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Preparation, physicochemical and microbiological evaluations of italian type salami fermented with kefir

Elaboração, avaliações físico-químicas e microbiológica de salame tipo italiano fermentados a base de kefir

Preparación, evaluaciones fisico-químicas y microbiológicas del salami italiano fermentado a base de kéfir

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Abstract

Industrialized meat product, known as salami, is composed of some types of meat, such as pork, beef or pork and beef together, added ingredients and bacon. This research aimed to elaborate Italian salami using the fermentation process with standard starter bacteria, such as control, water and milk kefir, and evaluate the physicochemical and microbiological characteristics of the final product. The use of kefir grains aimed to analyze the possibility for their use in the production of salami as starter cultures from their symbiotic association of yeasts, acid-lactic bacteria, acetic bacteria and also their probiotic and antimicrobial action. The microbiological and physicochemical composition indicates that kefir is a product with probiotic characteristics, because it has in its composition living microorganisms capable of improving the intestinal microbial balance producing beneficial effects on the health of the individual who consumes it. At the end of the analyses, it was observed that the salami based on standard starter bacteria culture presented greater diversity of fungi, and the fungus *Exophiala spp* was present in the 3 types of salamis. The samples showed absence of pathogenic bacteria such as *Salmonella* ssp, *Shigella* ssp and *E.coli*. There was little variation in the

physical-chemical characteristics of the salamis evaluated. It was concluded that kefir can replace industrial fermentation culture without affecting the microbiological and physicochemical pattern of salami.
Keywords: Innovation; New product; Diversity.

Resumo

Produto cárneo industrializado, conhecido como salame, é composto por alguns tipos de carne como suína, bovina ou suína e bovina juntas, adicionados de ingredientes e toucinho. Esta pesquisa teve por objetivo realizar a elaboração de salames tipo italiano utilizando o processo de fermentação com bactérias starter padrão, como controle, kefir a base de água e de leite, e avaliar as características físico-químicas e microbiológica do produto final. A utilização dos grãos de kefir teve como finalidade analisar a possibilidade para a sua utilização na produção de salames como culturas starter a partir de sua associação simbiótica de leveduras, bactérias ácido-láticas, bactérias acéticas e também a sua ação probiótica e antimicrobiana. A composição microbiológica e físico-química indica que o kefir é um produto com características probióticas, pois possui em sua composição microrganismos vivos capazes de melhorar o equilíbrio microbiano intestinal produzindo efeitos benéficos à saúde do indivíduo que o consome. Ao final das análises foi observado que o salame a base de cultura bactérias starter padrão apresentou maior diversidade de fungos, e o fungo *Exophiala spp* estava presente nos 3 tipos de salames. As amostras apresentaram ausência de bactérias patogênicas como a *Salmonella* ssp, *Shigella* ssp e *E.coli*. Verificou-se pouca variação nas características físico-químicas dos salames avaliados. Conclui-se que o kefir pode substituir a cultura fermentativa industrial sem afetar o padrão microbiológico e físico-químico do salame.
Palavras-chave: Inovação; Novo produto; Diversidade.

Resumen

El producto cárnico industrializado, conocido como salami, se compone de algunos tipos de carne, como carne de cerdo, ternera o cerdo y ternera juntos, agregados con ingredientes y tocino. Esta investigación tuvo como objetivo realizar la preparación de salami tipo italiano utilizando el proceso de fermentación con bacterias iniciadores estándar, como control, kéfir a base de agua y leche, y evaluar las características fisico-químicas y microbiológicas del producto final. El uso de kéfir en grano tuvo como objetivo analizar la posibilidad de su uso en la producción de salami como cultivos iniciadores a partir de su asociación simbiótica de levaduras, bacterias ácido del ácido láctico, bacterias acéticas y también su acción probiótica y antimicrobiana. La composición microbiológica y fisico-químicas indica que el kéfir es un producto con características probióticas, ya que posee en su composición microorganismos vivos capaces de mejorar el equilibrio microbiano intestinal produciendo efectos beneficiosos sobre la salud del individuo que lo consume. Al final de los análisis se observó que el salami basado en un cultivo estándar de bacterias iniciadoras tenía una mayor diversidad de hongos, y el hongo *Exophiala spp* estaba presente en los 3 tipos de salami. Las muestras mostraron ausencia de bacterias patógenas como *Salmonella* ssp, *Shigella* ssp y *E.coli.* Hubo poca variación en las características físico-químicas de los salamis evaluados. Se concluye que el kéfir puede reemplazar el cultivo de fermentación industrial sin afectar el patrón microbiológico y fisico-químico del salami.
Palabras clave: Innovación; Nuevo producto; Diversidad.

