

# **ENERGY BALLS: INTEGRATION OF BEE POLLEN WITH NATURAL INGREDIENTS FOR A NUTRITIOUS AND FUNCTIONAL FOOD**

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“Renda-se, como eu me rendi. Mergulhe no que você não conhece, como eu  
mergulhei.”

**Clarice Lispector**

## **Abstract**

The growing interest in natural foods has driven the development of products that combine nutritional value, practicality, and sustainability. In this context, this work aimed to develop and characterize formulations of energy balls based on natural ingredients from the Trás-os-Montes region (bee pollen, dried fig, almond flour, honey, and olive oil), focusing on the use of pollen as a protein source. Furthermore, this work was developed under the Bio4Med Project, specifically in the Task entitled "*Olivais de Mel - Honey Olive Groves*", which aims to value beekeeping products obtained from hives located in olive groves. After some preliminary tests, three formulations were developed with different proportions of bee pollen (10%, 20%, and 30%), which were evaluated for physicochemical, microbiological, and sensory parameters. The results showed that only the formulation with 30% of pollen met the criterion of being a protein source, as at least 12% of the energy value of the food was provided by protein. As expected, the formulation with 30% pollen presented the highest protein content. Sensory analyses indicated good acceptance by consumers, especially for the formulation with 10% pollen, which was considered more balanced in terms of flavor and texture. From a microbiological perspective, all formulations were safe for consumption. These data confirm the potential of bee pollen as an innovative ingredient in ready-to-eat foods, expanding its applications in the food industry.

**Keywords:** bee pollen; protein source; ready-to-eat food; Trás-os-Montes.

## Resumo

O crescente interesse por alimentos naturais tem impulsionado o desenvolvimento de produtos que aliem valor nutricional, praticidade e sustentabilidade. Neste contexto, este trabalho teve como objetivo desenvolver e caracterizar formulações de bolas energéticas à base de ingredientes naturais da região de Trás-os-Montes (pólen apícola, figo seco, farinha de amêndoa, mel e azeite de oliva) com foco na utilização do pólen como fonte proteica. Adicionalmente, este trabalho foi desenvolvido no âmbito do Projeto Bio4Med na Tarefa intitulada "Olivais de Mel", na qual se pretende valorizar produtos apícolas obtidos a partir de colmeias colocadas em olivais. Após a realização de alguns testes preliminares, foram desenvolvidas três formulações com diferentes proporções de pólen apícola (10%, 20% e 30%), que foram avaliadas quanto a parâmetros físico-químicos, microbiológicos e sensoriais. Os resultados demonstraram que só a formulação com 30% de pólen atendeu ao critério de ser uma fonte de proteína, uma vez que pelo menos 12% do valor energético do produto foi fornecido pela proteína. Como esperado, a formulação com 30% de pólen apresentou o maior teor proteico. As análises sensoriais indicaram boa aceitação por parte dos consumidores, especialmente para a formulação com 10% de pólen, considerada mais equilibrada em sabor e textura. Do ponto de vista microbiológico, todas as formulações apresentaram-se seguras para o consumo. Esses dados confirmam o potencial do pólen apícola como ingrediente inovador em alimentos prontos para consumo, ampliando suas aplicações na indústria alimentícia.

**Palavras-chave:** pólen apícola; fonte de proteína; alimentos prontos para consumo; Trás-os-Montes.

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# 1. Introduction

In recent decades, there has been a significant increase in public concern regarding health, quality of life, and food sustainability. The rise in non-communicable chronic diseases such as type 2 diabetes, obesity, and cardiovascular conditions has driven the search for dietary strategies that promote a healthier lifestyle (BALASUNDARAM and DALEY, 2025; HUANGFU et al., 2024). In this context, natural and energy-rich minimally processed foods have gained prominence both in the market and in scientific research. Moreover, products developed from ingredients with bioactive properties may provide health benefits, such as antioxidant, anti-inflammatory, and prebiotic effects (BOURKE et al., 2024; ROSENTAL et al., 2021).

According to Bourke et al. (2024), the food industry has been responding to consumer demands for healthy, convenient, and functionally appealing snacks, particularly those focused on protein supplementation, which also contribute to weight control and satiety. As a result, so-called “energy balls” or “snack balls” have emerged as a modern alternative for nutritious, functional, and portable snacks.

The formulation of healthy and sustainable products is closely linked to the valorization of regional ingredients (MATÍAS et al., 2024). In the present study, the selected ingredients include olive oil, known for its antioxidant properties and monounsaturated fatty acids beneficial to cardiovascular health; honey, a naturally energetic food rich in phenolic compounds with antimicrobial and antioxidant actions; dried fig, which adds dietary fiber, minerals, and a characteristic sweet flavor; and almond flour, which offers a healthy lipid profile and high nutritional density (CLEMENTE-SUÁREZ et al., 2023; GIAMPIERI et al., 2022; RIOLO et al., 2022; SANDHU et al., 2023). The combined application of these ingredients in new food products provides not only nutritional value but also cultural significance.

In addition to these ingredients, bee pollen stands out for its high content of proteins, essential amino acids, B-complex vitamins, and antioxidant compounds (KACEMI and CAMPOS, 2023). Although its bioactive properties are well documented, bee pollen is still rarely incorporated into ready-to-eat food products, with its use being more common in yogurts and baked goods (BAKY et al., 2023; KARABAGIAS et al., 2018; SOKMEN, 2022).

Current studies have mainly focused on the incorporation of bee pollen in bread, fermented beverages, or food emulsions. Therefore, the use of pollen as a functional

ingredient in protein-rich snacks, in combination with other ingredients from the Trás-os-Montes region, represents an innovative and promising approach in the context of new product development. This aligns with the goals of the Bio4Med project, which aims to promote the sustainable use of local ingredients with functional value.

## **2. Literature Review**

### **2.1 Energy balls**

The energy balls are sweet, non-baked snacks typically formulated with natural ingredients and designed to offer a compact source of energy and nutrition. Their composition is rich in proteins, carbohydrates, dietary fibers, and unsaturated fats, making them a convenient and functional option for consumption throughout the day. They are perfect for sports both before and after physical activity, because the proteins and carbohydrates help build lean muscle and boost energy (ALSUHEBANI et al., 2025).

The composition of energy balls allows for adaptations based on nutritional needs and dietary restrictions. These snacks typically include a base of dried fruits, nuts, seeds, and whole grains, which contribute essential micronutrients such as vitamins, minerals, and bioactive compounds. Importantly, their no-bake preparation helps preserve heat-sensitive constituents, such as polyphenols and omega fatty acids, which enhances their antioxidant and anti-inflammatory properties (GARG et al., 2023).

Furthermore, the preparation method is simple and preserves the nutritional integrity of these ingredients - energy balls do not need to be baked. As a result, their bioactive compounds, such as polyphenols and fatty acids, remain stable, ensuring greater antioxidant activity and anti-inflammatory benefits.

Skoczek-Rubińska and Bajerska (2021) suggest, in their study, that energy-dense snacks contribute significantly to daily caloric intake and can impact weight management, like these balls that have several ingredients together, it has a high caloric value and can therefore help in weight loss processes and in athletes' diets, as it provides a feeling of satiety and appetite control between meals.

### **2.2 Commercial energy balls**

To analyze the potential nutritional values of energy balls and support the development of new formulations, it is essential to research the current market for this niche. Below is a description of the ingredients and nutritional declaration for commercial energy balls from widely distributed brands in Europe, highlighting their ingredients as shown in Table 1. This analysis helps identify common formulation trends and nutritional targets that are currently valued by both consumers and manufacturers.

**Table 1-** Formulations and nutritional profile of commercial energy balls

<b>Commercial energy balls</b>	<b>Ingredients</b>	<b>Nutritional declaration (per 100g)</b>
Govinda – Fig	Apricots, Dates, Figs, Walnuts, and Hazelnuts	Calories: 309kcal; Lipids: 6.5g (0.6g saturated); Carbohydrates: 58g (54g sugars); Proteins: 3.3g; Dietary Fibers: 11g; Salt: 0.02g
Govinda – Coconut	Coconut, Dates, and Agave	Calories: 596kcal; Lipids: 56g (56g saturated); Carbohydrates: 20g (19g sugars); Proteins: 5.6g; Dietary Fibers: 11g; Salt: 0.02g
Bettery – Apple & Cinnamon	Maltitol, Dates, Almond Butter, Oat flour, Pea protein, Cinnamon, Apple Powder, and Salt	Calories: 413kcal; Lipids: 8.3g (1.7g saturated); Carbohydrates: 45.2g (13.7g sugars); Proteins: 18.3g; Dietary Fibers: 19.7g; Salt: 0.4g
Bettery – Banana & Peanut Butter	Maltitol, Dates, Peanut butter, Oat flour, Chocolate, Banana Powder, and Salt	Calories: 412kcal; Lipids: 9.1g (1.9g saturated); Carbohydrates: 45.5g (13.69g sugars); Proteins: 17.7g; Dietary Fibers: 12.2g; Salt: 0.4g
Bettery – Cocoa & Hazelnut	Maltitol, Dates, Pea protein, Oat flour, Chocolate, Hazelnut, Coconut, and Salt	Calories: 399kcal; Lipids: 7.8g (5.3g saturated); Carbohydrates: 52.7g (12.83g sugars); Proteins: 16.3g; Dietary Fibers: 6.5g; Salt: 0.4g
Continente – Peanut & Banana	Dates, Peanut paste, Oat flakes, Banana powder, and natural	Calories: 387kcal; Lipids: 14g (3.8g saturated); Carbohydrates: 51g (39g sugars); Proteins: 11g; Dietary Fibers: 6.3g; Salt: 0g
Continente – Hazelnut & Cocoa	Dates, Hazelnut paste, Cocoa powder, and roasted Hazelnuts	Calories: 405kcal; Lipids: 16.9g (2.1g saturated); Carbohydrates: 48.2g (42.1g sugars); Proteins: 10g; Dietary Fibers: 9.8g; Salt: 0g
Continente – Almond	Dates, Almond, Oat flour, and Almond paste	Calories: 405kcal; Lipids: 16.3g (1.5g saturated); Carbohydrates: 50.9g (41.8g sugars); Proteins: 9.1g; Dietary Fibers: 9.4g; Salt: 0.01g
Origens – Date, Cocoa & Coconut	Dates, Peanut butter, Pea protein, Agave jelly, Oat flakes, Cocoa nibs, Coconut fat, and Himalayan salt	Calories: 329kcal; Lipids: 7.3g (1.6g saturated); Carbohydrates: 42g (41.7g sugars); Proteins: 17.2g; Dietary Fibers: 12.8g; Salt: 0.65g
Origens – Brownie	Dates, Peanut butter, Pea protein, Agave jelly, Oat flakes, Cocoa, and Coconut fat	Calories: 345kcal; Lipids: 9.4g (3.6g saturated); Carbohydrates: 43g (41g sugars); Proteins: 16.9g; Dietary Fibers: 10.6g; Salt: 0.51g

**Table 1 (Continuation)** - Formulations and nutritional profile of commercial energy balls

<b>Commercial energy balls</b>	<b>Ingredients</b>	<b>Nutritional declaration (per 100g)</b>
Pingo doce – Peanut Butter	Dates, Peanut butter, Cocoa, and Peanuts	Calories: 382kcal; Lipids: 14g (2g saturated); Carbohydrates: 48g (44g sugars); Proteins: 11g; Dietary Fibers: 10g; Salt: 0.015g
Pingo doce – Coconut & Peanut	Date paste, Coconut, Peanuts, Cocoa powder	Calories: 414kcal; Lipids: 19g (11g saturated); Carbohydrates: 46g (42g sugars); Proteins: 9.3g; Dietary Fibers: 11g; Salt: 0.02g
Salutem – Apple & Cinnamon	Dates, Raisins, Apple, Sunflower seeds, Coconut, Cinnamon	Calories: 338kcal; Lipids: 5.2g (2.8g saturated); Carbohydrates: 65g (58g sugars); Proteins: 3.1g; Dietary Fibers: 10g; Salt: 0.03g
Salutem –Hazelnut & Cocoa	Dates, Raisins, Sunflower seeds, Hazelnuts, Cocoa, grated Coconut	Calories: 378kcal; Lipids: 12g (2g saturated); Carbohydrates: 57g (49g sugars); Proteins: 5.8g; Dietary Fibers: 11g; Salt: 0.01g
Ippie – Banana & Peanut	Dates, Oat, Banana, Peanuts, Cocoa, Peanut butter, Agave jelly	Calories: 408kcal; Lipids: 12.8g (6.6g saturated); Carbohydrates: 62.1g (46.4g sugars); Proteins: 6.7g; Dietary Fibers: 8.3g; Salt: 0.3g
Ippie – Coconut & Peanut	Dates, Oat, Peanuts, grated Coconut, Agave jelly	Calories: 409kcal; Lipids: 13.9g (5.8g saturated); Carbohydrates: 58.7g (44.4g sugars); Proteins: 8.4g; Dietary Fibers: 7.9g; Salt: 0.2g
Ippie – Cocoa & Hazelnut	Dates, Hazelnut paste, roasted Hazelnut, Cocoa powder, and nibs	Calories: 388kcal; Lipids: 15.6g (2.1g saturated); Carbohydrates: 50g (39.6g sugars); Proteins: 7.2g; Dietary Fibers: 9.9g; Salt: < 0.25g

Analyzing these commercial energy balls, it is observed that these products have a variety of ingredients in their composition, allowing them to serve different consumer segments, in addition to having a high energy value, providing between 300 and 600kcal in a 100g portion. Additionally, it is evident that many of these products contain substantial amounts of dietary fiber, which contributes to their value as a healthy and functional alternative.

In relation to the ingredients, dates are one of the most common components in the formulations shown, serving as a base to give the product its sweetness and characteristic flavor. There are different types of oilseeds, such as almonds, hazelnuts, and peanuts, which provide functional characteristics. Additionally, vegetable butters and

flours, like those made from oats, contribute to the product's texture. There is also the use of sweetening agents, such as maltitol and agave syrup, as options to replace conventional sugar. In addition, ingredients such as banana, cocoa, coconut, cinnamon, and apple are used to add flavor and diversify the options available on the market.

Among the formulations analyzed, some stand out for their different nutritional characteristics. For example, the 'Govinda – Coconut' formulation has a high lipid content due to the addition of coconut, while 'Bettery – Apple & Cinnamon' stands out for its high protein content, thanks to the addition of pea protein.

The diversity of formulations available on the market demonstrates the versatility of energy balls and their adaptability to meet the needs of the market and the target audience. In this sense, the formulation proposed in this study seeks to align itself with the observed trends: figs, which can provide the sweetness and characteristic flavor of dates in commercial products; almonds and olive oil, which provide functional characteristics and texture; honey, which can replace industrialized sweeteners; and finally pollen, which is an innovative natural source of carbohydrates and proteins making this food not only energetic, but also more protein-rich, which can be important in athlete's diets and also older people diets.

### **2.3 Homemade energy balls**

To determine the potential acceptability of energy balls to the target audience, it is also important to analyze whether homemade recipes for this product already exist, as it is already available on the market. Table 2 presents several recipes along with their respective references, which is crucial for understanding the main ingredients and preparation methods used in the homemade production of energy balls, thereby allowing for an understanding of the different nutritional and sensory preferences.

