1=certainly would not buy; 5 = certainly would buy) (Stone & Sidel, 2004).

2.4 Statistical analysis

The results obtained on physicochemical properties (composition, instrumental color, texture profile analysis) and from the sensorial test were expressed as the mean values and the standard error of the mean. These data were analyzed statistically using mixed model ANOVA analyses and the means were compared using the Tukey test ($p < 0.05$).

All results were submitted to principal component analysis (PCA) to investigate correlations between them. The means of the variable were inserted in columns (dependent variables) and the different treatments of sausages in rows (cases), and the data were standardized before the analysis, applying a correlation matrix without factor rotation. Statistical analyses were performed using Statistic© v. 7.0 (StatSoft, Tulsa, USA).

3 Results and discussion

3.1 Thermal properties

Table 2 presents the thermal properties of the resistant starch used.

The resistant starch presented a high gelatinisation temperature range showing that there was no gelatinisation of the starch granules in the sausage processing, which would lead to loss of resistance of this starch. Cassava starch is the starch most used in sausage formulations in Brazil, where the gelatinisation temperature for cassava starch is between 67.1 to 70.1 °C (Gomand et al., 2010). Studies carried out with eight different cassava starch genotypes showed a gelatinisation temperature range between 72.7 and 78.3 °C (Rolland-Sabaté et al., 2013).

3.2 Physicochemical characterization

Table 3 shows the results of the centesimal composition, emulsion stability, instrumental colour and texture profile analysis (TPA) of the different sausage treatments.

The moisture levels between treatments presented a significant difference ($p < 0.05$), where the contents varied between 58.5 and 62.8%. T_2 presented higher moisture value amongst treatments possibly due to the higher amount of water added in the formulation. All treatments have a moisture content within the

Table 2. Resistant starch - thermal properties.

Sample	T_0* (°C)	T_p* (°C)	T_f* (°C)	ΔH* (J/g)
AR	110.9 ± 1.0	127.1 ± 0.6	142.3 ± 0.1	8.53 ± 0.2

*T_o: initial temperature; T_p: peak temperature; T_f: final temperature; ΔH: enthalpy variation. Averages ± standard deviation. n=3.

Table 3. Sausage treatment's centesimal composition, instrumental colour and texture profile analysis.

	T_0*	T_1*	T_2*	p	SEM[4]
Centesimal composition					
Moisture (%)[1]	58.5[c]	60.9[b]	62.8[a]	0.0000	0.6220
Lipids (%)[1]	19.5[a]	17.6[b]	15.4[c]	0.0031	0.6403
Protein (%)[1]	12.9	11.9	12.1	0.0469	0.1857
Ash (%)[1]	3.5[a]	3.3[b]	3.3 [b]	0.0006	0.0343
Carbohydrates (%)	4.0	8.7	6.5	-	-
Calories (kcal/ 100 g)	242.7	240.7	212.6	-	-
pH[1]	6.10[c]	6.16[b]	6.21[a]	0.0007	0.0177
Emulsion stability (%)[1]	99.5	98.8	99.1	0.5060	0.2309
Colour parameters					
L*[2]	51.1[b]	54.0[a]	50.8[b]	0.0000	0.2842
a*[2]	13.3[a]	12.4[b]	13.2[a]	0.0000	0.0851
b*[2]	13.6[a]	13.4[b]	13.7[a]	0.0025	0.0346
Texture profile analysis					
Hardness (N)[3]	9.7[ab]	11.9[a]	8.7[b]	0.0271	0.5081
Cohesiveness[3]	0.7[a]	0.5[b]	0.7[a]	0.0002	0.0017
Springiness[3] (mm)	0.8[b]	0.8[a]	0.8[a]	0.0012	0.0071
Chewiness (N.mm)[3]	5.2	5.2	4.5	0.4541	0.2547

*T_0: treatment without resistant starch addition; T_1: treatment with 25% reduction of pork back fat and the addition of 4% resistant starch; T_2: treatment with 50% reduction of pork back fat and the addition of 4% resistant starch. [1]n=3, Carbohydrate content was calculated by the difference; [2]n=12; [3]n=11; [4]SEM - Standard error of measurements. Averages with different letters in the same line indicate statistical differences ($p \leq 0.05$) by the Tukey test.

limit established by the Brazilian legislation – 65% (Brasil, 2000). A study carried out with the substitution of bacon by wheat fibre in proportions of 10, 15 and 20% for sausages of the Frankfurter type presented values above those reported in this study, with moisture content between 58.14 and 68.13% (Choe et al., 2013). Similar values are reported by Méndez-Zamora et al. (2015), who stated that the addition of inulin and pectin fibres in Frankfurter type sausages influenced the increase in moisture due to the water retention capacity of the fibres.