1. Introduction

The search of the population for new products for consumption motivates the food industries to commit themselves to the development of new alternatives with greater added value. The meat industry also seeks to be innovating, through new formulations of products with greater health benefits beyond the functional appeal, promoting an increasing demand and demand of the consumer market, thus there is a need for research that gives this support to these industries (Oliveira, et al, 2013; Tonollo, 2016).

Meat products or processed meats are the result of the need to preserve meat since ancient times. Meat, because it has high nutritional value, a high amount of free water and favorable pH is susceptible to microbial development favoring its deterioration. The conventional processes of meat conservation are salting, drying and fermentation, the latter dates back to the Babylonians reaching modern times through salamat (Terra, 2004).

In the fermentation process, microbial cultures are used for the development of the special flavor of the final product, for this we use initiating colonies called starter, these accelerate the maturation process and improve conservation by reducing the pH, developing an acidic flavor characteristic of fermented products, presenting two factors that makes the product differentiated from other sausages, the low moisture content and presence of acids (Leroy & de Vuyst, 2004). As an attractive alternative for the food industry, the total or partial replacement of starter-starter crops with others, for example kefir, can offer sensory, technological and nutritional advantages in addition to contributing to the safety of the elaborated product (Carasi et al, 2014; Muthukumarasamy & Holley, 2007).

The kefir is a probiotic mixture, has its origin from the Caucasian mountains of Russia, resulting from double fermentation: lactic and alcoholic. The beverage, after fermentation contains lactic, formic, succinic and propionic acids, CO_2, ethyl alcohol, in addition to different aldehydes and traces of isoamyl alcohol, acetone and a variety of folates. The final product also consists of a suspension of symbiont microorganisms formed by acidophilic bacteria and yeasts, the association of all substances in the final product have several beneficial health effects presenting characteristics peculiar to kefir (Magalhães et al, 2011; Miguel et al, 2010; Moreira et al, 2008).

For the preparation of kefir, cow's milk is generally used, but the milk of buffalo, goat and sheep can be used, presenting as final product yellow grains composed of a heteropolysaccharide complex called kephyde. Water kefir is also found on the market, which is cultivated with brown sugar or fruit juices, obtaining a microbial composition product similar to the grains grown in milk, these have an ochre and brown coloration, being composed of dextran (Gulitz et al, 2011; Hsin-Hui et al, 2012; Otles & Cagindi, 2003; Weschenfelder, 2011; Witthunhn et al, 2004). Some research shows that kefir consumption improves digestion, as it is able to favor the multiplication of probiotic bacteria in the intestine and because it has biotherapeutic substances, due to these factors, stimulation of immune system cells occurs. (Foligne et al, 2010; Guzel-Seydim et al, 2012; Liu et al, 2002; Moreno de Leblanc et al, 2007; Vinderola et al, 2006; Wróblewska et al, 2009).

Thus, this research aimed to develop Italian type salamis using the fermentation process with standard starter bacteria, such as control, water and milk kefir, and the realization of evaluations, physicochemical and microbiological characteristics of the final product.

2. Materials and Methods

2.1 Drawing of salamis

The experiment was carried out at the Food Technology Laboratory of the Don Bosco Catholic University.

Three formulations of fermented meat were prepared, one control from standard starter culture (0.2%), one with fermentation with water kefir and one with milk kefir. The meat preparations contained 60% pork, 40% beef, and 5g of sodium chloride, 5g of colour fixator, 10g of special condiment for salami and water kefir (0.2%) and milk kefir (0.2%) were added.

The meat was ground in an industrial grinder and the condiments added after grinding. The inlay in synthetic wrap was performed by electric inlay and the salamis were packed in fermentation rooms for 45 days. After the fermentation period, physicochemical and microbiological analyses were performed.

2.2 Physical-chemical analysis

The centesimal composition was performed following the methodology described by AOAC (2002), being determined: the moisture content, by the drying method of the samples until constant weight, in an oven at 105 °C; the ashes in the samples, after complete carbonization in incineration in the muffle at 550 °C until the obtention of a coal-free residue, with

grayish-white coloration; lipids, by the content of soluble substances in ethyl ether, by means of the Soxhlet lipid extractor apparatus; and the amount of protein, by the micro Kjeldahl method, multiplying the value by factor 6.25.

Water activity (aW) and color of ready-made products were analyzed.

Regarding color analysis, we have:

- **L:** luminosity (the maximum value of L is 100, and represents a perfect diffuse reflection, while the minimum value is zero and constitutes black.);

- **a:** red color intensity ("a" has no specific numerical limits. It ranges from red "+a" to green "-a");

- **b:** yellow color intensity ("b" has no specific numerical limits. Ranges from yellow "+b" to blue "-b");

- Δ**E:** total color difference.