In general, homemade energy balls are made with a base of oat flakes, combined with nuts, dried fruits, seeds, and natural sweeteners. Additionally, ingredients such as cocoa and coconut are used to enhance flavor and aroma.

The production process for these energy balls follows similar basic steps in the different recipes: Initially, the dry ingredients are crushed until they reach a homogeneous texture, followed by the addition of the wet ingredients. The mixture is processed until it forms a moldable dough, which is then shaped into small spheres. These spheres can be finished with optional toppings. Some recipes also recommend storing the energy balls

in airtight, refrigerated containers, which is important to ensure the texture and flavor are preserved.

**Table 2** - Homemade energy balls recipes and respective references

Homemade energy balls	Ingredients	Preparation method	Reference
Peanut, Oat, and Banana Energy Balls	Oat flakes, roasted Peanuts, crushed Banana, Agave syrup, Cocoa nibs, Dates	Grind roasted peanuts and oat flakes until they resemble flour. With the processor running, add banana, date, and agave syrup and blend until crumbly. Form balls with the dough. Dip the balls in cocoa nibs. Store in a zip bag in the refrigerator.	<a href="https://www.24kitchen.pt/receita/bolas-energeticas-de-amendoim-aveia-e-banana">https://www.24kitchen.pt/receita/bolas-energeticas-de-amendoim-aveia-e-banana</a>
Almond, Cocoa, and Coconut Energy Balls	Almonds, Dates, Coconut oil, Cocoa, dehydrated Coconut, Salt	Grind the almonds until you create a fine, powdery paste. Add the dates and continue to grind. Add coconut oil, cocoa, and salt and mix. Make balls and cover them with grated coconut. Place in the refrigerator.	<a href="https://menshealth.pt/nutricao/energy-balls-receitas/117381/">https://menshealth.pt/nutricao/energy-balls-receitas/117381/</a>
Low FODMAP Energy Balls	Oat flakes, Peanut butter, Dates, Water	Grind dates. Add the peanut butter and grind again. Add the oat flakes, cocoa, and water and mix until you obtain a moldable paste. Form balls with your hands and store them in an airtight container.	<a href="https://www.mygutfeeling.pt/bolas-energeticas-low-fodmap/#google_vignette">https://www.mygutfeeling.pt/bolas-energeticas-low-fodmap/#google_vignette</a>
Date and cocoa Energy Balls	Nuts, Dates, Cocoa, Chia seeds, grated Coconut	Grind the walnuts until a sandy consistency. Add the remaining ingredients until a homogeneous mixture is formed. Shape the balls with your hands. Cover the energy balls with grated coconut.	<a href="https://anarachid.pt/bolinhas-energeticas-de-tamaras-e-cacau/">https://anarachid.pt/bolinhas-energeticas-de-tamaras-e-cacau/</a>

## 2.4 Alternative sources of proteins

The growing demand for alternative protein sources reflects a global transformation in the food system, driven by concerns about sustainability, health, and animal welfare. Intensive animal protein production is associated with significant environmental impacts, including greenhouse gas emissions, soil degradation, biodiversity loss, and excessive consumption of water and energy (AKINMEYE et al., 2024). In light of these challenges, unconventional protein sources (such as plants, microorganisms, insects, and cultivated meat) have gained prominence due to their lower ecological footprint and potential to meet the increasing food demand of a population estimated to reach 9.8 billion by 2050 (WOOD and TAVAN, 2022).

In addition to environmental benefits, the partial replacement of animal proteins with alternative sources is associated with health benefits, such as a reduced risk of cardiovascular diseases, type 2 diabetes, and certain types of cancer. Among the most prominent plant-based options are bee pollen, quinoa, almond, hemp, and legumes, which

have rich and balanced nutritional profiles in essential amino acids (PIHLANTO et al., 2017).

In the financial context, the expansion of the alternative protein sources market is also beneficial, with projections reaching 290 billion dollars by 2035, driven by flexitarian consumers and marketing strategies of major food industries seeking to meet this new demand (NGUYEN et al., 2022). However, the success of large-scale adoption depends not only on technological and nutritional innovations but also on consumer acceptance, which is influenced by cultural factors, perception of naturalness, convenience, cost, and information about the environmental and ethical benefits of these sources (AKINMEYE et al., 2024).

As in the present work, it will be used bee pollen as protein source, this product will be discussed in more detail in the next section.

## **2.5 Bee pollen**

Bee pollen is a food widely recognized for its nutritional and bioactive profile, composed of substances beneficial to health. Produced by bees by collecting pollen from flowers and adding salivary substances and nectar, it is stored in the hive as a source of nutrition for the colony. Its chemical composition varies according to its botanical origin, climatic conditions, and species of bees involved in the collection process (BARROS et al., 2024).

The process of obtaining pollen by bees, as shown in Figure 1, is specialized and involves collecting pollen grains directly from the anthers of flowers during foraging. Worker bees use their hind legs to collect pollen, mixing it with nectar and salivary secretions to form small pellets that are transported back to the hive (QIAO et al., 2024).

To collect pollen, beekeepers use pollen traps placed at the entrance of the hives, collecting this material without compromising the nutrition of the colony. These traps retain part of the pollen transported by the bees, which is removed and dried to enhance its durability, thereby maintaining its nutritional and bioactive properties and facilitating its application in food products and functional supplements (QIAO et al., 2024).

Bee pollen's composition is primarily based on carbohydrates, proteins, lipids, and dietary fiber, as well as a wide range of micronutrients and bioactive compounds. Table 3 presents the average nutritional composition of bee pollen, based on Aylanc et al. (2023), and used as a reference in this study to evaluate the nutritional potential of the developed formulations.

Among carbohydrates, monosaccharides such as glucose and fructose predominate, as well as structural polysaccharides that provide protection to pollen grains. In terms of protein, pollen is considered one of the richest sources of essential amino acids (KACEMI and CAMPOS, 2023).

The protein content of bee pollen can comprise up to 61% of its composition, making it one of the richest natural foods in this macronutrient, and containing a significant portion of the essential amino acids necessary for human nutrition (QIAO et al., 2024). As a result, this food has been incorporated into several food products, aiming to enrich their nutritional composition and enhance their health benefits.



**Figure 1 - Bee Pollen Production**  
**Source – Qiao et al. (2024)**

**Table 3 - Nutritional composition of bee pollen (multiflower)**

Nutritional declaration (per 100g)	Bee pollen
<b>Calories (kcal)</b>	398.5
<b>Lipids (g)</b>	4.4
<b>Carbohydrates (g)</b>	66.35
<b>Dietary fiber (g)</b>	4.9
<b>Protein (g)</b>	20.95
<b>Water (g)</b>	6.45

**Source - Adapted from AYLANC et al. (2023)**

In relation to food formulations, bee pollen has been gaining ground in bakery and confectionery, as its addition to products such as bread, cookies, and energy bars improves not only the nutritional value but also the sensory properties of these foods. For

example, the incorporation of pollen into cookies contributed to an increase in the content of bioactive compounds and antioxidants, in addition to modifying the texture, making them softer, and intensifying the color of the products, making them more attractive to consumers. However, higher concentrations affected expansion and made the cookies denser, underscoring the need for balance when incorporating pollen into a formulation (SOKMEN et al., 2022).

Similarly, the inclusion of pollen in bread formulations (BAKY et al., 2023) has demonstrated improvements in protein quality and amino acid profile, making it a viable alternative for the development of functional foods. Pollen addition also favored moisture retention, resulting in a softer and more structured crumb.

Furthermore, bee pollen has been successfully incorporated into dairy-based products, such as yogurts and fermented beverages, thereby enhancing their antioxidant capacity and protein content (KARABAGIAS et al., 2018). These results reinforce the potential of bee pollen to be incorporated into various food matrices, providing functional and nutritional benefits.

The bioactive properties of pollen are generally attributed to its content of phenolic, including flavonoid compounds, which confer its antioxidant activities. These have been linked to pollen's ability to reduce oxidative stress and fight inflammation, crucial factors in preventing chronic diseases such as cardiovascular and metabolic disorders. Furthermore, pollen has antimicrobial, immunomodulatory, and hepatoprotective activities, in addition to being beneficial for gastrointestinal health (MĂRGĂOAN et al., 2019).



**Figure 2 - Bee pollen**  
**Source – Mundo Boa Forma (2021)**

From a nutritional point of view, pollen is used as a food supplement, being included *in natura* in human meals (Figure 2) due to its balanced composition of macro

and micronutrients, being considered a "superfood". It also stands out in promoting health in various age groups, including pregnant and breastfeeding women, as it can help meet part of their daily mineral needs. Furthermore, pollen has been an aid in recovery from illnesses and as a complementary source of nutrients for those deficient in these compounds (ALCALÁ-OROZCO et al., 2024).

Finally, bee pollen also stands out for its contribution to cardiovascular health, as it can reduce capillary fragility and improve heart function, in addition to having beneficial effects on lipid metabolism regulation. These benefits are reinforced by its content of unsaturated fatty acids, such as linoleic and oleic acids, which play important roles in cellular health and protection against disease (KACEMI and CAMPOS, 2023).

Despite the numerous benefits attributed to bee pollen, it is important to highlight that its consumption can trigger allergic reactions, particularly in individuals who are already sensitive to environmental pollens.

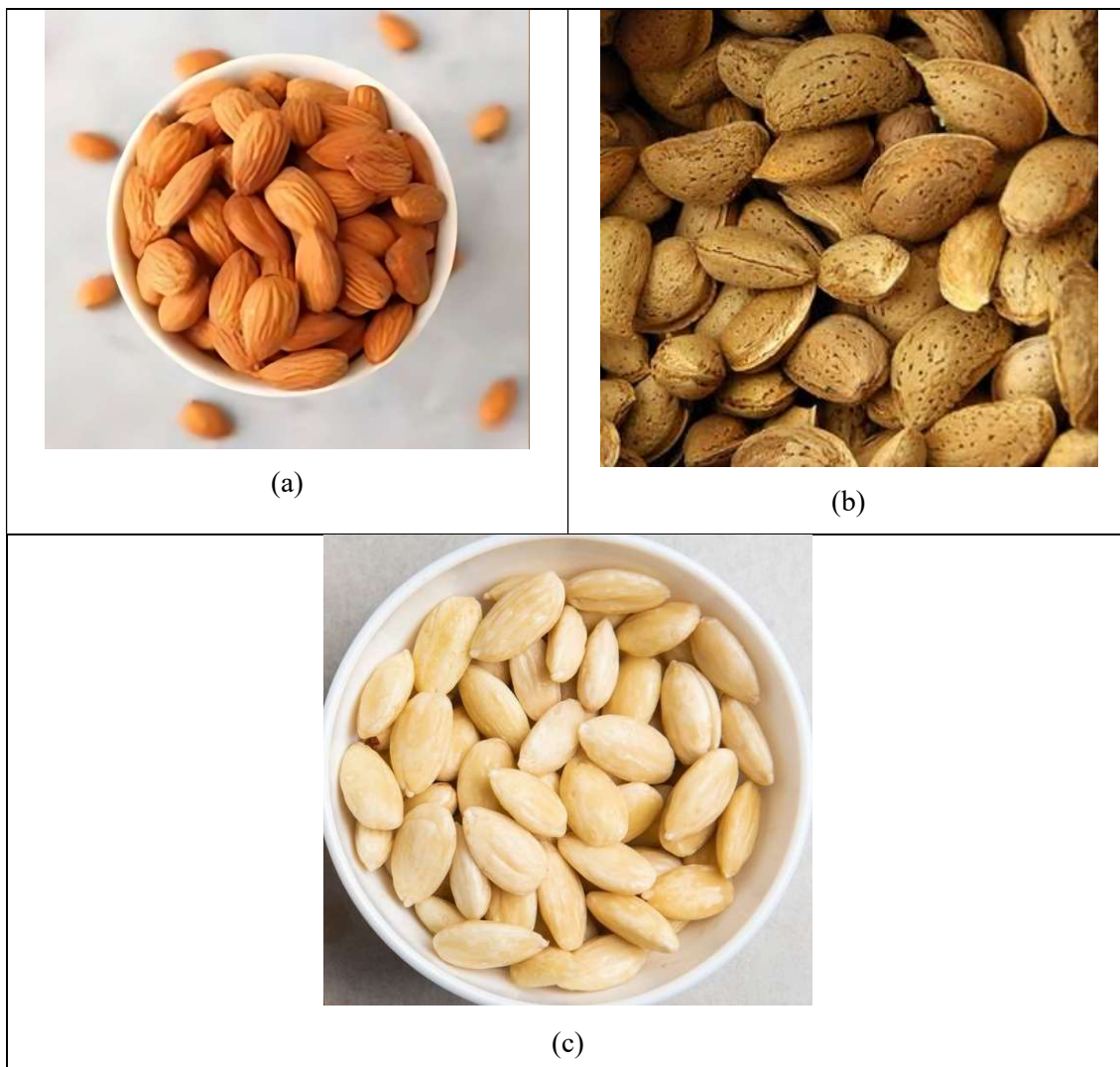
Clinical studies indicate that these reactions can range from mild manifestations, such as oral itching, to more severe episodes, including anaphylaxis, particularly in children or adults with a history of allergic rhinitis or pollinosis (MARTÍN-MUÑOZ et al., 2010). The origin of these reactions is often related to the presence of allergenic proteins from botanical families such as Asteraceae, Fabaceae, and Rosaceae, which comprise part of the pollen collected by bees (MATUSZEWSKA et al., 2022).

Cross-sensitization between plant proteins and the allergenic content of pollen may occur even in individuals who have never had previous contact with bee products. Nevertheless, the prevalence of adverse reactions to bee pollen in the general population is considered low. Studies, such as that by Denisow and Denisow-Pietrzyk (2016), have emphasized that when consumed with caution and under supervision in predisposed individuals, the product remains safe and promising as a dietary supplement.

## **2.6 *Prunus dulcis* (Almond)**

The almond (*Prunus dulcis*) belongs to the Rosaceae family, an ancient nut crop that originated in the Mediterranean climate of the Middle East, and it is now cultivated in arid and hot regions due to its ability to adapt to adverse soils and climatic conditions (ALGHAMDI and ALSABEHI, 2024). Botanically, almonds are considered drupes, not true nuts, and are made up of three parts: the nucleus, the intermediate shell, and the outer green shell, as can be seen in Figure 3 (JAVAID et al., 2019).

There are two main types of almonds: sweet (*Prunus dulcis*) and bitter (*Prunus amara*). While the first is edible and used in human food, the second contains toxic glycosides, such as amygdalin, which can release cyanide, limiting its consumption (ÖZCAN, 2023).



**Figure 3** – (a) Almond with intermediate skin; (b) Almond with outer skin; (c) Almond's nucleus

**Source** – (a) Tua Saúde (2024); (b) Squesito (2025); (c) Tudo Gostoso (2022)

Almonds are rich in nutrients, including lipids, proteins and fiber (Table 4), as well as vitamins (mainly vitamin E) and minerals such as calcium [Ca], magnesium [Mg] and potassium [K], also standing out for their high concentration of unsaturated fatty acids, such as oleic and linoleic acids, and bioactive compounds such as polyphenols, phytosterols and tocopherols, which perform antioxidant and anti-inflammatory functions (CLEMENTE-SUÁREZ et al., 2023).