A significant reduction in the percentage of lipids was observed for T_1 (17.6%) and T_2 (15.4%) compared to T_0 (19.5%), showing that the reduction of the addition of back fat by 25% and 50% for T_1 e T_2, respectively, influenced this result. The results of this work corroborate the study by Choe et al. (2013), stating that the lipid content decreased as the highest percentage (20%) of wheat fibre was added in Frankfurter type sausages. Thus, it is possible to affirm that the reduction of 25% of pork back fat in T_1 and 50% in T_2 contributed to the decrease in the caloric value of the sausage treatments which were 240.7 and 212.6 kcal/100 g of sausage respectively. Choe et al. (2013) demonstrated that the caloric value of Frankfurter type sausages was influenced by fat reduced and added wheat fibre.

There was no significant difference for the protein content ($p \leq 0.05$) amongst treatments and values ranged between 11.9 and 12.9%.

The percentage of carbohydrates found in the present study showed values that varied between 4.0 and 8.7%, taking into account the values allowed by the Brazilian legislation (Brasil, 2000). The presence of resistant starch as a fat substitute increased the carbohydrate content in the treatments, with T_1 having the highest carbohydrate value (8.7%) and considering that cassava starch was not the only source of carbohydrate used in the formulations. According to Daguer et al. (2011), sausage products have added starch used as binders, although these ingredients should be classified as fillers due to their cost reduction in the manufacturing process. They also help in the product's water retention, consequently decreasing the meat concentration of the product.

There was a significant difference for the ash content ($p < 0.05$) between treatments where the contents varied between 3.25 and 3.45%. Similar values of ash content were described by Borrajo et al. (2016) in sausages with added wheat fibre.

The addition of resistant starch in sausages increased pH values ($p \leq 0.05$) where the average varied from 6.10 to 6.21. The addition of resistant starch and the partial reduction of fat in sausage did not influence the emulsion stability, ($p \leq 0.05$). Possibly the other raw materials used contributed to a positive result in emulsion stability. Choe et al. (2013) reported improve yield by adding three different levels of wheat fibre (10, 15 and 20%) in Frankfurter type sausages. A proportional inverse relationship with cooking loss was observed, explained by the high water and fat retention capacity present in the fibres.

The partial reduction of fat was approximately 10% and 21% for T_1 and T_2, respectively. According to the Brazilian legislation, sausages made with beef, pork or poultry must have the following chemical composition: maximum moisture of 65%, minimum protein of 12% and maximum lipid of 30% (Brasil, 2000).

Regarding the luminosity values (L*), these vary in the scale, from 0 to 100, indicating the light reflectance. The higher the values, the lighter the colour of the sausages (Ramos & Gomide, 2017). The values of luminosity (L*) in the treatments varied between 50.8 and 54.0. T_1 was the lightest value possibly due to the raw material used. T_2 presented a value similar to T_0 showing that the resistant starch did not influence this parameter. The colour parameters of sausages with added resistant starch were also determined by Sarteshnizi et al. (2015) who obtained values for luminosity (L*) of 47.42 when 2% of resistant starch was added.

For the parameter a* (red and green intensity) and for parameter b* (yellow and blue intensity) (Table 3) there was a difference between the treatments but the resistant starch did not compromise this result. Méndez-Zamora et al. (2015) presented similar values to the ones in this study for parameters a* and b* and affirms that the addition of pectin and inulin in sausages influence the red and yellow tonality.

From the TPA (Table 3) it was possible to calculate the texture parameters for each of the sausage treatments. There was no difference for chewiness ($p \leq 0.05$) among the treatments. However, for hardness, cohesiveness and springiness the treatments differed from each other ($p \leq 0.05$). T_1 was the treatment with the highest average for hardness (11.87). In the studies carried out on the elaboration of sausages with added wheat fibre, the authors report that the value of hardness increased due to the presence of 20% added wheat fibre (Choe et al., 2013).

For the cohesiveness parameter, T_1 presented the lowest value for the parameter (0.53). However, the values for this parameter are all low which characterizes that all the treatments are of easy compression and rupture in the teeth. This decrease in hardness and increased cohesiveness was also observed by Sarteshnizi et al. (2015) with the addition of resistant starch in sausages. Values below those found in this study were determined by Choe et al. (2013), with values ranging from 0.27 to 0.34 for cohesiveness in sausages with the addition of wheat fibre and reduction of back fat.

In the springiness parameter, treatments T_1 and T_2 had different values ($p \leq 0.05$) from T_0 as the latter was the sample with the highest springiness. Méndez-Zamora et al. (2015) reports the positive effect on springiness with the addition of inulin and pectin in Frankfurter type sausages, and attributed the increase in the springiness in sausages to the addition of dietary fibres and their functional capacity as a substitute for fat by water retention.

Table 4. Sensory acceptance for sausages treatments.

Attributes	T_0*	T_1*	T_2*	p	SEM
Appearance	6.7	6.7	6.7	0.000	0.1035
Colour	6.7	6.4	6.6	0.000	0.1041
Odour	7.2	7.1	6.9	0.000	0.0896
Flavour	7.3	6.8	6.8	0.000	0.1166
Texture	7.4	6.8	7.0	0.000	0.1030
Overall acceptance	7.2	6.8	6.9	0.000	0.0966

*T_0: treatment without resistant starch addition; T_1: treatment with 25% reduction of pork back fat and addition of 4% resistant starch; T_2: treatment with 50% reduction of pork back fat and addition of 4% of resistant starch. SEM - Standard error of measurements. There was no significant statistical difference ($p > 0.05$).