2.3 Microbiological analysis

Microbiological analyses were performed according to the recommendation and requirements of RDC no. 12, of January 2, 2001 (Brazil, 2001). The methodology for performing microbiological analyses was based on Normative Instruction Nº 62, of August 26, 2003, from the Ministry of Agriculture, Livestock and Supply, which formalizes the official analytical methods for microbiological analyses for control of animal products and water (Brasil, 2003). The following microbiological analyses were performed: *Salmonella* ssp count, thermotolerant coliforms and *Shiguella* ssp.

2.4 Methodological report for research

The data presented followed the protocols of teaching, research and scientific methodology, as described by Estrela (2018).

2.5 Data analysis

The data obtained were submitted to analysis of variance and their means were compared by the Tukey test, a level of 5% significance. The results were arranged in tables.

3. Results and Discussion

Meat and its derivatives present a compensation system between protein, lipid and moisture levels (Rech, 2010). Table 1 shows the differences in the centesimal composition between salamis produced with standard starter bacteria and with water and milk kefir.

Table 1. Chemical composition of Italian type salamis using fermentation with standard starter bacteria, kefir based water and milk.

Parameters	Standard salami starter	Salami Kefir Water	Salami Kefir Milk	CV	P
Moisture	50.91b	50.45b	53.83A	1,49	<0,0001
Protein	25.22b	25.15b	25.64a	1,46	<0,0001
Lipid	19.90b	20.93a	20.99a	3,06	0,0034
Ashes	5.30A	4.98ab	4.92B	5,17	0,0195

Values followed by equal letters, in the column, do not differ from each other by the Tukey test, at 5% probability. Source: Authors.

Despite the slight increase in humidity among the products, there was no significant difference (p>0.05) in relation to the 3 types of salamis elaborated, with starter culture, kefir water and milk. The treatments obtained statistically equal results for moisture, protein, lipids and ash. Humidity was above the 35% indicated for Italian salami, reaching 50.91% in salami with starter culture, 50.45 for salami with kefir water and 53.83% for kefir milk salami. These moisture results were above the values found in the study carried out by Rech (2010), in which he obtained values between 34.66 and 34.99% in Italian salamis. In the work of Hoz et al., (2004) values for humidity similar to the work performed were obtained, with values between 45 and 46.68%, corroborating the present study.

The values found for lipids were relatively low when compared to the maximum of 35% established by the legislation, ranging from 19.90 to 20.99%. In the study by Rech (2010) a variation between 30.01 and 31.73% was observed, while Nobile et al., (2009) reported values between 23.64 and 29.84%.

The analysis of the protein content of the 3 formulations of salami sat at 25%, according to the legislation. Similar results were reported by Rech (2010), who presented on average 25% protein in Italian salamats. In the studies by Mendonza et al. (2001) obtained 24% protein in the samples of dried fermented sausages.

In Brazilian legislation there is no defined pattern for ashes, so the results obtained presented an average of 4.92 to 5.30%. In the studies by Rech (2010) a variation between 5.48 and 5.93 was observed and Mendonza et al, (2001) found a variation of 5.1 to 7.1% of ash.

The 3 formulations obtained water activity values lower than the maximum level of 0.90, recommended by Brazilian legislation for Italian type salami (Brazil, 2000). Water activity is a determining factor for the shelf life of products, as it makes the environment unfavorable to the growth and multiplication of decaying and pathogenic microorganisms (Macedo et al, 2008). The salami produced with milk kefir showed higher water activity, which is probably associated with higher luminosity and humidity found, but all samples are in the mean evaluated by the Tukey test. (Table 2).

Table 2. Color and water activity of Italian type salamis using fermentation with standard starter bacteria, kefir based water and milk.

Analysis	Standard salami starter	Milk Salami Kefir	Kefir Water Salami	CV	P
L	18.79 b	21.22 a	21.31 a	3,64	0,0002
the	7,15	7,03	6,11	16,67	0,3201
b	2,51	2,71	2,61	26,32	0,8987
Aw	0.77 b	0.83 a	0.80 b	3,93	0,0191

Averages followed by distinct letters, in the line, differ from each other by the Tukey test at 5%. Source: Authors.

After 45 days, at the end of the maturation period, the color of the salami samples was monitored by means of a colorimeter. The analysis of L* values, referring to luminosity, it was observed that there was no significant difference (p>0.05), the color patterns showed higher *L in the salamis produced with kefir, both water and milk, which indicates higher luminosity of the product and may be associated with higher moisture content found in these products. Luminosity is considered a factor of attraction to the consumer, with color being the first attribute observed at the time of purchase (Gao, Li & Liu, 2014). The L* values presented in this study are below the values found by Campagnol et al, (2007) reported in their experiments at the end of the drying process, ranging from 37.22 to 38.70.