Vitamin E and other antioxidants found in almonds help combat oxidative stress, reducing the risk of inflammation and delaying the body's cellular aging, in addition to

playing a role in human brain health, muscle strengthening, and improving immunity throughout the body (HIGGINS et al., 2020).

Phenolic compounds are present in almond shells and seeds, helping to prevent degenerative diseases, such as cancer and cardiovascular disorders. In the shell, there is a high concentration of catechins, chlorogenic acid, and other flavonoids, which contribute to the almond's antioxidant properties (GARCIA-PEREZ et al., 2021).

Regular consumption of almonds is associated with several health benefits, including reducing LDL cholesterol and increasing HDL cholesterol, regulating blood pressure, controlling blood sugar levels, and potentially helping to manage type 2 diabetes. Furthermore, almonds can promote satiety, which is attractive for weight loss diets, due to their high protein and fiber contents (JAVAID et al., 2019; SINGAR et al., 2024).

**Table 4 - Nutritional composition of almond**

<b>Nutritional declaration (per 100g)</b>	<b>Almond</b>
<b>Calories (kcal)</b>	650
<b>Lipids (g)</b>	56.8
<b>Carbohydrates (g)</b>	7.1
<b>Sugars (g)</b>	5
<b>Dietary fibers (g)</b>	12.2
<b>Proteins (g)</b>	21.6
<b>Water (g)</b>	3

**Source - INSA (2025)**

The versatility of the almond as a food is demonstrated through its use both in its natural form and in derived products, as well as in vegan diets that use its milk, oil, flour, and pastes. Due to their attractive nutritional value, they are considered an essential component of a balanced diet (GARCIA-PEREZ et al., 2021).

## **2.7 Olive oil**

Described since ancient times as “liquid gold” by Homer, olive oil is known for its nutritional, sensorial and functional qualities, in addition to being one of the main foods used in the Mediterranean Diet, beneficial to human health, becoming a

fundamental component in healthy diets in various countries around the world (Figure 4) (CLODOVEO et al., 2014).

Another important characteristic of olive oil is its chemical composition, which is rich in monounsaturated fatty acids, such as oleic acid, known for its benefits to cardiovascular health. Additionally, it contains bioactive compounds, including polyphenols and tocopherols, that exhibit antioxidant and anti-inflammatory properties. Polyphenols contribute to protection against inflammatory and metabolic diseases, while tocopherol (vitamin E) acts as a good antioxidant, preventing the oxidation of lipids (TARABANIS et al., 2023).



**Figure 4 - Olive oil**  
**Source – Portugal Global (2024)**

The consumption of olive oil in the daily routine is associated with a reduced risk of cardiovascular diseases, including atherosclerosis and hypertension, with an improvement in the lipid profile, with a reduction in LDL (low-density lipoprotein) cholesterol levels and an increase in HDL (high-density lipoprotein), and the prevention of neurodegenerative diseases such as Alzheimer's due to its ability to reduce oxidative stress and increase cognitive health (RIOLO et al., 2022).

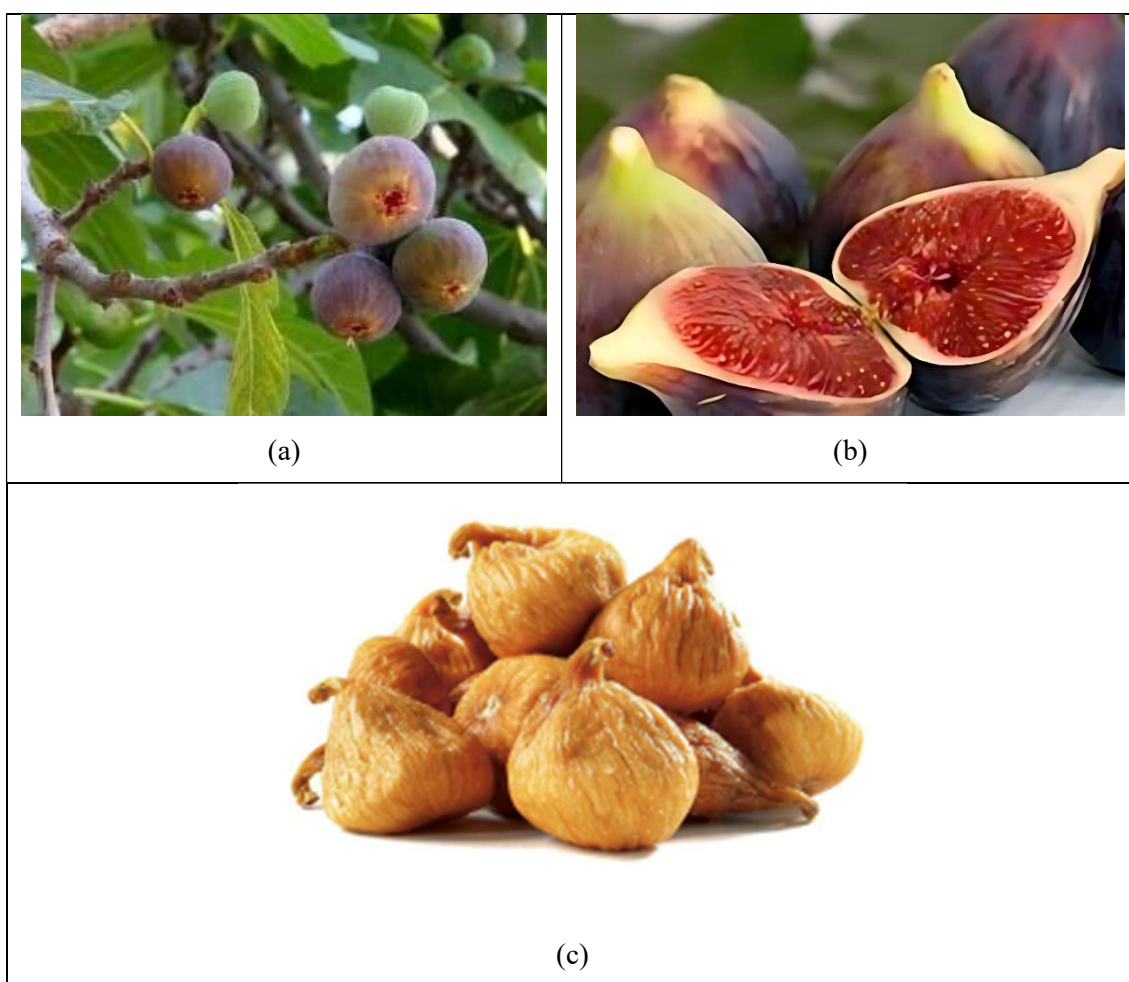
From a sensorial point of view, olive oil stands out for its aromas and flavors, which vary according to its origin and the methods used in its production. Flavors, such as fruity, bitter and spicy, are related to the presence of bioactive compounds, such as polyphenols, influencing consumer acceptance, such as oleocanthal, which is responsible for the sensation of spiciness characteristic of a high-quality olive oil, having anti-inflammatory properties like those of the medication ibuprofen (CHAJI et al., 2024).

In Mediterranean countries, the consumption of organic olive oil has grown, being associated with the reduction of environmental impact and support for more sustainable production systems, playing an important role in valuing cultural traditions, as it promotes

the preservation of agricultural practices, in addition to stimulating local economic development (PERŠURIĆ and DAMIJANIĆ, 2021).

## 2.8 *Ficus carica* (Fig)

The fig (*Ficus carica* L.), belonging to the Moraceae family and demonstrated in Figure 5, has been cultivated for over 6000 years in regions with a hot and dry climate, particularly in Turkey, the world's largest producer, followed by Egypt and Morocco. However, despite being popularly recognized as a fruit, the fig is classified as a closed inflorescence, where hundreds of unisexual flowers develop and form small fruits. There are four main types of figs (Caprifigs, Smyrna, San Pedro, and Common) and more than 800 varieties grow around the world (KHAN et al., 2020).



**Figure 5** – (a) Fresh fig on the tree; (b) Fresh open fig; (c) Dried fig  
**Source** – (a) (b) Valpaços (2025); (c) Frutifique (2025)

In addition to being rich in sugars (Table 5), figs are a rich source of bioactive compounds, including polyphenols and carotenoids, as well as minerals such as calcium [Ca], magnesium [Mg], and potassium [K]. These compounds provide fruit with

antioxidant, anti-inflammatory, and antimicrobial properties, as well as metabolic and cardiovascular benefits. Figs are also a good source of dietary fiber, contributing to digestive health and dietary satiety (SANDHU et al., 2023).

**Table 5 - Nutritional composition of dried fig**

<b>Nutritional declaration (per 100g)</b>	<b>Dried fig</b>
<b>Calories (kcal)</b>	270
<b>Lipids (g)</b>	0.6
<b>Carbohydrates (g)</b>	58.3
<b>Sugars (g)</b>	58.3
<b>Dietary fibers (g)</b>	11
<b>Proteins (g)</b>	2.3
<b>Water (g)</b>	-

**Source - INSA (2025)**

Those with darker skin have a higher concentration of polyphenols and antioxidant capacity compared to those with lighter skin. In addition to this analysis, the peel also has a higher content of phenolic compounds than the pulp of the closed inflorescence, but lower than that of the plant's leaves – characteristics that may vary with the stage of maturation and processing of the inflorescence (SANDHU et al., 2023).

This food is sold both fresh and dried. Fresh figs are perishable, which is why they are processed into products such as jellies and nectars. On the contrary, dried figs contain higher concentrations of sugars, fiber, and bioactive compounds due to the reduction of water in the drying process (ARVANITI et al., 2019).

Figs have been linked to significant cardiovascular, digestive, and metabolic health benefits. Its inclusion in the diet can improve blood glucose levels, promote intestinal health, and reduce markers of oxidative stress. Furthermore, its regular consumption is beneficial for higher-quality diets and lower body mass indices, leading to weight loss (RASOOL et al., 2023).

Although dried figs are more marketed for their shelf life, fresh figs offer a sensory profile with greater juiciness and less sweetness. In this work, dried figs were chosen because of their consistency and sweetness.

## 2.9 Honey

Honey (Figure 6) is a natural product, originating from flower nectar, transformed and stored by honeybees in the hive's combs. It is recognized as a functional food due to its nutritional and therapeutic properties. Honey must be free of substances foreign to its natural composition and cannot contain additives such as sugars or physical and biological contaminants (BERENBAUM and CALLA, 2021).



**Figure 6** – Bee honey  
**Source** – Agronegócios (2021)

According to the Portuguese Law (Law-Decree nº 214/2003), legislation in force, the legal classification of honey is determined based on its botanical origin, mode of production and presentation, thus standardizing the types of honey available on the market, ensuring transparency in commercialization and maintenance of the specific characteristics of each variety, like can be seen in Table 6.

To a large extent, the chemical composition of bee honey is made up of simple sugars, such as fructose and glucose, including, to a lesser extent, complex mixtures of carbohydrates, amino acids, organic acids, enzymes, minerals, aromatic compounds, pigments, and pollen grains. These components confer honey with antioxidant, antimicrobial, immunomodulatory, healing, and anti-inflammatory properties, thereby adding value to the food (GIAMPIERI et al., 2022).

As for its quality, it is influenced by factors such as the floral origin, climatic conditions, extraction and processing methods, in addition to storage, and evaluated through parameters such as pH, diastase activity, and hydroxymethylfurfural (HMF) content. Regarding its moisture content, the percentage must be analyzed, and the value should be as favorable as possible to prevent fermentation and preserve the product's

properties (TRISHA et al., 2023). In Table 7, it is indicated the nutritional composition of honey mentioned by INSA (2025).

**Table 6 - Classification of honey**

<b>Origins</b>	<b>Type of honey</b>	<b>Characteristics</b>
Botanical	Nectar honey or flower honey	Honey obtained from plant nectar
	Honeydew honey	Honey obtained mainly from excretions of plant-sucking insects (Hemiptera) that remain on living parts of plants or from secretions originating from living parts of plants
Method of production and/or presentation	Honey in combs	Honey stored by bees in the sealed alveoli of combs recently built by the bees themselves or from thin sheets of engraved wax made exclusively with wax bee and not containing brood, sold in whole combs or in sections of combs
	Honey with comb pieces	Honey that contains one or more pieces of honey in combs
	Drained honey	Honey obtained by draining uncapped combs that do not contain brood
	Centrifuged honey	Honey obtained by centrifuging uncapped combs that do not contain brood
	Pressed honey	Honey obtained by compressing combs that do not contain brood, without heating or with moderate heating of 45 °C (maximum)
	Filtered honey	Honey obtained by a process of eliminating organic or inorganic materials foreign to its composition that removes an important part of the pollen

**Source** - Directive 2001/110/EC, Law-Decree No. 214/2003.

Furthermore, preservation techniques such as high-pressure processing (HPP) help to preserve the antioxidant properties of honey and maintain its microbiological quality during long periods of shelf time, being an alternative to traditional thermal

pasteurization carried out on this food, which in some cases compromises the enzymatic activity and bioactive compounds in honey (SCEPANKOVA et al., 2024).

Honey is also used in traditional and modern medicine due to its therapeutic properties, such as the effectiveness of natural antioxidant activity, contributing to the neutralization of free radicals in a human's body, in addition to its antimicrobial potential against pathogens that can cause diseases in people, in addition to its application in wound treatments that brought good results, due to the healing and antimicrobial properties (SCEPANKOVA et al., 2024).

**Table 7 - Nutritional declaration of honey**

<b>Nutritional declaration (per 100g)</b>	<b>Honey</b>
<b>Calories (kcal)</b>	314
<b>Lipids (g)</b>	-
<b>Carbohydrates (g)</b>	78
<b>Sugars (g)</b>	78
<b>Dietary fibers (g)</b>	-
<b>Proteins (g)</b>	0.5
<b>Water (g)</b>	18.5

**Source - INSA (2025)**

In Portugal, honey is a product of high cultural and economic importance, with several regions holding the Protected Designation of Origin (DOP), a seal that attests to the quality and intrinsic connection of the product to its production territory. Currently, the country has nine varieties of honey certified with PDO, highlighting the richness and diversity of the national beekeeping heritage. Among these, two produced in the Trás-os-Montes region stand out: Honey of *Terra Quente* and Honey of *Parque de Montesinho*.

Honey of *Parque de Montesinho* is produced in the areas of Bragança and Vinhais. This honey is distinguished by its peculiar flavor and aroma, resulting from the region's characteristic flora, which includes species such as heather, chestnut, and rosemary. The production of this honey is directly linked to the environmental conditions of the Montesinho Natural Park, an area renowned for its extreme temperatures and native vegetation (DGADR, 2005).

Honey of *Terra Quente* is characterized by nectar predominantly sourced from rosemary, heather, eucalyptus, lavender, and broom. According to its specification document, a minimum of 15% rosemary pollen contributes to its distinct and recognizable flavor. This honey is produced across various municipalities in the region, like Mirandela, Vila Flor, Moncorvo, Freixo de Espada à Cinta, Mogadouro, Alfândega da Fé, and Macedo de Cavaleiros (DGADR, 2006).

