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Pea Antipasto Enriched with Probiotic Bacteria: Development and Sensory Acceptance

Antipasta de Guisantes Enriquecida con Bacteria Probiótica: Desarrollo Sensorial y Aceptación

Antepasto de Ervilha Enriquecido com Bactéria Probiótica: Desenvolvimento e Aceitação Sensoria

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Abstract

The incorporation of probiotic bacteria in pea-based products can encourage the consumption of this legume, contributing to the production of functional foods. The objective of this work was to develop pea-based antipasto, enriched with the probiotic *Lactiplantibacillus plantarum*, as well as to evaluate the microbiological quality and sensory characteristics of the product. The prepared antipasto contained peas, olive oil, onion, salt and oregano. After preparation, the addition of probiotic bacteria was performed and the product was kept refrigerated at 8 °C to carry out analyzes of filamentous fungi and yeasts, Enterobacteriaceae and Escherichia coli and sensory attributes of appearance, aroma, flavor and texture by 50 consumers not trained. Analyzes were performed at times 0 and after 14 days of product storage. The results of filamentous fungi and yeasts, Enterobacteriaceae and *E. coli* were satisfactory and the product was safe for consumption. Average scores ranging from 7.0 (I liked it moderately) to 8.0 (I liked it a lot) were attributed to the attributes evaluated in the sensory analysis. The antipasto showed good purchase intention, demonstrating high market potential. The pea is a promising alternative for the elaboration of probiotic non-dairy products with functional appeal, and can be inserted in food preparations.

Keywords: Legume; Appetizer; Lactobacilli; Acceptability; Purchase Intent.

Resumen

La incorporación de bacterias probióticas en productos a base de guisantes puede incentivar el consumo de esta leguminosa, contribuyendo a la elaboración de alimentos funcionales. El objetivo de este trabajo fue desarrollar un antipasto a base de guisantes, enriquecido con el probiótico *Lactiplantibacillus plantarum*, así como evaluar la calidad microbiológica y las características sensoriales del producto. El antipasto preparado contenía guisantes, aceite de oliva, cebolla, sal y orégano. Luego de la preparación se realizó la adición de bacterias probióticas y el producto se mantuvo refrigerado a 8 °C para realizar análisis de hongos filamentosos y levaduras, Enterobacteriaceae y Escherichia coli y atributos sensoriales de apariencia, aroma, sabor y textura por parte de 50 consumidores no capacitados. Los análisis se realizaron en los tiempos 0 y después de 14 días de almacenamiento del producto. Los resultados de hongos filamentosos y levaduras, Enterobacteriaceae y *E. coli* fueron satisfactorios y el producto fue seguro para el consumo. A los atributos evaluados en el análisis sensorial se les atribuyeron puntuaciones promedio que van desde 7,0 (me gustó moderadamente) hasta 8,0 (me gustó mucho). El antipasto mostró buena intención de compra, demostrando alto potencial de mercado. La arveja es una alternativa promisoria para la elaboración de productos no lácteos probióticos con atractivo funcional, pudiendo ser insertada en preparaciones alimenticias.

Palabras clave: Legumbre; Aperitivo; lactobacilos; Aceptabilidad; Intención de Compra.

Resumo

A incorporação de bactérias probióticas em produtos à base de ervilha pode estimular o consumo dessa leguminosa, contribuindo para a produção de alimentos funcionais. O objetivo deste trabalho foi desenvolver antepasto à base de ervilha, enriquecido com o probiótico *Lactiplantibacillus plantarum*, bem como avaliar a qualidade microbiológica e as características sensoriais do produto. O antepasto preparado continha ervilhas, azeite, cebola, sal e orégano. Após o preparo, foi realizada a adição de bactérias probióticas e o produto foi mantido refrigerado a 8 °C para realização das análises de fungos filamentosos e leveduras, Enterobacteriaceae e Escherichia coli e atributos sensoriais de aparência, aroma, sabor e textura por 50 consumidores não treinados . As análises foram realizadas nos tempos 0 e após 14 dias de armazenamento do produto. Os resultados para fungos filamentosos e leveduras, Enterobacteriaceae e *E. coli* foram satisfatórios e o produto é seguro para consumo. Pontuações médias variando de 7,0 (gostei moderadamente) a 8,0 (gostei muito) foram atribuídas aos atributos avaliados na análise sensorial. O antepasto apresentou boa intenção de compra, demonstrando alto potencial de mercado. A ervilha é uma alternativa promissora para elaboração de produtos probióticos não lácteos com apelo funcional, podendo ser inserida em preparações alimentícias.

