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# Application of Lactic Fermentation Processes for the Use of Vegetable Matrices and Apiculture Products as a Contribution to the Colombian Caribbean Region Economy

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There is a pressing need to prioritize the development of food products that incorporate various functional elements while carefully considering factors such as production costs, food safety, and environmental impact. Colombia has climatic characteristics that allow it to have varied agricultural and beekeeping production, but there is little innovation in the development of value-added products. The beekeeping development in the municipalities of Montes de María has not received the attention it deserves, despite its importance as a sustainable productive dynamic. In this research, fermented drinks were developed using vegetable matrix sesame (Sesamum indicum L.) seeds, pollen, and honey. In lactic fermentation, a decrease in pH was evident, with pH levels between 4 and 4.5, with the addition of pollen. The percentage of lactic acid (g/L) was influenced by the sesame Sesamum indicum L. seed content. The highest value was presented by the drink with the lowest amount of sesame seeds (0.75 g of lactic acid/L). Volumetric productivity, with values between 54.60 % and 82.09 %, compared to other substrates. The sensory evaluation revealed a higher level of acceptability of drinks containing pollen and honey after the fermentation process, thus, the inclusion of pollen and honey improved the attributes of the drinks.

## 1. Introduction

Currently, there is a growing interest in finding techniques to obtain food products that meet the nutritional and quality requirements of consumers. Foods offer the body nutritional advantages and specific impacts on human well-being due to the presence of components that positively affect the physiological functions of the body (Todorov et al., 2022). Consequently, the food category called functional foods is linked to improving health and preventing diseases (Fuentes et al., 2015). Colombia has a diverse climate that allows extensive agricultural and beekeeping production. However, the country has not yet fully exploited the potential of value-added products. In the Caribbean region, the capacity of the peasant population to generate goods, services, fair income, and employment opportunities is limited, which affects the region's economy, which depends largely on livestock and agricultural activities (Alzate Mora, 2020). Despite its importance, beekeeping in the Caribbean needs more attention from stakeholders (Production Chain of Bees and Beekeepers (CPAA), 2020). The honey and pollen extraction is influenced by bee species, climate, floral variety, honey maturation, and pestling techniques (Nunes et al., 2022).
Honey is a solution composed of sugars such as fructose and glucose, along with compounds essential for life such as vitamins, proteins, organic acids, minerals, enzymes, and others. It represents one of the byproducts of the hive (Machado De-Melo et al., 2018). Bee pollen is emerging as an important food product due to its high nutrients concentration and bioactive compounds, a source of proteins, carbohydrates, dietary fiber, lipids, predominantly polyunsaturated fatty acids, and various vitamins and minerals (Basso et al., 2019), In addition,

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it includes notable amounts of phenolic acids, flavonoids, carotenoids, phytosterols and other antioxidant compounds (Mora-Adames et al., 2021).
Fermented beverages are generated through meticulous management of microbial growth and enzymatic modifications of their components (Marco et al., 2017). Recently, these beverages have received renewed attention due to numerous concerns related to dietary approaches and the presence of vitamins, prebiotics, and probiotics (Septembre-Malaterre et al., 2018). Lactic fermentation is carried out by lactic acid bacteria, which can grow in foods due to their metabolic activities (Cante et al., 2022). These bacteria use carbohydrates to generate valuable metabolites, including amino acids, enzymes, vitamins, organic acids, and alcohol (Singh et al., 2017). Lactic fermentation has been carried out using vegetable matrices and mixtures (Ramos et al., 2022). Protein is predominantly found in plant-based seeds, which also have nutrients that offer a source of high-quality fats and easily absorbed carbohydrates, along with fiber, vitamins, and minerals (Väkeväinen et al., 2020).
In the Caribbean region, there is a wide variety of crops native to Colombia, demonstrating the rich agricultural diversity. One of those vegetable crops that stands out for its important annual production is sesame (*Sesamum indicum L.*). Throughout history, Colombia has had a long tradition of growing sesame, particularly in the Caribbean region, with a focus on the departments of Bolívar, Córdoba, Sucre, and Magdalena. This region alone represents more than 60 % of the country's total sesame production (Agronet, 2021).
Bolívar is emerging as the department with the highest concentration of planting and production activities related to sesame seeds, with an area of more than 3,800 ha, followed by the Sucre department with 400 ha dedicated to this crop (Congress of the Republic of Colombia, 2021). Sesame (*Sesamum indicum L.*) is considered a superfood, it contains around 15 essential amino acids, and 80% of the lipid content (polyunsaturated fats), minerals such as bioavailable calcium, iron and zinc which play a role in the assimilation of carbohydrates, proteins and fats (Hernández-Monzón et al., 2019).
The use of bee products and vegetables matrices through the implementation of lactic fermentation procedures as a contribution to the economic growth of the Colombian Caribbean region, the focus is aimed at providing support to the community of farmers on a small scale that often faces challenges in meeting demand due to the high availability of similar products in the market. From a social point of view, research and development of innovative products that incorporate locally sourced and sustainable components should be encouraged. This approach aims to encourage the creation of new investment prospects and avenues of employment for farmers, beekeepers, and people residing in the Colombian Caribbean region.