## **3. Objectives**

### **3.1 General Objective**

The general objective of the present work was to develop and characterize formulations of energy balls based on natural and functional ingredients from the Trás-os-Montes region — olive oil, almond flour, dried fig, honey, and bee pollen — by evaluating their physicochemical, microbiological, and sensory properties, with a focus on the use of bee pollen as a protein source.

### **3.2 Specific Objectives**

The specific objectives of the present work were:

- To produce different formulations of energy balls incorporating olive oil, almond flour, dried figs, honey, and bee pollen, collected from olive groves studied in the Bio4Med Project;
- To evaluate the physicochemical composition of the selected formulations, with emphasis on the protein content associated with the bee pollen;
- To characterize the sensory profile of the samples through consumer testing;
- To contribute to the valorization of products from the Trás-os-Montes region within the scope of the Bio4Med project.

## 4. Materials and Methods

### 4.1 Chemicals and reagents

Ethanol, methanol, sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), sodium hydroxide (NaOH), hydrochloric acid (HCl), and petroleum ether were purchased from Fisher Scientific (Pittsburgh, PA). Kjeldahl catalyst tablets were purchased from Panreac Applichem (Barcelona, Spain).

### 4.2 Formulations

#### 4.2.1 Raw materials collection

The formulations of energy balls were developed using functional ingredients from the Trás-os-Montes region, with an emphasis on bee pollen as a source of protein. Bee pollen was collected at Quinta do Valongo, Mirandela, at the end of May 2024. The samples were collected with pollen traps placed in front of the beehive entrance. After, the pollen was cleaned of debris and kept in plastic bags at -18 °C until their delivery to the laboratory. The samples were freeze-dried using a lyophilizer (FreeZone 4.5 model 7750031, Labconco, USA) and were stored within a temperature range between +2 °C and +5 °C, until further analysis.

Honey was collected within the scope of the Bio4Med project, originating from Terra Quente, Mirandela (Figure 7). It was harvest in July 2024.



**Figure 7** - (a) Bee pollen collected at Quinta do Valongo; (b) Honey from Terra Quente  
**Source** – Own authorship (2025)

The other ingredients (olive oil, figs, and almond flour) were purchased at a supermarket.

#### 4.2.2 Tested formulations

The formulation of the energy balls involved several experiments, during which different ingredients were tested. Taking into account the nutritional declarations of these foods (INSA (2025) or labels of the commercial products), shown in Table 8, different proportions of the ingredients were tested, using Excel software, aiming to achieve the assumption that at least 12 % of the energy value of the food is provided by protein, the criterion needed to use the claim that a food is a source of protein (Regulation (EC) N° 1924/2006).

**Table 8** - Nutritional declaration of ingredients used in formulations

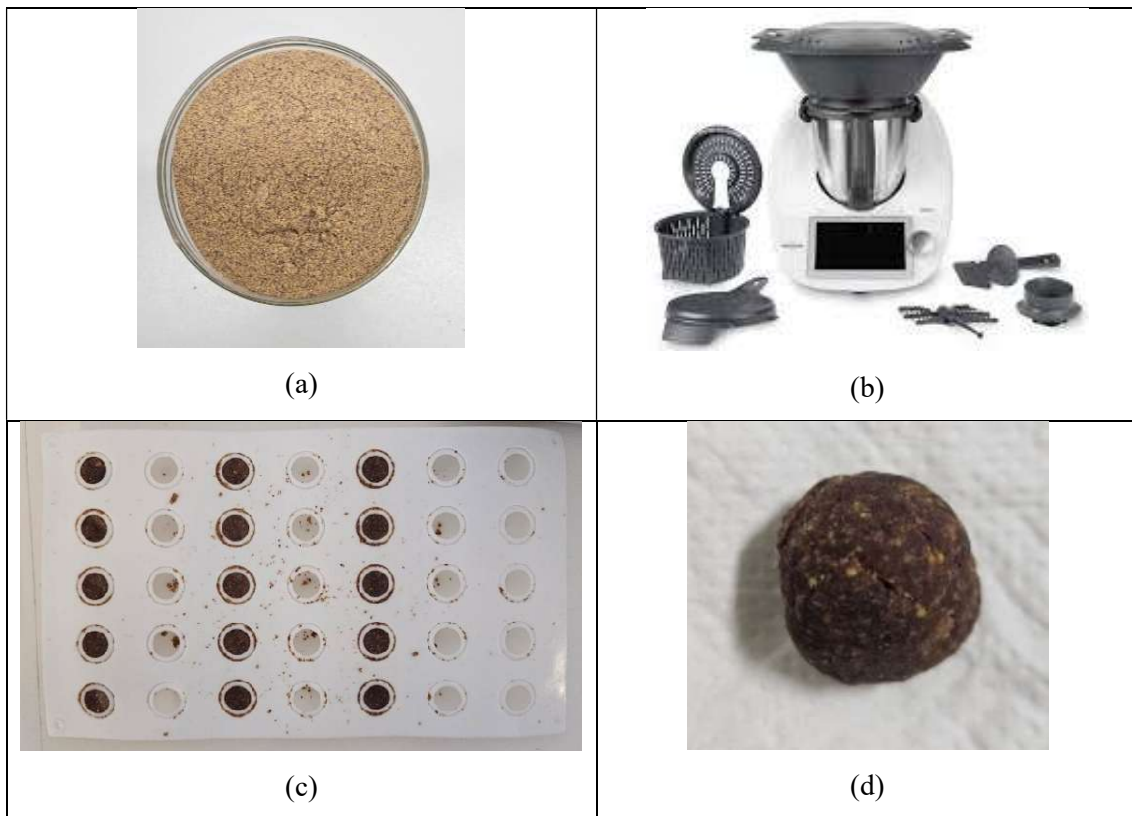
Nutritional Declaration (per 100g)	Almond flour <sup>1</sup>	Dried fig <sup>1</sup>	Dried pea <sup>2</sup>	Honey <sup>2</sup>	Olive oil <sup>1</sup>	Bee pollen <sup>3</sup>
Calories (kcal)	622	294	330	314	819	398.5
Lipids (g)	54	1.4	1.3	-	91	4.4
Carbohydrates (g)	4.5	60	49.4	78	-	66.35
Sugars (g)	3.8	60	2.3	78	-	-
Dietary fibers (g)	8	10	15	-	-	4.9
Proteins (g)	25	3.9	22.7	0.5	-	20.95
Salt (g)	0.03	0.11	9.3	-	-	-
Water (g)	-	-	-	18.5	-	6.45

Source – (1) Pingo Doce supermarket (2025); (2) INSA (2025); (3) Adapted from AYLANC et al. (2023)

The dried figs and olive oil were purchased from the Pingo Doce supermarket, and almond flour was not used at this stage, as the focus was on concentrating the protein content in the bee pollen. With all ingredients available (Table 9), a Bimby® was used to mix and grind them, followed by shaping the mixture into balls using a chocolate mold, as shown in Figure 8, to test formulation 1.

**Table 9** – Definition of tested energy balls formulations (data expressed in percentage of each ingredient used to produce the energy balls)

Formulations	Almond flour	Dried fig	Dried pea	Honey	Olive oil	Bee pollen
1	0%	30%	0%	8%	2%	60%
2	40%	30%	0%	8%	2%	20%
3	0%	30%	40%	8%	2%	20%
4	20%	30%	20%	8%	2%	20%



**Figure 8** – (a) Ground bee pollen (b) Bimby® (c) Energy ball mold (d) First formulation test  
**Source** – (a) (c) (d) Own authorship (2025); (b) PCGuia (2025)

However, after sensory tests, an overly intense flavor and a scratchy sensation in the throat were identified, attributed to the high concentration of pollen. Therefore, as shown in Table 9, three new alternative formulations were proposed, incorporating almond flour (purchased at the Pingo Doce supermarket) and/or dried pea (purchased at a local greengrocer, represented in Figure 9) as complementary sources of protein.



**Figure 9** – (a) Dried pea; (b) Ground dried pea  
**Source** – Own authorship (2025)

The formulations with dried pea exhibited a more brittle texture, as shown in Figure 10 (left-to-right representation, respectively, of the formulations 2, 3, and 4 corresponding to Table 9). Additionally, further sensory tests were conducted, revealing a bitter taste. These formulations were considered less pleasant, with a preference for the formulation that included only almond flour (formulation 2).



**Figure 10** – Formulations tested with almond flour and dried pea  
**Source** – Own authorship (2025)

Therefore, formulation 2 (preferred) served as the basis for three new formulations, in which at least 12% of the energy value of the food was provided by protein, as shown in Table 10.

**Table 10** - Definition of final formulations

Formulations	Protein <sup>1</sup> (%)	Almond flour	Dried Fig	Honey	Olive oil	Bee pollen
A	12.3	45.00%	33.75%	9.00%	2.25%	10.00%
B	12.8	40.00%	30.00%	8.00%	2.00%	20.00%
C	13.3	35.00%	26.25%	7.00%	1.75%	30.00%

<sup>1</sup>Percentage of the energy value provided by protein.

The visual aspects of the energy balls with the final formulations are represented in Figure 11. In general, the texture was not brittle, there was no bitterness, and the pollen flavor was not overpowering.



**Figure 11** - Formulations A, B e C  
**Source** - Own authorship (2025)

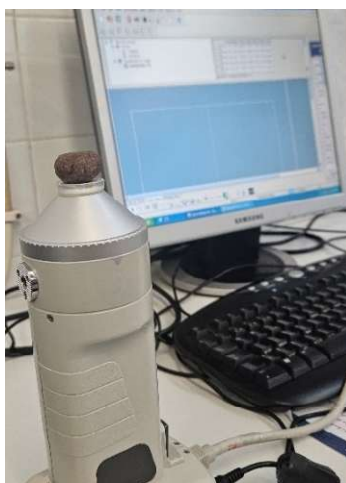
### 4.3 Pollen palynological analysis

The palynological analysis was performed by the LabApis Laboratory at Trás-os-Montes and Alto-Douro University. The homogenized bee pollen (about 1 g) was placed in separate vials with distilled water and vortexed vigorously to soften the samples slightly. Then, 200  $\mu\text{L}$  were taken for each sample from the resulting mixture and centrifuged at 1000 g for 5 minutes. The obtained pellet was subjected to acetolysis according to the method reported by Louveaux et al. (1978) and Von Der Ohe et al. (2004). Pollen's identification and counting were performed using an optical microscope. More than 1200 grains per preparation were counted following the criteria of Vergeron (VERGERON, 1964).

### 4.4 Physical analysis

#### 4.4.1 Color

Color analysis of formulations A, B, and C was carried out using a portable colorimeter as shown in Figure 12, following the CIELAB system, which provides the parameters  $L^*$  (lightness),  $a^*$  (+red- $\bar{}$ green component), and  $b^*$  (+yellow- $\bar{}$ blue component).



**Figure 12** – Colorimeter  
**Source** – Own authorship (2025)

The  $L^*$  parameter ranges from 0 (black) to 100 (white), while  $a^*$  and  $b^*$  can take positive or negative values, indicating, respectively, a predominance of reddish or greenish tones, and yellowish or bluish tones, respectively.

Based on these values, the software calculated the derived parameters *Chroma* ( $C^*$ ), which the higher the  $C^*$  value represents color saturation or intensity, ranging from 0 to 130, and Hue angle ( $h$ ), which indicates the hue of the color, represented by an angle ranging from  $0^\circ$  to  $360^\circ$ , corresponding to different shades on the color wheel ( $0^\circ$  = red,  $90^\circ$  = yellow,  $180^\circ$  = green,  $270^\circ$  = blue).

This analysis enabled the evaluation of not only the visible coloration but also its nuances and intensity, which are essential for visually characterizing the formulations.

#### 4.4.2 Texture

The texture analysis of the energy balls was performed using a texture analyzer (Stable Micro Systems, TA.XT plus model) (Figure 13), applying the Texture Profile Analysis (TPA) technique. This method simulates human mastication by performing two consecutive compressions on the sample, allowing the evaluation of its mechanical properties under controlled deformation.

The balls were previously molded in triplicate into a standardized spherical shape with a radius of 2 cm to ensure reproducibility of the results. The test was conducted using a flat cylindrical probe, operating at a constant pre-test, test, and post-test speed, up to a defined deformation of 50% of the original sample height.

The following parameters were evaluated:

- **Hardness:** maximum force recorded during the first compression, representing the sample's resistance to deformation.
- **Fracturability:** force at the point where the sample breaks or fractures during the first compression.
- **Adhesiveness:** work required to overcome the adhesive forces between the sample and the probe, reflecting how much the food "sticks."
- **Elasticity:** the ability of the sample to return to its original height after deformation (measured by the distance between compressions).
- **Stickiness:** negative force generated during the separation of the probe from the sample, related to the sticky sensation in the mouth.
- **Cohesiveness:** the ratio between the area under the second compression and the area under the first, reflecting the internal integrity of the sample.

- **Resilience:** the ability of the sample to recover its original shape immediately after the first deformation.

These parameters are essential for understanding the sensory acceptability of the product, particularly in terms of firmness, consistency, elasticity, and ease of chewing, characteristics highly valued in functional snacks.



**Figure 13 - Texture analyzer**  
**Source – Own authorship (2025)**

## 4.5 Chemical analysis

### 4.5.1 Moisture

The moisture content was determined for the bee pollen and for formulations A, B, and C, using the AOAC 925.45 methodology, which involved an automated moisture analyzer (Figure 14). Initially, the samples were removed from refrigeration, homogenized with a spatula, and weighed in triplicate on an analytical balance, with approximately 1.65 g of each sample.



**Figure 14 – Sample on the moisture analyzer**  
**Source - Own authorship (2025)**

Each sample was evenly spread on a cut piece of aluminum foil, previously placed in the device's chamber, as illustrated in Figure 14. Drying was carried out at 105 °C for about 2 minutes, until mass stabilization, which was the time required for the equipment to indicate the final moisture content.

#### 4.5.2 Ash

The determination of ash content in bee pollen and formulations was carried out according to the AOAC 923.05 methodology. First, clean and dry porcelain crucibles were prepared and placed in a muffle furnace at 600 °C for approximately 30 minutes to remove any residual moisture and organic matter, then stored in a desiccator to keep them thoroughly dry. After cooling in the desiccator, the crucibles were weighed, followed by the addition of approximately 1 g of the sample in triplicate, and then weighed again.

The crucibles were returned to the muffle furnace for about 30 minutes, which was sufficient for complete carbonization of the organic matter, followed by cooling and reweighing. This process was repeated until the samples reached a constant mass.