3.3 Sensory analysis

The appearance, colour, odour, flavour, texture and overall acceptance attributes of the sausages treatments are presented in Table 4.

The results of the sensory acceptance did not present any significant difference among the treatments ($p \leq 0.05$) for the appearance, colour, flavour, texture, aroma and overall acceptance attributes. Thus, all treatments were well accepted by consumers, contributing to the reduction of fat in sausages. Grizotto et al. (2012) carried out a study with the addition of two types of okara flour in Frankfurter type sausages with partial (1.5%) and total (4.0%) replacement of texturized soy protein and concluded that the addition of okara flour did not influence sensory acceptance. In their research, Choe et al. (2013) worked with Frankfurter sausages substituting bacon with wheat fibre in proportions of 10, 15 and 20%, and all the samples were well accepted.

3.4 Principal component analysis

The principal components analysis (PCA) showed that the first main component explained 62.31% of the data variation and the second main component explained 37.69% (Figure 1), where the two eigenvectors were both important in the explanation of the variance of the data, totalling 100% of the variation observed for sausage treatments and evaluated parameters.

The location of each sausage treatment suggests what the evaluated parameters were and presented greater content or characterization of the sausages of this study. The first main component was explained by the presence of T_2, characterized by the parameters of carbohydrates, moisture and pH positively correlated. For T_1, hardness and luminosity (L*) parameters were positive. For T_0, protein and ashes parameters characterized this

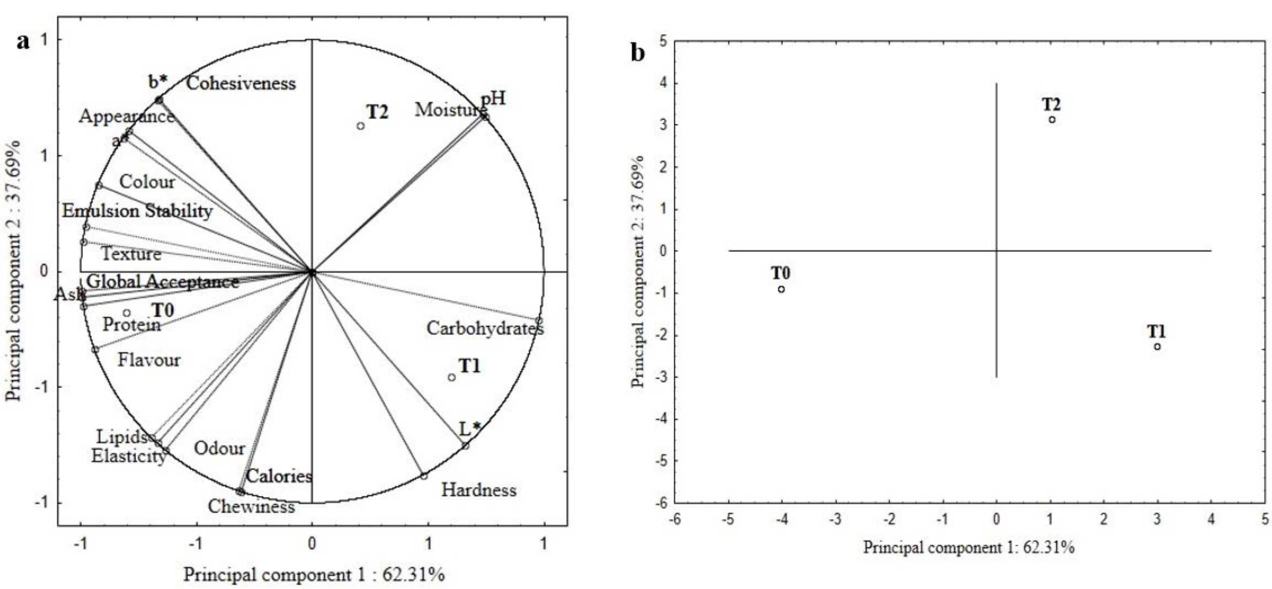

Figure 1. Principal components analysis among physical, sensory, texture profile analysis. (a) variables projection; (b) samples projection. T_0: treatment without resistant starch addition; T_1: treatment with 25% reduction of pork back fat and addition of 4% resistant starch; T_2: treatment with 50% reduction of pork back fat and addition of 4% of resistant starch.

treatment, besides being represented in the component by the overall acceptance. Therefore, the principal component analysis (PCA), corroborates the data presented in Tables 3 and 4.

4 Conclusion

The addition of resistant starch as a fat substitute in sausages did not influence the centesimal composition, texture profile analysis and sensory analysis, indicating it to be a promising ingredient in the making of healthier meat products.

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