## 2. Materials and methods

### 2.1 Materials

In this research, sesame (*Sesamum indicum L.*) was used for experimental development acquired directly from peasant farmers in the Colombian Caribbean region, as well as honey and bee pollen provided by beekeepers in the Montes de María re, Colombia. The fermented food product was obtained using the commercial mixed culture of Danisco (Danisco, Denmark), to produce fermented dairy beverages by direct inoculation, Danisco VEGE 053 LYO 200 DCU with *Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus delbrueckii subsp. lactis, Lactobacillus acidophilus (NCFM®), Bifidobacterium lactis* (HN019TM).

### 2.2 Lactic fermentation process and parameter monitoring

The process of obtaining sesame (*Sesamum indicum L.)* beverage is shown in **Errore. L'origine riferimento non è stata trovata.**. It began with cleaning the seeds with plenty of drinking water; subsequently, the seeds were hydrated in a 1:10 ratio for 12 h, then the hydrated seeds went through a grinding process in which they were mixed with water at 100 °C for 0.083 h in an electric blender at maximum speed (Moncada et al., 2019). The above mixture was filtered and pasteurized at a temperature of 75 °C for 0.33 h and then cooled in water to 4 °C, then heated to 40 °C.
The product obtained was homogenized using 0.02 g/kg of the lyophilized commercial culture VEGE 053 LYO 200 DCU, and then mixed for 0.17 h. Then, the mixture was placed in 0.18 L glass bottles, which in turn were placed in MV Power model SNJ-159B electric incubators, for 10 h at 40 °C. After 10 h, the fermented product was homogenized, tempered, packaged, and refrigerated at 4 °C. In the fermentations, two factors were used for the sesame/water ratio (%w/w), which were S1:3 and S1:5. In addition, bee pollen was added to these same ratios in a ratio of 1:100 (% w/w). At the end of the experiments, bee honey was added in a ratio of 10:100 (%w/w), named S1:3P and S1:5P.

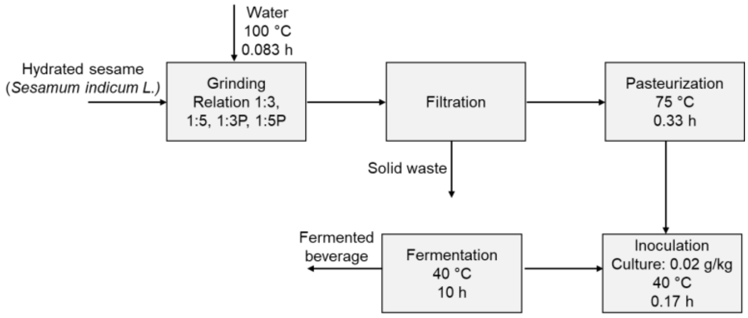


*Figure 1: Diagram of the lactic fermentation process of the sesame-based beverage, P:pollen.*