The ash content was calculated using the following equation (ISO/FDIS, 2023):

$$X = \frac{(M_3 - M_1)}{M_2} \times 100 \quad (\text{Eq.1})$$

X: the ash content [%]

M<sub>1</sub>: mass of the dry and empty ash dish [g]

M<sub>2</sub>: mass of the samples [g]

M<sub>3</sub>: mass of the dry ash dish with the ashes [g]

#### 4.5.3 Crude fat

Lipid extraction was carried out using the Soxhlet method with petroleum ether as the solvent (Figure 15), as described in AOAC 989.05 methodology. Approximately 3 g of each sample was placed in cellulose cartridges and subjected to a continuous extraction cycle for 6 hours at 70°C. After the process, the solvent was initially removed using a rotary evaporator. To ensure complete evaporation of any residual petroleum ether, the samples were then placed in an oven at 105 °C until a constant mass was reached. Lipid quantification was performed gravimetrically by weighing the dry tube and the residue in the tube.



**Figure 15 – Soxhlet system**  
**Source – Own authorship (2025)**

The total lipids were calculated using the following equation (ISO/FDIS, 2023):

$$X = \frac{(M_2 - M_1)}{M_3} \quad (\text{Eq. 2})$$

X: Total lipid [%];

M<sub>1</sub>: mass of the empty tube, which is dried until a constant mass is achieved [g];

M<sub>2</sub>: mass of the tube with fat after extraction and drying [g];

M<sub>3</sub>: mass of the sample [g].

#### 4.5.4 Total proteins

The protein content of the pollen and formulations A, B, and C was determined using the Kjeldahl method, as described by AOAC 920.87. Initially, the samples were removed from refrigeration, homogenized with a spatula, weighed in triplicate on an analytical balance, using approximately 0.5 g per replicate, and placed into digestion tubes.

During the digestion step (Figure 16), the following reagents were added to each tube: 2 Kjeldahl catalyst tablets, 15 mL of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), and 2.5 mL of distilled water for the blank sample only. The tubes were then placed in a digestion block at 400 °C for a total digestion time of approximately 1 hour. After cooling, 25 mL of distilled water was added to each tube, before proceeding to the distillation step.

Distillation was carried out using the PRO-NITRO equipment (automatic nitrogen analyzer). Before analyzing the samples, distilled water was used as a control (blank). For analysis, each tube was shaken and then submitted to individual reading. A correction factor of 5.6 was used, based on ISO/FDIS 24382:2023(E).

The total protein content was calculated using the equation (ISO/FDIS, 2023):

$$N = \frac{(M_s - M_b)}{m} \times 5.6 \times 100 \quad (\text{Eq. 3})$$

N: Total protein content [%];

M<sub>s</sub>: Mass of N (nitrogen) in the sample [mg];

M<sub>b</sub>: Mass of N in the blank [mg];

m: mass of the sample [mg].



**Figure 16 - Digestion step**  
**Source – Own authorship (2025)**

#### 4.5.5 Carbohydrates

The carbohydrate content of the samples was obtained by differential calculation, based on the following equation defined in the literature (AOAC, 2000):

$$\% \text{ Carbohydrates} = 100 - (\% \text{Moisture} + \% \text{Ash} + \% \text{Proteins} + \% \text{Lipids}) \quad (\text{Eq. 4})$$

#### 4.5.6 Calories

The energy value, expressed in kcal, was calculated per 100 g of sample using the following equation (TOMASSI and MERENDINO, 2006):

$$\text{Calories} \left( \frac{\text{kcal}}{100\text{g}} \right) = 4 \times (\% \text{Proteins} + \% \text{Carbohydrates}) + 9 \times (\% \text{Lipids}) \quad (\text{Eq.5})$$

## 4.6 Microbiological analysis

Microbiological analysis was performed to assess the hygienic-sanitary quality of the bee pollen and formulations A, B, and C. The procedure began with the homogenization of 5 g of each sample (in triplicate) in 45 mL of sterile peptone water. Serial dilutions of  $10^{-1}$ ,  $10^{-2}$ , and  $10^{-3}$  were prepared, from which volumes of 0.1 mL were plated according to the type of medium used, except for the plates with Compact Dry selective medium, where 1 mL was inoculated.

All procedures were conducted under aseptic conditions, and the results were expressed in colony-forming units per gram of sample (CFU/g), based on the average of the triplicate samples analyzed.

The samples were analyzed using the following selective media and conditions:

- **Plate Count Agar (PCA, LiofilChem, Italy):** used for the enumeration of total mesophilic microorganisms, according to ISO 4833-2 (2013), with incubation under aerobic conditions at 30 °C for 72 h.
- **Baird-Parker Agar (BP, LiofilChem, Italy):** used for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species), incubated at 37 °C for 48 hours.
- **Dichloran Glycerol Agar 18% (DG18, LiofilChem, Italy):** selective medium for the growth of molds and yeasts, according to ISO 21527-2:2008, incubated at 25 °C for 5 days.
- **The ready-to-use Compact Dry EC:** dehydrated chromogenic plates used for the simultaneous identification of total coliforms (red or red violet and blue colonies) and *Escherichia coli* (blue colonies), with readings taken after incubation at 37 °C for 24-48 hours.

## 4.7 Sensory analysis

A sensory test was conducted with 50 untrained consumers to evaluate the acceptance and preference of formulations A, B, and C (Figure 17), which were identified by randomly assigned three-digit numbers. The test was applied using a structured questionnaire, attached as an appendix to this thesis, which included general questions about the consumers followed by questions regarding the acceptance of formulations A, B, and C, using a 7-point hedonic scale. This scale assessed the attributes of appearance, aroma, flavor, texture, and overall acceptance. At the end, the tasters were asked about

their preference for the samples, and they were asked to indicate which sample they preferred.



**Figure 17 – Sensory Analysis**  
**Source – Own authorship (2025)**

#### **4.8 Statistical analysis**

The program used in the statistical analysis was Minitab (version 14, Minitab Ltd., Coventry, UK). The normality and homogeneity of the variances were assessed by the Shapiro–Wilk and Levene tests, respectively. Most of the time, the data were found to be normal. When homogeneity of variances was observed, ANOVA was applied. Then, in case of significant differences between samples ( $p < 0.05$ ), the Tukey test was applied. In situations where no homogeneity of variances was observed, ANOVA–Welch was used to detect significant differences between samples. In the case in which this occurred, the Games–Howell test was then applied.

In the discussion of the sensory analysis results, both absolute and relative frequencies were used. To verify differences between gender and age groups, the chi-square test was used. A significance level of 5% was considered. The data obtained from the sensory analysis (using a structured scale) were analyzed using non-parametric tests, specifically the Wilcoxon pairwise test. Spearman’s rank correlation coefficients were determined to evaluate how strongly two sensory parameters were correlated.

A principal component analysis (PCA) was also performed on the results of the three final formulations. The PCA score plot was used to differentiate the formulations, taking into account the physicochemical and sensory analysis data. The number of components to retain for data analysis was evaluated by: (i) the respective eigenvalues (must be  $>1$ ); (ii) Cronbach’s parameter (that should be positive); and (iii) the total percentage of variance (that should be as high as possible), explained by the number of components selected.

## 5. Results and Discussion

### 5.1 Palynological results

The palynological analysis of the bee pollen revealed a multifloral profile, with a predominance of pollen grains from the species *Jasione montana*, belonging to the family Campanulaceae, representing 42.04% of the total identified grains, indicating it as the main floral source for the bees during the collection period (end of May).

Among the 1,244 pollen grains counted, which can be analyzed in Table 11, a significant presence of other taxa was also observed, such as *Quercus* spp. (28.46%), *Echium* spp. (11.17%), and *Rubus* spp. (7.56%), confirming the heterogeneous floral origin of the pollen and allowing it to be classified as multifloral.

**Table 11** – Results of palynological analysis on bee pollen

Family/ Pollen type	Total pollen	
	Number	%
<b>ASTERACEAE</b>		
<i>Centaurea</i> spp.	2	0.16
Type <i>Anthemis arvensis</i>	7	0.56
Type <i>Crepis capillaris</i>	14	1.13
<b>BORAGINACEAE</b>		
<i>Echium</i> spp.	139	11.17
<b>CAMPANULACEAE</b>		
<i>Jasione montana</i>	523	42.04
<b>CISTACEAE</b>		
<i>Cistus</i> spp.	2	0.16
<b>FABACEAE</b>		
Type <i>Cytisus striatus</i>	24	1.93
<i>Vicia</i> spp.	20	1.61
<b>FAGACEAE</b>		
<i>Quercus</i> spp.	354	28.46
<b>JUGLANDACEAE</b>		
<i>Juglans regia</i>	5	0.4
<b>OLEACEAE</b>		
<i>Olea europaea</i>	31	2.49
<b>POACEAE</b>	1	0.08
<b>ROSACEAE</b>		
<i>Prunus</i> spp.	1	0.08
<i>Rubus</i> spp.	94	7.56
<b>OTHERS</b>	27	2.17
<b>TOTAL</b>	1244	100

Furthermore, even if the hives are placed in olive groves, the percentage of pollen grains coming from olive trees was reduced (2.49%) because olive flowers are "anemophilous", meaning that they are pollinated by the wind. Thus, the flowers do not

produce nectar, and for this reason, they are not visited much by bees. Some pollen grains may appear in the honey, but only occasionally, which explains the results obtained.

The multifloral nature of the pollen used in this study may contribute positively to its nutritional potential, considering that different floral origins are associated with a greater variety of health-promoting components.

## 5.2 Physical results

### 5.2.1 Color

The results of the color analysis are presented in Table 12.

**Table 12** – Results of color analysis on energy balls formulations (A, B and C) with parameters  $L^*$  (Lightness),  $a^*$  (red-green axis),  $b^*$  (yellow-blue),  $C^*$  (Chroma index) and  $h$  (hue angle)

Samples	$L^*$	$a^*$	$b^*$	$C^*$	$h$
A	$33.6 \pm 0.7^a$	$11.2 \pm 0.4^b$	$11.9 \pm 0.5^{a,b}$	$16.3 \pm 0.4^{a,b}$	$46.8 \pm 1.8^a$
B	$34.3 \pm 1.4^a$	$11.7 \pm 0.5^{a,b}$	$12.5 \pm 1.5^a$	$17.2 \pm 0.8^a$	$46.8 \pm 4.5^a$
C	$33.0 \pm 2.3^a$	$12.1 \pm 0.4^a$	$10.8 \pm 1.0^b$	$16.2 \pm 0.5^b$	$41.7 \pm 3.4^a$

Different letters in a column indicate significant differences between samples ( $p < 0.05$ ).

The  $L^*$  parameter, which indicates the lightness of the sample (0 = black and 100 = white), did not show significant statistical differences between the formulations, suggesting that the three formulations presented similar lightness values.

Regarding  $a^*$  parameter, which expresses the tendency towards reddish tones (positive values) or greenish tones (negative values), there was a significant difference between formulations A and C. Formulation C showed the highest value (12.1), followed by B (11.7) and A (11.2), suggesting an intensification of red tones as the proportion of pollen increases: ingredient naturally rich in phenolic compounds with reddish pigmentation.

As for the  $b^*$  parameter, related to the predominance of yellowish (positive values) or bluish (negative values) tones, formulation B obtained the highest value (12.5), which is higher than that of formulation C (10.80). These results reflect the influence of ingredients such as honey and almond flour, which contribute to more yellowish tones.

*Chroma* index ( $C^*$ ), which represents the saturation or intensity of the color. Formulation B was the most intense (17.2), followed by A (16.3) and C (16.2), revealing that formulations with higher almond and honey content provided more vivid colors, an aspect that is sensorially appealing to consumers.

Finally, the hue angle ( $h$ ), which defines the color tone ( $0^\circ = \text{red}$ ,  $90^\circ = \text{yellow}$ ), no significantly differences were observed between samples. All samples were located in the orange region of the chromatic circle, consistent with the combination of natural ingredients rich in pigments such as carotenoids and flavonoids.

Overall, the data revealed that subtle variations in formulation composition originated samples with dark color, with warm tones (reddish to orange) and with similar hue values.

### 5.2.2 Texture

The texture of Formulations A, B, and C was evaluated through Texture Profile Analysis (TPA), simulating human mastication with two consecutive compressions. The results of this analysis are presented in Figure 18, which shows a boxplot dispersion.

Regarding normality and homogeneity of variances among the groups, all variables showed normal and homogeneous distribution, with the exception of the adhesiveness parameter, which was not homogeneous, especially in sample C, which showed the greatest variability. In this case, it was applied the ANOVA-Welch test.

Hardness, corresponding to the maximum force recorded in the first compression, was significantly higher in formulation C, which had a median value of 736 g, compared to formulations A (336 g) and B (570 g), which differed statistically from each other. Sample C presented the highest median for hardness indicating to be the most resistant to deformation, possibly due to greater structural compaction promoted by ingredients such as bee pollen. However, softer products tend to be preferred by consumers who value a smoother chewing experience. This may suggest that formulation A will probably be preferred to the others in what concerns hardness.

The fracturability parameter showed a null value in all formulations, confirming that none of them suffered abrupt rupture under compression. This feature was not expected in products with a homogeneous and flexible texture, such as energy balls.

Regarding adhesiveness, high variability was observed in formulation C. This sample presented the most negative value (median equal  $-31.1 \text{ g.s}$ ), suggesting greater adhesion to the equipment probe. Formulations A and B, with less negative values (medians equal to  $-4.3 \text{ g.s}$  and  $-5.6 \text{ g.s}$ , respectively), showed lower adhesiveness.

Elasticity, which reflects the sample's ability to return to its original shape after deformation, also showed significant differences among all formulations. Formulation A

was the most elastic (0.34), followed by B (0.30) and C (0.24), suggesting that A has a more flexible structure and may provide a more pleasant chewing experience.