Palavras-chave: Leguminosa; Antepasto; Lactobacilos; Aceitabilidade; Intenção de compra.

Introduction

With the growing demand for functional foods, research has focused on the development of new products, enabling innovation in the food area and the creation of new market niches (1), such as those containing bioactive compounds and probiotics (2). Thus, the development of probiotic foods should be emphasized due to their proven efficacy and the adaptability of cultures to different food matrices (3). Probiotics are live microorganisms that, when administered in adequate amounts, provide a health benefit to the host (4).

For decades, the probiotic market has focused on dairy products such as yogurt and other fermented products, but with the rise of vegetarianism and lactose intolerance, changes have occurred in this scenario (5). For this reason, raw and fermented vegetables (6; 7) and some legumes have been studied and may represent an excellent vehicle for probiotic bacteria (8; 9). Peas (*Psium sativum*) are the edible seeds of pods, very well known all over the world and, in general, have 20-25% protein, 40-50% starch and 10-20% fiber (10; 11). According to Pinheiro et al. (12), boiled peas have a reference value of 96 calories per 100 g serving. Although this legume is widely used in food and culinary preparations, there are few studies that evaluate the sensory characteristics of pea-based appetizers containing probiotic bacteria, such as antipasto, which are preparations consumed before meals. According to Capillas and Herrero (13), sensory analysis evaluates the properties (texture, taste, appearance, smell, etc.) of a food through the tasters' senses (sight, smell, taste, touch and hearing), and this type of analysis has been used for the purpose of accepting or rejecting food products, complementing technological and microbiological safety in the evaluation of food quality. In this context, the objective of this work was to develop a pea appetizer enriched with probiotic bacteria, contributing to the production of healthy and functional foods, and to evaluate its microbiological quality and sensory acceptability.

Materials and Methods

Preparation of antipasto

The split pea was purchased in 500 g packages and sent to the Vegetable Processing unit of the Food Science and Technology Department, where it was processed. To prepare the antipasto, 62% peas, 4% onions, 2% salt, 2% oregano and 30% olive oil were used. The pea was washed followed by immersion in water, remaining for at least 12 hours at 8 °C, to reduce the level of antinutrients, in addition to providing the grains with softness for processing (14). The onion was peeled, sliced, cooked with the pea for approximately 80 °C/30 min., and the mixture was ground in a domestic processor (Arno, Brazil) for approximately 15 seconds. After crushing, the mixture was added with salt, oregano and olive oil, until the antipasto was completely homogenized. The antipasto was then heat-treated until it reached approximately 80 °C/10 min, and then 80-100 grams of the product were placed in sterile, still warm glass jars, identified as the control treatment.

Inoculation of *L. plantarum* to antipasto

For each 400 g of prepared antipasto, under aseptic conditions, at 40 °C, a capsule of *L. plantarum* LP299V (Jarrow Formulas®) containing 10^{10} cells was added. After the addition of the probiotic, the antipasto were then filled into sterile glass bottles and kept for 24 hours at a temperature of $\pm$ 25 °C and, after this period, the product was stored in B.O.D. at 8 ºC for up to 14 days to carry out microbiological and sensory analyses. The control treatment consisted of antipasto without addition of probiotic bacteria.

Evaluation of the microbiological quality of antipasto

To evaluate the microbiological quality of antipasto, 25 g of both samples (control and containing *L. plantarum*), were homogenized in 225 mL of 0.85% NaCl saline solution (Synth, Diadema, São Paulo, Brazil), with serial dilutions being performed. For filamentous fungi and yeasts, Dicloran Glycerol 18 Agar (Acumedia, Michigan, USA – DG 18) was used, incubating the plates in B.O.D (Novatecnica NT 704, 31 Piracicaba, São Paulo, Brazil) at 25 °C ± 1 °C for 5 days. After this period, the count was performed and the results were expressed in CFU/g, following the methodology described by Beuchat and Cousin (15). Enterobacteriaceae and *Escherichia coli* were determined by the Most Probable Number (MPN) technique according to Kornacki and Johnson (16). Lauryl Sulfate Tryptose Broth (LST) was used for the presumptive test and Brilliant Green Bile Broth (BV) to confirm coliforms at 30 ºC (total coliforms) and EC Broth to confirm coliforms that ferment at 45 ºC (thermotolerant coliforms). The result was expressed in MPN per gram of antipasto. The analyzes were carried out at times 0 and after 14 days of storage at 8 °C. Product shelf life has been established at 14 days, since the product is not added of preservatives, packaged manually, and kept refrigerated. Furthermore, previous studies indicated sensory changes after this period.