Fermentation was monitored by taking samples of 0.015 L every 2 h, during which time the pH, titratable acidity (measured as g lactic acid/), and total soluble solids (measured as °Brix) were measured. The pH and volatile acidity were monitored while the fermentation process was carried out using a portable pH meter model HI 9813-6 brand Hanna instrument. The titratable acidity was carried out by the potentiometric method following the guidelines given in the official method AOAC 937.05. The titration was carried out with a previously prepared solution of 0.1 [M] NaOH, using 3 drops of 5 % phenolphthalein as an indicator. For all the samples that were worked, considering that the turning point of phenolphthalein occurs at a pH = 8.2 ± 0.1. Once the tests were completed, the acidity (g/L) of each replicate sample was determined using Eq. (1):

$$\%\,Acid\ lactic\ [g/L] = \left(\frac{V_g \cdot C \cdot F}{V_m}\right) \cdot 100 \qquad (1)$$

Where $V_g$ is the spent volume of NaOH in ml, $C$ is the concentration of NaOH [M], y $V_m$ m is the volume of the sample in ml, and F is the correction factor for lactic acid equivalent. At the end of the experiments, the volumetric productivity was quantified using Eq. (2), where the difference in concentration of lactic acid (g/L) in the samples is calculated between the time it took to carry out the fermentation:

$$Volumetric\ productivity = \left(\frac{x_e - x_s}{t}\right) \cdot 100 \qquad (2)$$

Where $x_e$ and $x_s$ correspond to the lactic acid concentrations at the beginning and end of fermentation, and $t$ is the fermentation time.

### 2.3 Sensory evaluation

The development process of new food products requires the application of sensory evaluation techniques, which have the purpose of acquiring valuable information related to the sensory characteristics and possible acceptability of experimental foods (Cruz et al., 2011). In this research, an analysis of sensory preferences was carried out, focusing specifically on the consumer, to determine the level of acceptance of the manufactured beverages. In addition, a descriptive analysis was carried out focused on the distinctive characteristics of the products. The sensory evaluation was carried out for the samples studied with the addition of honey, for which the letter "H" was added, leaving: S1:3H, S1:5H, S1:3PH, and S1:5PH. The survey was applied to a group of 15 people, randomly selected from the population of Cartagena de Indias in Bolívar, between 18 and 60 years of age. Seven specific attributes were evaluated in the surveys: appearance, consistency, color, characteristic smell, sour taste, sweet taste, characteristic flavor, and general acceptability. To facilitate a more precise evaluation, a quantitative scale was used with a five-point qualitative scale, in which the options ranged from: Like (1), Neither like nor dislike (4), and Dislike (5) (Benavides Martín, 2019).

## 3. Results and Discussion

### 3.1 Analysis of the parameters of the fermentation process

Figure 2 shows the variation of pH with respect to time for samples prepared from sesame (*Sesamum indicum L.*) seeds with different wet grain: water ratios and the addition of pollen. The pH variation was evaluated with respect to the fermentation time, thus in the samples with sesame (S1:3 and S1:5), the pH was between 6.3 and 4.8 in a 10 h fermentation, while in the samples with sesame (*Sesamum indicum L.*) and honey (S1:3P and S1:5P) the pH was between 5.4 and 4.4 in a 6-h fermentation, this makes a potential difference when adding pollen as the initial substrate of the fermentation with an ideal pH is a shorter time. A yogurt-type drink generally has pH values between 4 and 4.5, as for the fermentation time it will depend on the substrates used (Martínez et al., 2019). In the literature have been reported products obtained from rice flour and sesame (*Sesamum indicum L.*) seeds fermented with probiotic cultures using fermentation times between 2.5 to 3 h (Hernández-Monzón et al., 2019), and other substrates such as cereals (oats and rice) pH between 3.11 to 4.7 (Gallo et al., 2020), cooked and crushed mussel between 4.5 and 4.7 (Kitundu et al., 2021).