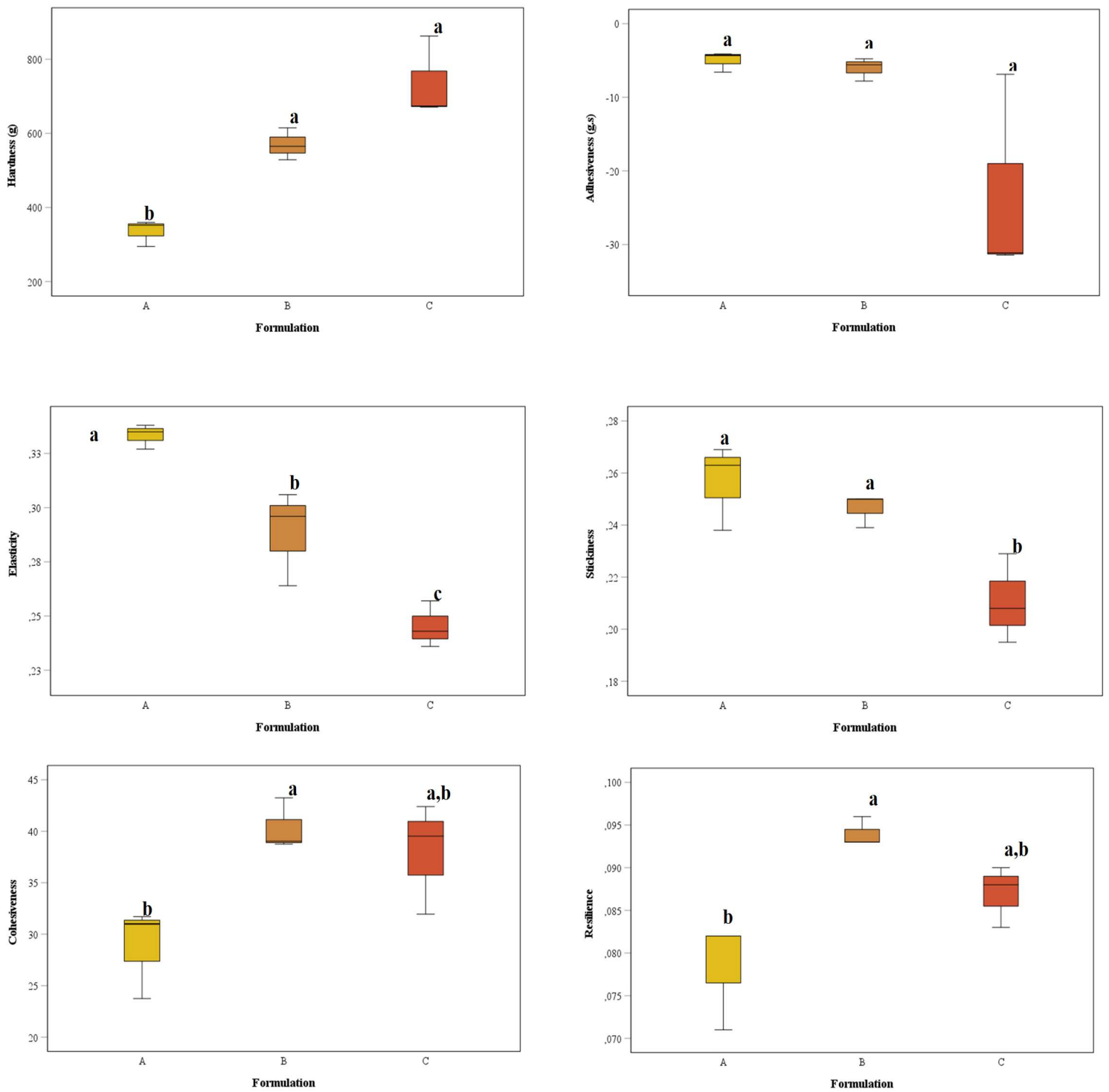
For the stickiness parameter, the results were similar between A and B, with values of 0.26 g and 0.25 g, respectively. Sample C presented a slightly lower value (0.21 g) and was statistically different from the others, indicating a less sticky mouthfeel, possibly associated with a higher presence of bee pollen, which may act as an absorbing agent.

Cohesiveness, related to the internal integrity of the sample, showed significant statistical differences among the formulations A and B. Sample B had the highest value (39.0) and was statistically superior to sample A (31.0), which showed the lowest cohesiveness. Sample C (39.5) had an intermediate value, statistically similar to both. This suggests that formulation B has a greater ability to maintain its structure after deformation—a desirable feature to prevent crumbling during consumption. Sample A, on the other hand, showed lower internal integrity, which may result in a more brittle texture, while sample C demonstrated a balance between structure and softness.

Resilience, which assesses the immediate recovery capacity of the sample after the first deformation, showed differences among the formulations. Sample B had the highest value (0.093), being statistically superior to sample A (0.082), which had the lowest value. Sample C (0.088) presented an intermediate result, statistically similar to both. These data suggest that sample B has greater immediate elastic recovery capacity, indicating a more structured and elastic texture in the short term. Sample A showed lower resilience, reinforcing its greater initial deformability. Formulation C, in turn, balances these two behaviors.

In summary, the results showed significant differences among the formulations, with emphasis on sample C, which exhibited greater hardness and lower elasticity and stickiness: characteristics that may be interpreted as positive or negative depending on consumer preference. Sample A stood out for its higher elasticity and lower cohesiveness, potentially being more attractive to consumers seeking softer products with a less dense

texture. Sample B, meanwhile, showed a balance between hardness and elasticity, potentially appealing to consumers who prefer an intermediate consistency.



**Figure 18** – Results of texture analysis of energy balls formulations (A, B, and C) with parameters Hardness, Adhesiveness, Elasticity, Stickiness, Cohesiveness, and Resilience  
**Source - IBM CORP. (2022)**

### 5.3 Chemical results

The chemical results obtained for the three final formulations are represented in Table 13. The results for the pollen regarding some determinations are also presented.

**Table 13** - Results of chemical analysis on energy ball formulations (A, B and C) and pollen sample (P)

Formulations	Moisture (%)	Ash (%)	Lipids (%)	Proteins (%)	Carbohydrates (%)	Calories (kcal/100g)	Energy Value of Protein (%)
P	7.05 ± 0.61 <sup>a</sup>	2.23 ± 0.05 <sup>a</sup>	6.62 ± 0.13 <sup>d</sup>	17.3 ± 0.3 <sup>a</sup>	66.8 ± 0.7 <sup>a</sup>	396 ± 3 <sup>d</sup>	17.5±0.4 <sup>a</sup>
A	2.44 ± 0.06 <sup>c</sup>	2.27 ± 0.15 <sup>a</sup>	26.01 ± 0.01 <sup>a</sup>	11.9 ± 0.7 <sup>c</sup>	57.3 ± 0.6 <sup>c</sup>	511 ± 1 <sup>a</sup>	9.3±0.5 <sup>d</sup>
B	2.88 ± 0.14 <sup>b,c</sup>	2.08 ± 0.14 <sup>a</sup>	23.70 ± 0.30 <sup>b</sup>	13.2 ± 0.2 <sup>b</sup>	58.2 ± 0.2 <sup>c</sup>	499 ± 1 <sup>b</sup>	10.6±0.2 <sup>c</sup>
C	3.49 ± 0.07 <sup>b</sup>	2.05 ± 0.20 <sup>a</sup>	20.29 ± 0.24 <sup>c</sup>	13.9 ± 0.3 <sup>b</sup>	60.2 ± 0.3 <sup>b</sup>	479 ± 2 <sup>c</sup>	11.6±0.2 <sup>b</sup>

Different letters in a column indicate significant differences between samples (p<0.05).

#### 5.3.1 Moisture

The bee pollen exhibited a moisture content of 7.05%, a value considered adequate for preservation according to Food and Agriculture Organization of the United Nations (FAO) criteria, which recommend that dried pollen should contain less than 10% moisture to ensure its microbiological stability and food safety (KRELL, 1996). This low water content suggests that the pollen was efficiently dehydrated, which inhibits the growth of spoilage or pathogenic microorganisms during storage.

As for the developed formulations, the moisture contents varied significantly among the groups. Formulation C showed the highest value among the three (3.49%), followed by B (2.88%) and A (2.44%). Statistical analysis revealed that Formulations A and B did not differ significantly from each other; however, Formulation A was distinct from Formulation C.

As pollen has a high moisture content and Formulation C has the highest pollen percentage (30%), this can explain the results obtained. However, all formulations maintained moisture levels below 4%, a level that, as indicated in the literature, prevents microbial growth and ensures stability, extending the shelf life of ready-to-eat products (BARBOSA-CÁNOVAS et al., 2020).

### **5.3.2 Ash**

The pollen showed an average ash content of 2.23%, reflecting its nutritional potential, as the mineral fraction contributes essential elements such as calcium, magnesium, iron, potassium, and zinc.

Regarding the developed formulations, no significant statistical differences were observed among the groups, showing the energy balls a mineral content of around 2%.

### **5.3.3 Lipids**

The results obtained are presented in Table 13, where it can be observed that the bee pollen sample (P) showed an average lipid content of 6.62%, a value consistent with those found in the literature, which range from 0.41% to 13.5% for dried bee pollen samples, values found in a systematic review of 100 articles (THAKUR and NANDA, 2020).

Among the energy ball formulations, the lipid values were considerably higher than those of the pollen, namely: 26.01% (A), 23.70% (B), and 20.29% (C), due to the presence of ingredients naturally rich in fats, such as olive oil and almond flour.

Formulation A had the highest lipid content, which can be explained by a higher proportion of these ingredients due to its lower bee pollen content. Although high lipid values are commonly associated with greater caloric intake, it is important to highlight that the fat composition in the formulations consists mainly of unsaturated fatty acids, due to the nature of the ingredients used.

These results are consistent with the goal of developing an energetic product, where lipids contribute not only to nutritional value but also to texture and palatability.

### **5.3.4 Proteins**

Regarding the total protein content analyses, the results are summarized in Table 13 where it can be seen that the bee pollen (P) showed an average protein content of 17.3%. This value falls within the range reported in the literature for dried pollen samples, which varies between 4.5% and 40.7%, with a global average of approximately 21.3% (THAKUR and NANDA, 2020). The results obtained in this study demonstrate that the pollen used has a relevant protein content, although slightly below the estimated global average, which can be explained by variations in botanical origin and environmental conditions during collection.

In the energy ball formulations, protein contents ranged from 11.9% (Formulation A) to 13.9% (Formulation C), with Formulation B showing an intermediate value of

13.2%. These values reflect the incorporation of pollen as a functional ingredient, but are also influenced by the proportions of the other components in the mixture, such as dried fig and almond flour, which have lower protein contents compared to pure pollen.

Among the formulations, sample C presented the highest protein content, while formulation A had the lowest, suggesting that variations in ingredient composition impact the final nutritional profile, which may be directly related to the increased proportion of pollen. However, only in Formulation C, a value of 12% of the energy value of the food was provided by protein, which is the criterion required to use the claim that a food is a source of protein (Regulation (EC) No. 1924/2006). When performing a t-test to compare the energy values provided by the protein of Formulation C with the theoretical value of 12%, no significant differences were observed ( $p=0.078$ ).

Overall, the results highlight the potential of pollen as a natural protein ingredient that can enrich functional foods. In addition to its quantitative content, pollen is also recognized for containing essential amino acids in balanced proportions, which reinforces its applicability in formulations aimed at health and energy performance.

### **5.3.5 Carbohydrates**

In Table 13, it is observed that the pollen sample (P) showed the highest carbohydrate content (66.8%). Among the formulations with a mixture of ingredients, the percentage of carbohydrates increased progressively with the addition of pollen: Formulation A (10% pollen) presented 57.3%, followed by B (20%) with 58.2%, and C (30%) with 60.2%.

This trend suggests that, despite the presence of other carbohydrate-rich ingredients such as dried fig and honey, bee pollen contributed significantly to the total carbohydrate content of the formulations. As the bee pollen proportion increased, the final carbohydrate value also rose, indicating that pollen, in addition to proteins and lipids, contains a relevant fraction of carbohydrates in its composition.

### **5.3.6 Calories**

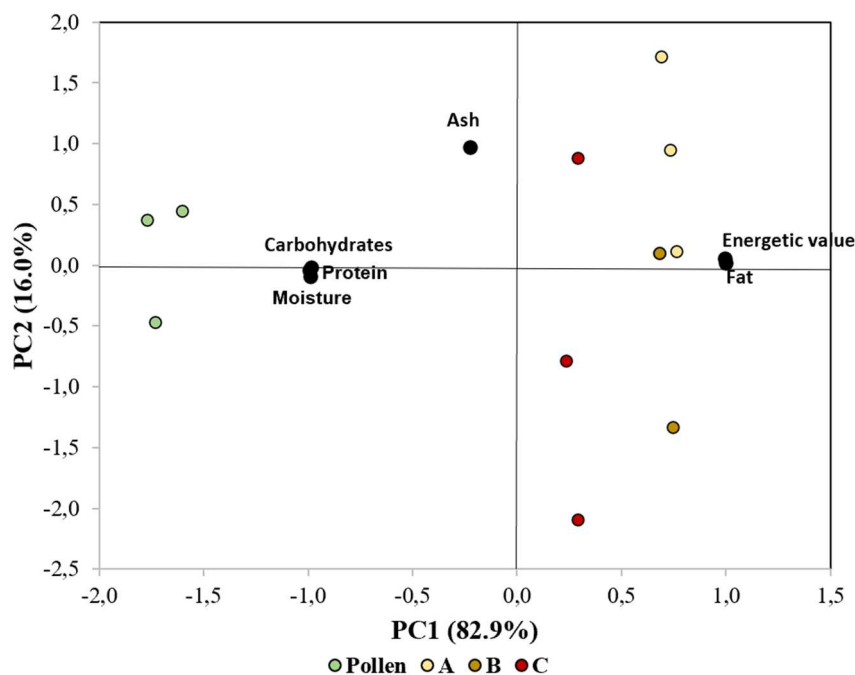
In Table 13, it is observed that the formulation with the highest energy value was formulation A (511 kcal/100g), followed by B (499 kcal/100g), C (479 kcal/100g), and finally the pollen sample (P), with 396 kcal/100g.

Formulation A presented the highest lipid content (26.01%) and also considerable values of proteins (11.9%) and carbohydrates (57.3%), which justifies its higher energy value.

Bee pollen, despite presenting the highest carbohydrate content among the samples (66.8%) and the highest protein content (17.3%), had the lowest lipid content (6.62%), which significantly impacted its total caloric value, since lipids are the macronutrient with the highest energy density. In formulations B and C, there is a tendency for lipid reduction as the amount of pollen increases (from A to C), which is also reflected in the progressive decrease in caloric value.

### 5.3.7 Principal Component Analysis

A Principal Component Analysis (PCA) was applied to classify the pollen and the three formulations into groups. Figure 19 shows the scores of the first two principal components for the pollen and the energy balls of the three formulations. The first two principal components explained 98.9% of the total variation (PC1 = 82.9% and PC2 = 16.0%, respectively).



**Figure 19** – Principal component analysis plot of pollen and energy balls of the three formulations, considering the chemical properties

PC1 was mainly correlated positively with the fat and energetic value. On the contrary, it correlated negatively with protein, moisture, and carbohydrates. PC2 was positively correlated with ash. The results of the pollen and the three formulations are depicted in Figure 19, each represented by a different color. The pollen samples were those that were better separated from the energy ball formulations, while there was some



## 5.4 Microbiological results

The results of the microbiological analyses of bee pollen and formulations A, B, and C are shown in Table 14.

Bee pollen sample showed the highest total microbial load, with  $1.6 \times 10^6$  CFU/g of microorganisms at 30°C, which classifies it as “questionable”, according to INSA guidelines for ready-to-eat foods, considering that this product is included in subgroup 2D (raw composite foods) (Table 15). This high load is consistent with the raw and untreated nature of the pollen, which may be exposed to environmental microorganisms during the collection and storage process. However, despite the high microbial load, no colonies of *Staphylococcus aureus* (*S. aureus*), total coliforms, *Escherichia coli* (*E. coli*), molds, or yeasts were detected in the pollen sample, indicating the absence of pathogenic or spoilage microorganisms under the tested conditions.