Sensory analysis

Prior to the analysis, the project was submitted and approved by the Ethics Committee in Research with human of IF Sudeste MG (CAAE number 57432222.2.0000.5588).

Sensory analysis was performed by 50 consumers who appreciate peas being employees and students of the Rio Pomba campus in individual booths with white light, under controlled conditions. Samples of 10 g of antipasto were served together with crackers and salt, in plastic plates coded with three random digits. Consumers received a form containing a nine-point hedonic scale ranging from "extremely liked" (score 9) to "extremely disliked" (score 1), to assess the overall impression, along with questions related to appearance attributes (pleasant, dark green , light green, homogeneous and unpleasant), aroma (pleasant, mild, unpleasant, characteristic and aromatic), flavor (pleasant, mild, characteristic, spicy, vinegary, bitter and buttery) and texture (pleasant, unpleasant, characteristic, compact, firm and sandy) in order to describe the antipasto.

Most consumers were female (65.5%), aged between 18 and 25 years (62%) and undergraduate students (59.9%). The majority (75.6%) reported liking peas, but they had no habit of consuming (85.1%) the legume.

The intention to purchase the product was based on the general impression of consumers and was assessed using a 5-point Attitude Scale (5 = would definitely buy; 4 = probably would buy; 3 = maybe yes/maybe no; 2 = probably would buy; 1 = I definitely wouldn't buy it). The analyzes were carried out at times 0 and at 14 days of antipasto storage at 8 °C.

Global Impression was evaluated using a randomized block design (RBD) with a factorial scheme, considering the addition or not of probiotics at times 0 and 14 days. The responses to the sensory analysis factors Appearance, Aroma, Flavor and Texture were also evaluated, using dynamic tables and the results of the sums expressed in radar graphs constructed in Microsoft Excel. The data obtained were subjected to analysis of variance (ANOVA) and means compared by Tukey's test at 5% significance. The analyzes were performed using the ExpDes.pt Package (17) for the R software (R-Core Team, 2021).

Results and discussion

Evaluation of the microbiological quality of antipasto

There were $7.0x10^1$ to $9.0x10^1$ CFU/g of filamentous fungi and yeasts and < 1.0 X 10^1 CFU/g of Enterobacteriaceae and *E. coli*, making the appetizer safe for consumption, which reflects good manufacturing practices adopted in the processing.

Sensory evaluation of pea antipasto

For the overall impression of the antipasto, mean scores between 7.0 (I liked it moderately) and 8.0 (I liked it a lot) were obtained (Table 1), which suggests good acceptance and consumer satisfaction. There was no difference ($p>0.05$) between treatments or between the times immediately after preparation (time 0) and after 14 days of refrigerated storage (Table 1).

Table 1: Average results of the evaluation of the global impression of the antipasto and at different times, carried out by the 50 consumers.

Treatments	Global impression
Control antipasto	7,61 a
Probiotic antipasto	7,62 a
C.V. (%)	15,51 %

Times	Global impression
0	7,55 a
14	7,69 a

Means followed by the same letter do not differ from each other by the Tukey test at 5% probability. To describe the antipasto of the control treatment and added of *L. plantarum* at times 0 and 14 days, based on questions related to attributes appearance; aroma; flavor and texture a spider graph was constructed (Figure 1). The center of the graph is considered null or low for a given attribute and, as the points representing the characteristics of each sample