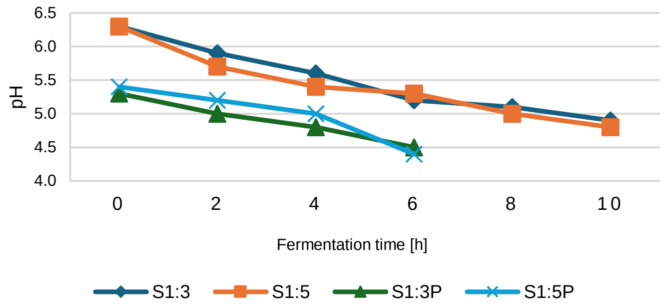


*Figure 2: pH variation with respect to fermentation time*

The monitoring of lactic acid production (% lactic acid g/L) is graphically represented in Figure 3, which shows the gradual increase in lactic acid production over time in the fermentations. When evaluating samples containing sesame (*Sesamum indicum L.*) seeds, it is evident that the production of lactic acid is notably higher in the case of S1:5 (0.75 % g of lactic acid/L), as well as in samples containing both sesame (*Sesamum indicum L.*) seeds as pollen, specifically S1:3P (0.69 % g of lactic acid/L). It is worth mentioning that lactic acid values in yogurt generally exceed the threshold of 1.5 % g lactic acid/L. However, in vegetable fermentations, high values such as 1.25 g/L were discovered (Gallo et al., 2020) with regular levels ranging between 0.5 and 0.8 g/L in both vegetables and fruits (Benavides Martín, 2019).

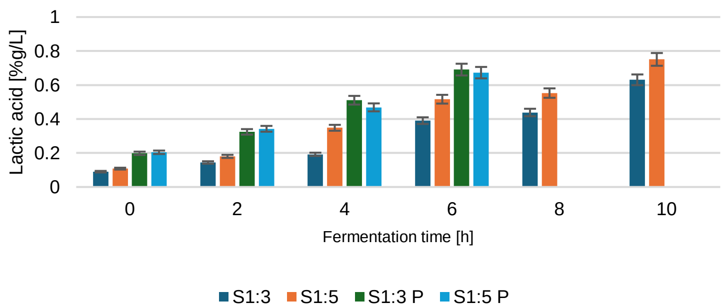


*Figure 3: Lactic acid variation content with respect to fermentation time*

Table 1 provides an overview of the volumetric productivity (% g/L h) for all beverages brewed after 10 hours (beverages containing sesame only) and 6 hours of fermentation (beverages containing sesame and pollen). It is evident that the highest volumetric productivities were achieved in the case of samples S1:3P and S1:5P. It is crucial to note that, although the productivities of samples S1:3 and S1:5 are comparable to the productivity of yogurts made with whey of 54 % productivity, they are lower than the productivity observed in yogurts at milk base (greater than 90 %) (Videa and Videa, 2019).

*Table 1: Volumetric productivity of sesame-based beverage*

| Sample | Fermentation time [h] | Initial concentration Lactic acid [g/L] | Final concentration Lactic acid [g/L] | Volumetric productivity [% g/L*h] |
|---|---|---|---|---|
| S1:3 | 10 | 0.90 | 6.30 | 54.60 |
| S1:5 | 10 | 1.08 | 7.50 | 64.27 |
| S1:3P | 6 | 1.98 | 6.90 | 82.09 |
| S1:5P | 6 | 2.04 | 6.72 | 78.08 |

The data presented in Table 1 clearly demonstrate that the addition of pollen to sesame (*Sesamum indicum L.*) seed mixtures leads to an increase in productivity. Consequently, the mixture with the highest proportion of sesame (*Sesamum indicum L.*) seeds exhibits the highest volumetric productivity, which can be justified by the low pH levels it reaches in a shorter period, thus achieving the desired value expeditiously.