Table 14 – Results of microbiological analysis on bee pollen (P) and energy ball formulations (A, B and C)

Samples	Microorganisms at 30°C (CFU/g)	<i>S. aureus</i> (CFU/g)	Total coliformes (CFU/g)	<i>E. coli</i> (CFU/g)	Molds (CFU/g)	Yeasts (CFU/g)
P	$1.6 \times 10^6$	< 10	< 10	< 10	< $10^2$	< $10^2$
A	$2.5 \times 10^5$	< 10	< 10	< 10	< $10^2$	< $10^2$
B	$2.8 \times 10^5$	< 10	< 10	< 10	$3.3 \times 10^2$	$1.1 \times 10^2$
C	$2.8 \times 10^5$	< 10	< 10	< 10	$1.1 \times 10^2$	$3.3 \times 10$

In the energy ball formulations, a significant reduction in the total microbial count was observed, with values of  $2.5 \times 10^5$  CFU/g (A),  $2.8 \times 10^5$  CFU/g (B), and  $2.8 \times 10^5$  CFU/g (C) (Table 14). These results classify all formulations as satisfactory, being well below the limit of  $10^6$  CFU/g allowed for products in group 2D (Table 15). The reduction observed compared to raw pollen demonstrates the effectiveness of processing, such as homogenization, the use of ingredients with antimicrobial activity (such as honey), along with the possible reduction in water activity due to the addition of honey, factors that contribute to the decrease in total microbial load. Furthermore, none of the formulations showed growth of *S. aureus*, total coliforms, or *E. coli*, indicating the adequacy of good manufacturing practices and the microbiological safety of the products.

As for filamentous fungi (molds) and yeasts, only formulations B and C showed slight growth. Sample B showed  $3.3 \times 10^2$  CFU/g of molds and  $1.1 \times 10^2$  CFU/g of yeasts, while sample C showed  $1.1 \times 10^2$  CFU/g of molds and  $3.3 \times 10$  CFU/g of yeasts. Although

these values are below the limits considered critical for non-sterile products, their presence may be associated with the use of minimally processed natural ingredients, such as dried figs, or environmental exposure during the shaping of the balls.

**Table 15** – Guidelines for microorganisms in ready-to-eat foods of subgroup 2D

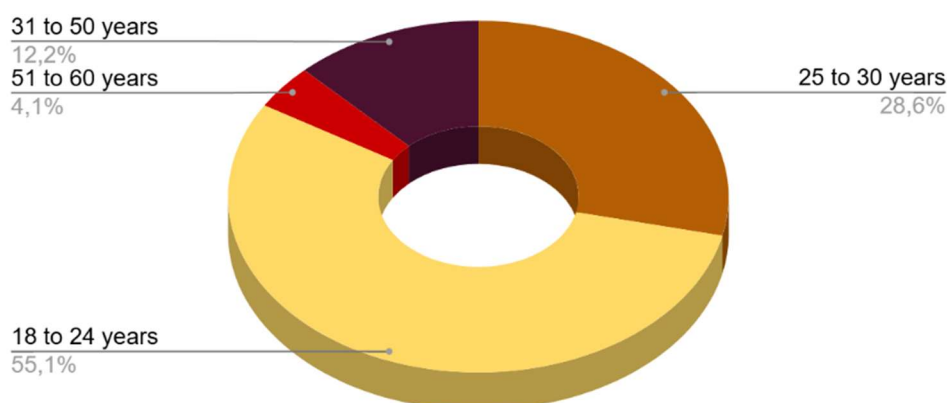
Microorganisms	Satisfactory	Questionable	Unsatisfactory
Microorganisms at 30°C	<10 <sup>6</sup>	10 <sup>6</sup> – ≤10 <sup>7</sup>	>10 <sup>7</sup>
<i>S. aureus</i>	<10 <sup>2</sup>	10 <sup>2</sup> - ≤10 <sup>4</sup>	>10 <sup>4</sup>
<i>E. coli</i>	< 10	10 – ≤10 <sup>2</sup>	≥ 10 <sup>2</sup>
Molds	< 5 x 10 <sup>2</sup>	5 x 10 <sup>2</sup> - ≤10 <sup>6</sup>	>10 <sup>6</sup>
Yeasts	<10 <sup>5</sup>	10 <sup>5</sup> - ≤ 10 <sup>6</sup>	>10 <sup>6</sup>

Source - (INSA, 2019)

Overall, the microbiological results obtained demonstrate that the formulations presented good hygienic-sanitary quality standards, especially considering the absence of pathogenic microorganisms, and are considered safe for consumption. The slight presence of fungi in some samples indicates the need for greater attention to handling, storage conditions, and humidity, especially if the goal is to extend shelf life without the use of preservatives.

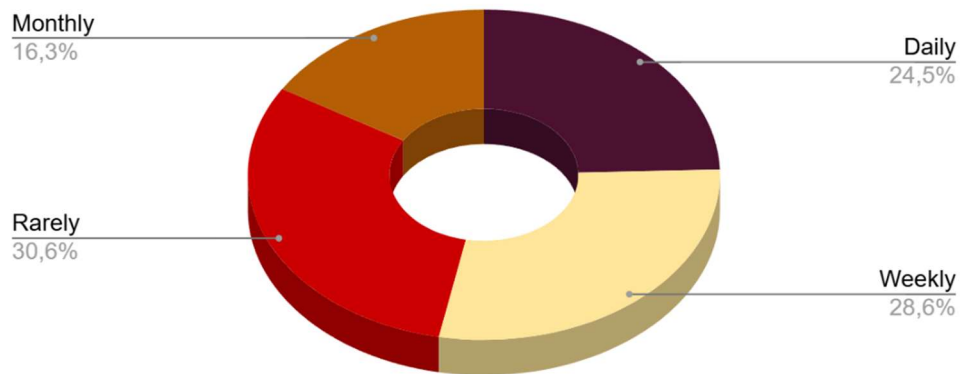
## 5.5 Sensory results

Only 48 consumers had their data considered in the statistical analysis, since two participants did not submit their responses correctly, despite having completed the tasting. In Figure 21, it can be seen that the majority of participants were between 18 and 24 years old (55.1%), followed by those aged between 25 and 30 years (28.6%), with a predominantly female audience (61.2%).



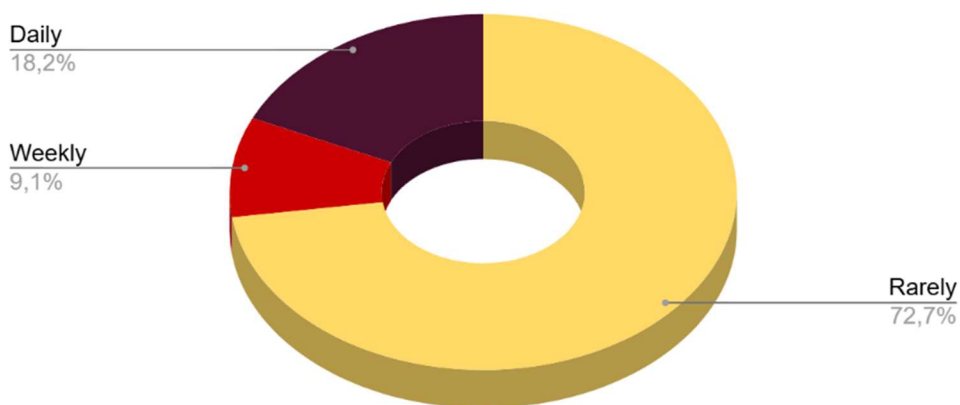
**Figure 21** – Age range of consumers

Regarding the consumption of natural, energy, and protein products, Figure 22 shows that the majority of participants reported rare (30.6%) or weekly (28.6%) consumption, while only 24.5% consumed such products daily.



**Figure 22** – Consumption of natural, energy, and protein products

Despite this, 57.1% had never heard of “energy balls,” and 77.6% had never consumed them, indicating that the product remains relatively unknown, even within the fitness community. Among those who had tried them, 72.7% stated that they consumed them rarely, as represented in Figure 23.

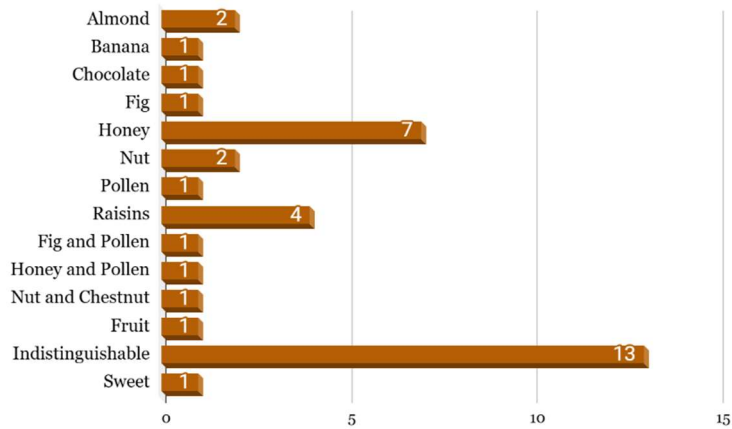


**Figure 23** – Consumption of energy balls among the consumers who reported their ingestion

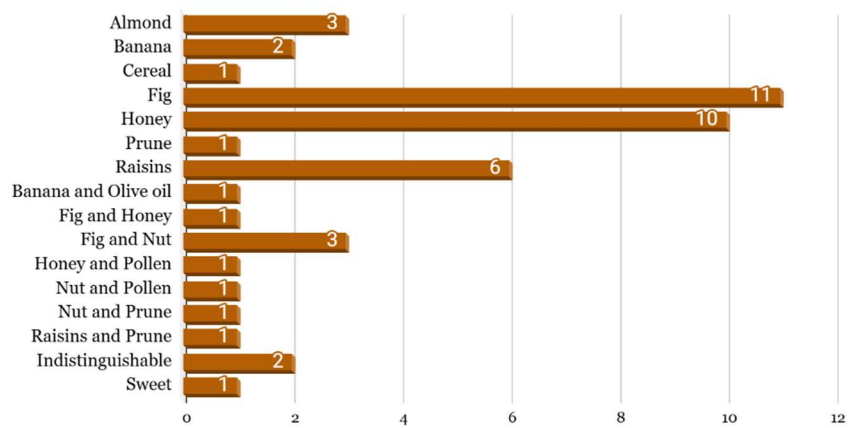
### 5.5.1 Identification of aromas and tastes

As shown in Figures 24 to 29, although a variety of aromas and flavors were identified, the results indicated that the main perceived ingredients were fig and honey. Some consumers referred to nuts, such as almonds, or combinations like honey and pollen, fig and pollen, as well as other perceptions, including herbal notes and tea, all of which evoke a natural profile and are thus linked to pollen, honey and almonds. This

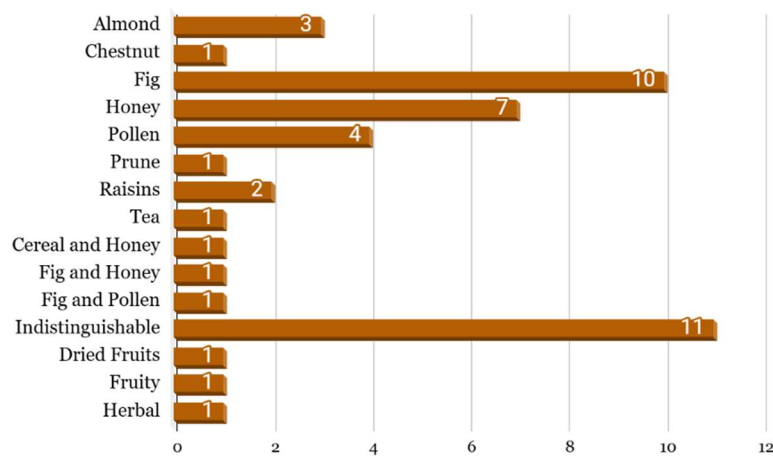
scenario demonstrates that the combination of ingredients in the energy balls achieved good congruence between the formulations and consumers' perception.



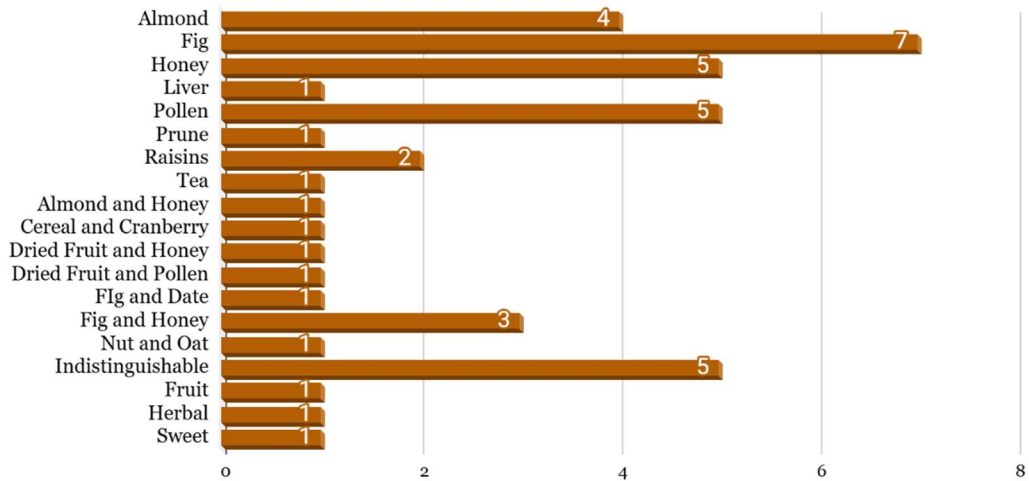
**Figure 24** – Answers about the more intense aroma in formulation A



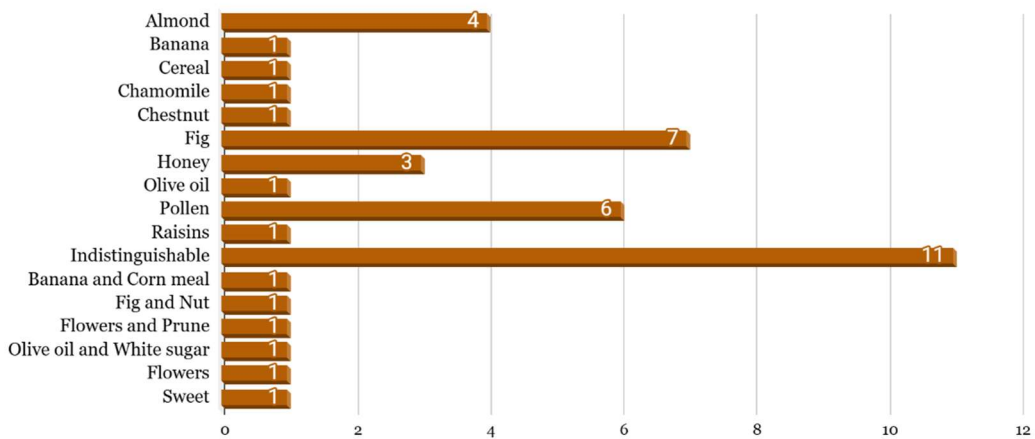
**Figure 25** – Answers about the more intense taste in formulation A



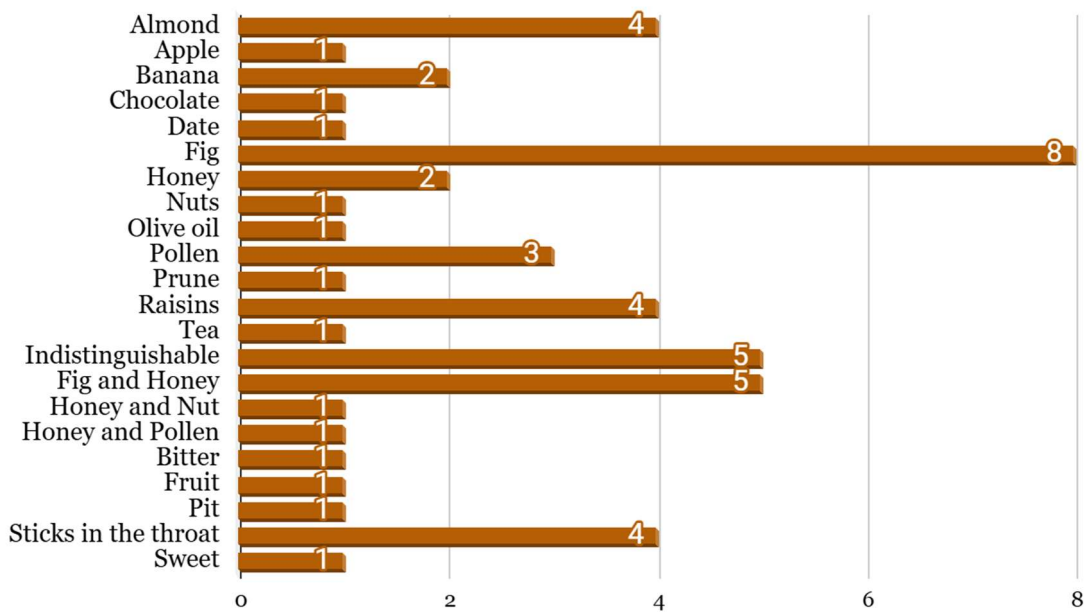
**Figure 26** – Answers about the more intense aroma in formulation B



**Figure 27** – Answers about the more intense taste in formulation B



**Figure 28**– Answers about the more intense aroma in formulation C



**Figure 29** – Answers about the more intense taste in formulation C

### 5.5.1 Statistical analysis

When analyzing the data obtained during the sensory analysis regarding consumers' experiences with energy balls, no significant differences were detected between the four age classes in their knowledge of this product ( $p = 0.306$ ) (Table 16). It was expected that the younger should know better what energy balls are; however, this was not observed.