In this report, the values found a* and b* with the mean values of 7.15; 7.03 and 6.11 for a* and 2.51; 2.72 and 2.61 for b*, for the formulations of starter culture, kefir milk and water, respectively, did not differentiate between salamis, so all presented similar patterns of red and yellow. In Ruiz's work (2011) it was observed that there was a reduction in the value of a* for some Italian-type salami treatments. The attribution of the event may be related to the effect of lactic acid on the different chemical stages of myoglobin, because this acid can partially or totally denature the heme prosthetic group, because with the oxidation of the nitrosomyoglobin pigment the intensity of red color is observed due to the conversion of this into metamyoglobin (Wójciak et al., 2012). And for the yellow color index in the work of Nobile et al., (2009) higher results were reported for b* 3.46 and 4.91, in this experiment the salape were prepared with pork fat substitutes, however the authors report that there was no great evidence of the influence of this substitution on color parameters, possibly this occurred due to the typical heterogeneity in the color of these products.

Table 3 shows the results of *salmonella* spp., thermotolerant coliforms and *Shigella* ssp for each formulation.

Table 3. Microbiological analysis of Italian type salamis using fermentation with standard starter bacteria, water-based kefir and milk.

Bacteria	**Standard salami starter**	**Kefir Water Salami**	**Milk Salami Kefir**
Salmonella SSP	Absent	Absent	Absent
Thermotolerant coliforms	Absent	Absent	Absent
Shigella ssp	Absent	Absent	Absent

Source: Authors.

In the results obtained, absence of *Salmonella* ssp was observed in 25g of the sample, the presence of thermotolerant coliforms and absence of *Shigiella* ssp were not detected. Among the most common bacteria in processed products, in addition to *Salmonella* ssp, is also *Shigella* ssp (Germano & Germano, 2003; Vieira et al., 2004), which although not mentioned in Brazilian legislation can generate problems of food infections.

These values found in microbiological analyses reveal that the processing of the 3 formulations of italian type salamis were performed with sanitary quality, a fact that is not always found, especially in small factories in Brazil, with disapproval in microbiological characteristics (Dalla Santa, 2008), so the 3 formulations met the microbiological standards regulated through Resolution nº12, of January 2, 2001, of the National Health Surveillance Agency (Brazil, 2001).

Three types of fungi were found in the samples, in which traditional salami samples showed a greater variety of fungi found and the fungus *Exophiala* spp was found in the 3 types of salami, as we can see in Table 4.

Table 4. Analysis of fungi in Italian type salamis using fermentation with standard starter bacteria, kefir based water and milk.

Fungi	Standard salami starter	Kefir Water Salami	Milk Salami Kefir
Exophiala spp	Present	Present	Present
Aspergillus spp	Present	Absent	Absent
Lichtheimia spp	Present	Absent	Absent

Source: Authors.

The genus Exophiala is classified as a dimorphic fungus, composed of leveduriform colonies, presenting brown or black color and with dry aspect (Hoffmann, 2011).

Fungi of the genus Aspergillus are classified as filamentous and can be found in all seasons, in soil, vegetables or decomcomposition matter, thus occurring the dispersion of the coheads, which is the infecting form. The infection caused by the fungus is called aspergillosis, which is considered opportunistic, because patients with immunological dysfunction are more vulnerable than other individuals (Amorim et al., 2014; Kousha, Tadi & Soubani, 2011; Raja & Singh, 2006).

Lichtheimia species grow well in artificial media at temperatures between 30°C and 37°C, and can be found on a wide variety of substrates such as agricultural products, hay and straw, as well as food products, including processed and unprocessed ones such as flours and fermented soybeans (Schwartze & Jacobsen, 2014).

4. Conclusion

It was concluded that the use of kefir as initial fermentation bacteria for salami reached the expected result, being the suitability for use as fermenter, and also making available the probiotic characteristics for the fermented carthon produced product. The physical-chemical analysis showed similarity between the data obtained maintaining standardization between the samples.

The kefir grains of water and milk were able to ferment the bovine product, in the same way as the initial bacteria, usually used for its fermentation. The microbiota that grows in salamas is one of the main responsible for the formation of the characteristics of the final product. The results were satisfactory, resulting in the absence of *Salmonella* ssp, thermotolerant coliforms and *Shigella* ssp. The presence of fungal growth in some samples shows us a negative point, but they are opportunistic fungi, which generally do not cause problems for human health. Thus, for further research, it is suggested the study of sensory analysis of products to be observed the acceptance of final products.

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