### 3.2 Sensory analysis

The acceptability of beverages based on sesame (*Sesamum indicum L.*), pollen, and the addition of honey was evaluated with the sensory analysis shown in Table 2. The scores obtained for the drinks showed an increasing trend with the reduction of sesame (*Sesamum indicum L.*) seeds content in the mix. Consequently, sample S1:5H presented a higher value with the addition of pollen, which is why sample S1:5PH, showed a higher value, mainly due to the sweet flavor provided by the inclusion of honey. The appearance showed good acceptability (value greater than or equal to 3) (Benavides Martín, 2019). However, it is worth noting that the color imparted by pollen played a crucial role in the evaluation process, as it significantly influenced the perception of the respondents. Furthermore, the consistency, color, and characteristic odor of the drinks were markedly improved in samples containing pollen (S1:3PH and S1:5PH). This resulted in higher viscosity and better physical characteristics compared to samples S1:3H and S1:5H. It is important to mention that samples S 1:3 H and S 1:5 H obtained low scores, mainly due to the detection of a bitter taste. However, this characteristic can be addressed by incorporating other solutions derived from plant matrices (Moncada et al., 2019) or using honey, as has been demonstrated in this research.

*Table 2: Sensory analysis results in sesame-based drinks and bee products*

| Sample | Appearance | Consistency | Color | Characteristic smell | Sour taste | Sweet taste | Characteristic flavor | General acceptability |
|---|---|---|---|---|---|---|---|---|
| S1:3H | 3.25 ± 0.8 | 2.73 ± 0.5 | 2.56 ± 0.7 | 3.21 ± 0.5 | 3.05 ± 0.8 | 2.58 ± 0.8 | 3.52 ± 0.8 | 2.87 ± 0.8 |
| S1:5H | 3.00 ± 0.3 | 2.65 ± 0.9 | 2.31 ± 0.8 | 3.05 ± 0.4 | 2.89 ± 0.7 | 2.75 ± 0.7 | 3.13 ± 0.6 | 3.09 ± 0.8 |
| S1:3PH | 4.05 ± 0.3 | 4.27 ± 0.3 | 3.24 ± 0.5 | 3.45 ± 0.7 | 3.22 ± 0.5 | 3.60 ± 0.3 | 4.15 ± 0.2 | 3.78 ± 0.4 |
| S1:5PH | 3.84 ± 0.6 | 3.49 ± 0.3 | 3.52 ± 0.3 | 3.67 ± 0.3 | 3.56 ± 0.3 | 3.84 ± 0.3 | 4.03 ± 0.4 | 4.02 ± 0.6 |

## 4. Conclusions

The growing demand for functional foods that offer benefits to people's health has become a prominent line of research. Within the Colombian Caribbean region, agriculture is the main driving force of its economy. However, there are numerous underutilized crops from which value-added products have not yet been obtained. In parallel, beekeeping has experienced significant growth in the region, with the emergence of new associations that prioritize bee well-being, environmental sustainability, and food security. In this context, this research project aimed to develop beverages through the lactic fermentation process, using sesame (*Sesamum indicum L.*) seeds and beehive products, such as pollen and honey to contribute to the economy of the region.

The fermentation process monitored pH levels between 4 and 4.5, which took 10 to 6 hours, depending on the specific mixture. Throughout the fermentation period, a visible decrease in pH levels from 6.3 to 4.4 was observed. It is worth noting that the addition of pollen to the drinks caused a more rapid decrease in pH levels, starting from a lower initial pH value. Furthermore, the percentage of lactic acid (g/L) was found to be relatively higher in the S1:5 and S1:3 P samples, underlining the important influence of sesame (*Sesamum indicum L.*) seeds content on this parameter. In terms of volumetric productivity, the beverages showed notable levels, ranging between 54.60 % and 82.09 %, compared to other substrates and even cow's milk. The sensory evaluation carried out revealed a higher level of acceptability of drinks containing pollen and honey after the fermentation process. The inclusion of pollen contributed to improving several attributes, while honey provided the desired sweetness to counteract the acidity produced during fermentation.

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