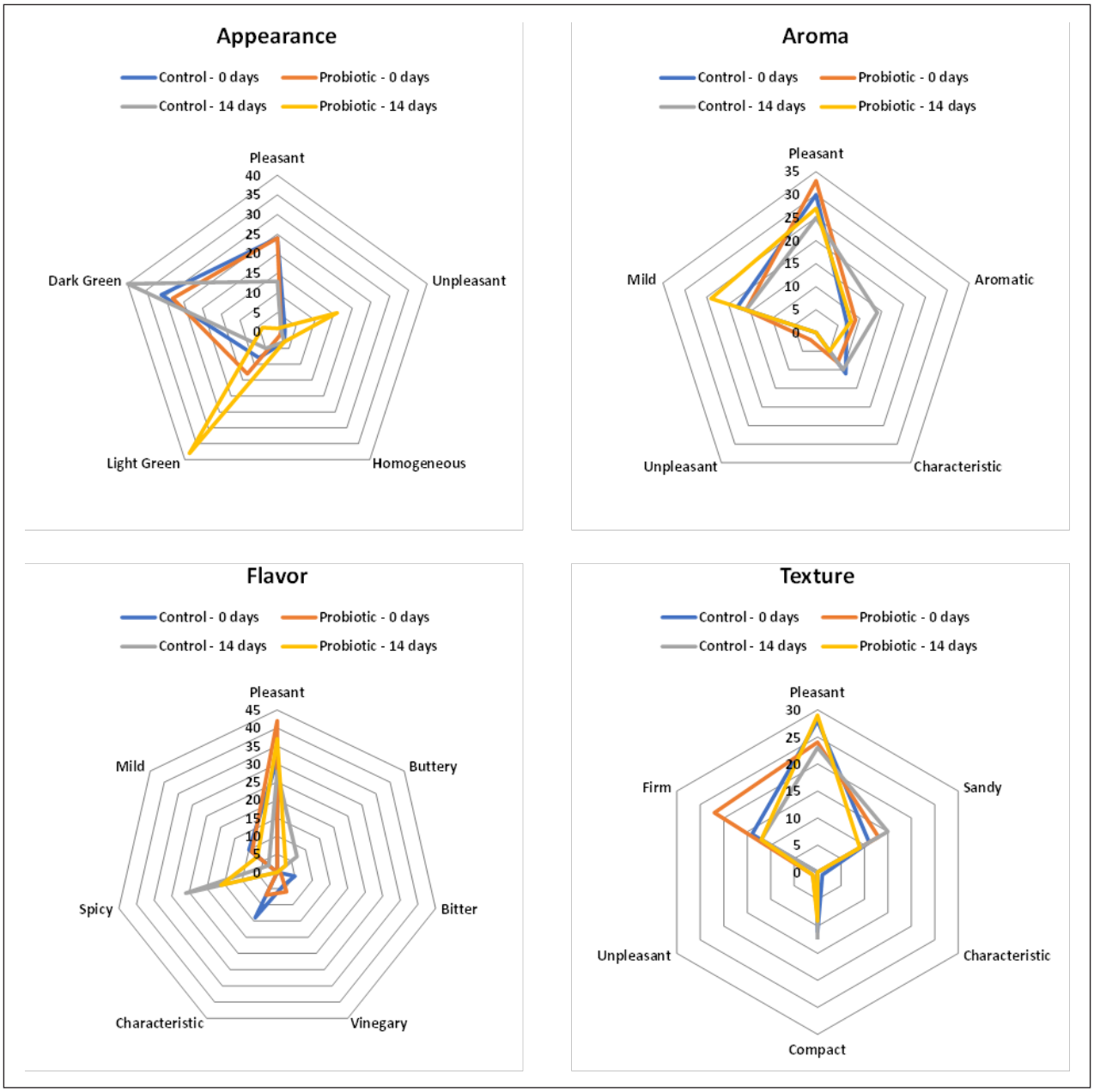

Figure 1: Sensory analysis of antipasto control and added of *L. plantarum* LP299V at times 0 and 14 days. The lines in the graphs represent the number of responses for each of the characteristics related to appearance, aroma, flavor and texture.

approach the ends, the intensity of the attributes increases.

For the appearance attribute, it was found that dark green was evidenced for most appetisers evaluated. However, at the end of the shelf life, the antipasto containing probiotic tended to light green, a characteristic pea color. Some consumers considered the appearance unpleasant at the end of the shelf life (Figure 1), probably due to the storage time promoting a loss of the product's original characteristics, which was visually noticed. Most consumers considered the antipasto aroma pleasant at times 0 and 14 and this attribute was also considered characteristic and mild by some tasters. The taste was also pointed out as pleasant by most tasters, with greater preference for antipasto added with probiotics (Figure 1).

Some tasters identified a spicy flavor in both appetizers, at the end of shelf life, which probably occurred because the spices enhance the product's flavor over time. Regarding to texture, at the beginning of the shelf life, time 0, most tasters attributed a pleasant texture to the antipasto, while at 14 days some considered the texture to be firm, sandy and compact (Figure 1). Thus, there was good acceptability of the product by consumers, highlighting the pleasant characteristic, noticeable in all evaluated attributes.

For purchase intention, both antipasto presented scores of 4.0 and 4.2 after analysis at time 0 and after 14 days, respectively, being between the hedonic terms "probably would buy" and "certainly would buy", on the scale of 5 points, indicating the purchasing attitude of consumers, as well as its high acceptability.

Conclusions

The pea antipasto presented satisfactory microbiological quality and were suitable for consumption. The product was well accepted by consumers and the addition of *L. plantarum* LP299V positively influenced the sensory quality, contributing to the elaboration of a functional appetizer, with a very good purchase intention, indicating that legumes such as peas can be explored in the elaboration of new products from vegetable matrix with market potential.

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