Concerning gender, no significant differences were detected between females and males regarding their knowledge of energy balls ( $p = 0.081$ ). Considering the consumption of energy balls, no significant differences were detected between the four age classes ( $p=0.654$ ). Again, it was expected that the younger should consume more energy balls; however, this was not observed. On the other hand, females reported a higher consumption of energy balls ( $p = 0.032$ ) than males, indicating that women are more receptive to these new products.

**Table 16** – Results of the statistical analysis performed on sensory data

		18 to 24 years	25 to 30 years	31 to 50 years	51 to 60 years	p-value <sup>1</sup>	Female	Male	p-value
<b>Knowledge in Energy Balls</b>	Yes	11	4	3	2	0.306	15	5	0.081
	No	16	9	3	0		14	14	
<b>Consumption of Energy Balls</b>	Yes	6	2	2	1	0.654	10	1	0.032
	No	21	11	4	1		19	18	
<b>Preferred Formulation</b>	A	18	7	4	0	0.104	17	12	0.931
	B	3	1	2	0		4	2	
	C	6	5	0	2		8	5	
<b>Purchase of Preferred Formulation</b>	Yes	19	7	5	2	0.802	22	11	0.42
	Maybe	7	5	1	0		6	7	
	No	1	1	0	0		1	1	
<b>Purchase value</b>	€0.7 – €0.89	9	5	1	0	0.41	6	9	0.202
	€0.9 – €1.09	12	4	3	1		14	6	
	€1.10 – €1.29	5	3	0	1		7	2	
	≥ €1.30	0	0	2	0		1	1	

<sup>1</sup>Two-Sided Asymptotic Significance for Pearson's Chi-Square

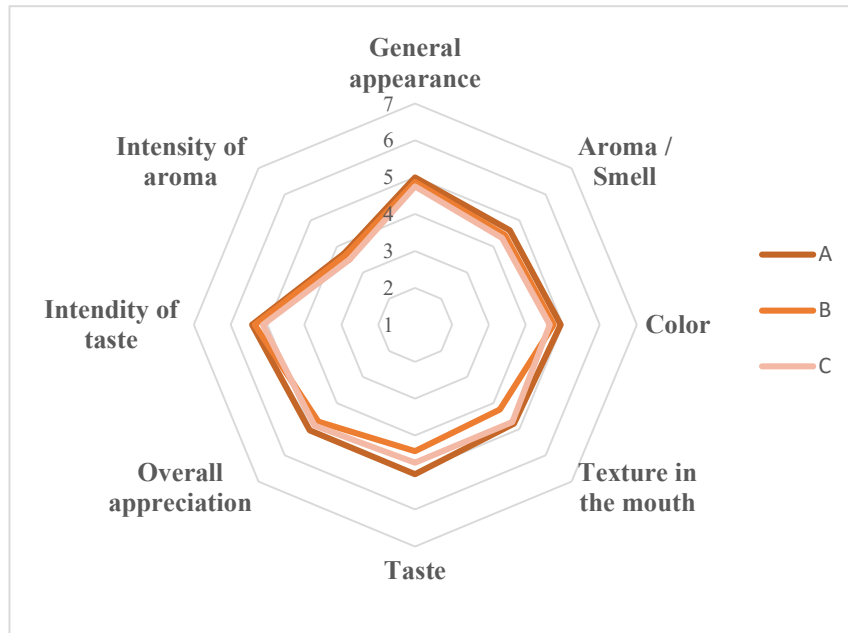
Regarding the properties evaluated during the sensory analysis, the results obtained are presented in Table 17. Significant differences were observed only in color, texture in the mouth, taste, and overall appreciation. The sample with the highest rate in those parameters was Sample A, which had the lowest percentage of pollen; however, in several parameters, this sample was not significantly different from Sample C, which had the highest pollen percentage. This means that this ingredient is not the only factor that consumers took into account, suggesting that the combination of other raw materials plays an important role in the sensory analysis.

Similar results to these could be observed in the radar charts designed for the three formulations (Figure 30).

**Table 17** – Results obtained in sensory analysis

Sensory Parameter	Value	Formulation		
		A	B	C
General Appearance	Median (Min-Max)	5.0 (1-7)	5.0 (2-7)	5.0 (1-7)
	Mean±SD	5.0±0.2	4.9±0.2	4.8±0.2
	Note:	Without significant differences (p-value <sup>1</sup> > 0.05)		
Aroma	Median (Min-Max)	5.0 (1-7)	4.0 (2-7)	5.0 (1-7)
	Mean±SD	4.6±0.2	4.4±0.2	4.3±0.2
	Note:	Without significant differences (p-value <sup>1</sup> > 0.05)		
Color	Median (Min-Max)	5.0 (2-7) <sup>a</sup>	5.0 (1-7) <sup>a,b</sup>	5.0 (1-7) <sup>b</sup>
	Mean±SD	4.9±0.2	4.8±0.2	4.6±0.2
	Note:	With significant differences between samples A and C (p-value = 0.0156)		
Texture in the mouth	Median (Min-Max)	5.0 (1-7) <sup>a</sup>	4.0 (1-7) <sup>b</sup>	5.0 (2-7) <sup>a</sup>
	Mean±SD	4.8±0.2	4.2±0.2	4.7±0.2
	Note:	With significant differences between samples A and B (p-value = 0.0166), and B and C (p-value = 0.0301)		
Taste	Median (Min-Max)	5.0 (1-7) <sup>a</sup>	5.0 (1-7) <sup>b</sup>	5.0 (2-7) <sup>a,b</sup>
	Mean±SD	5.0±0.2	4.4±0.2	4.7±0.2
	Note:	With significant differences between samples A and B (p-value = 0.0079)		
Aroma Intensity	Median (Min-Max)	3.5 (1-7)	3.5 (1-7)	4.0 (1-7)
	Mean±SD	3.7±0.3	3.7±0.2	3.5±0.2
	Note:	Without significant differences (p-value <sup>1</sup> > 0.05)		
Taste Intensity	Median (Min-Max)	6.0 (2-7)	6.0 (2-7)	5.0 (1-7)
	Mean±SD	5.4±0.2	5.3±0.1	5.1±0.2
	Note:	Without significant differences (p-value <sup>1</sup> > 0.05)		
Overall Appreciation	Median (Min-Max)	6.0 (1-7) <sup>a</sup>	5.0 (2-7) <sup>b</sup>	5.0 (2-7) <sup>a,b</sup>
	Mean±SD	5.0±0.2	4.7±0.2	4.8±0.2
	Note:	With significant differences between samples A and B (p-value = 0.0237)		

<sup>1</sup>p-value of the Wilcoxon pairwise test



**Figure 30** – Results of hedonic evaluation on energy balls formulations (A, B and C)

Table 18 presents the Spearman correlations calculated between the evaluated parameters, along with the corresponding significance levels. Several significant correlations were observed, and all of them were positive, suggesting that the observations have a similar rank between the two variables.

**Table 18** – Spearman correlations ( $\rho$ ) calculated between the evaluated sensory parameters

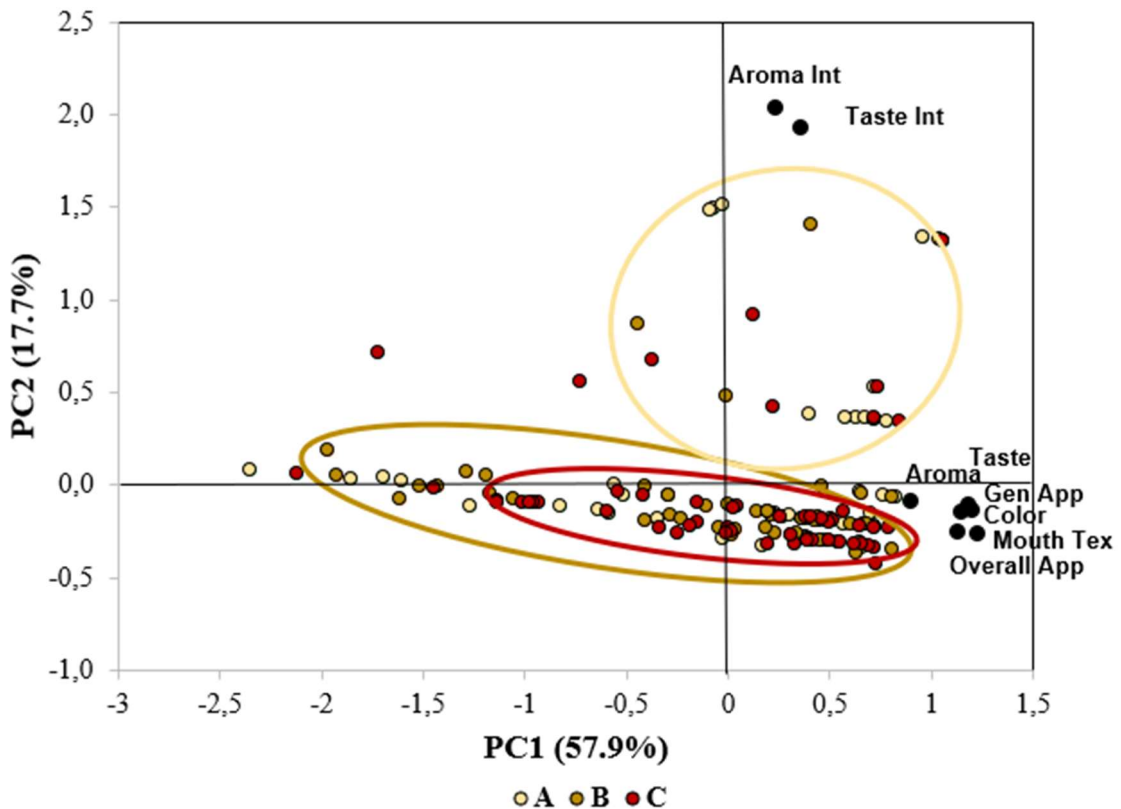
	General App.	Aroma	Color	Mouth Texture	Taste	Aroma Intensity	Taste Intensity	Overall App.
General App.	--	0.610**	0.799**	0.642**	0.668**	0.192*	0.337**	0.752**
Aroma	--	--	0.538**	0.404**	0.510**	0.259**	0.140	0.575**
Color	--	--	--	0.627**	0.623**	0.202*	0.258**	0.676**
Mouth Texture	--	--	--	--	0.828**	0.098	0.337**	0.836**
Taste	--	--	--	--	--	0.110	0.331**	0.683**
Aroma Intensity	--	--	--	--	--	--	0.288**	0.179*
Taste Intensity	--	--	--	--	--	--	--	0.331**

Note: \*\*The correlation is significant at the 0.01 level. \*The correlation is significant at the 0.05 level.

The aroma and color significantly affected the general appearance, beyond other parameters. It was interesting to note that the aroma was slightly affected by the aroma intensity ( $\rho = 0.259$ ). Similar behavior was observed for the taste, with the taste intensity ( $\rho = 0.331$ ). So, the aroma and taste are not only ranked by their intensities, but there are other parameters that influence them. The overall appreciation of the energy balls was positively correlated mainly with mouth texture ( $\rho = 0.836$ ), general appearance ( $\rho =$

0.752), taste ( $\rho = 0.683$ ), and color ( $\rho = 0.676$ ). Although the aroma, taste intensity, and aroma intensity also contributed to the overall appreciation, their contributions were lower ( $< 0.6$ ).

A Principal Component Analysis (PCA) was applied to classify the formulations into groups. Figure 31 shows the scores of the first two principal components for the energy balls of the three formulations. The first two principal components explained 75.6% of the total variation (PC1 = 57.9% and PC2 = 17.7%, respectively).

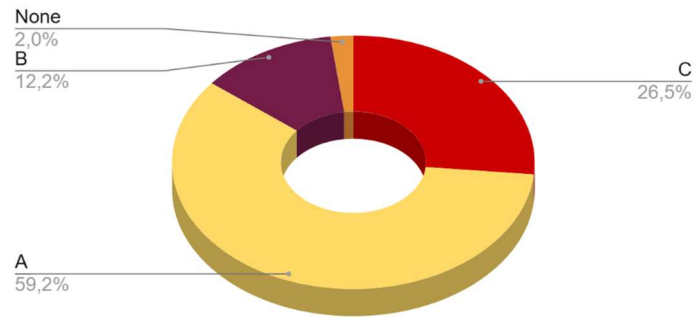


**Figure 31** – Principal component analysis plot of energy balls of the three formulations, considering the sensory data

PC1 was mainly correlated positively with the overall appreciation, general appearance, taste, color, and mouth texture. PC2 was highly correlated with aroma and taste intensities. The results of the three formulations are depicted in Figure 31, each represented by a different color. Although there was a high overlap in values between the three formulations, Formulation B presented a greater number of cases in which consumers evaluated the energy balls of this formulation less positively in terms of overall appreciation, general appearance, and taste compared to Formulations A and C. Both formulations presented better results.

Additionally, at the end of the sensory tasting, consumers were asked to indicate their preferred formulation, being the results shown in Figure 32. Formulation A was the

preferred option, followed by C, in line with the observations from PCA. No significant differences were detected among the four age classes ( $p = 0.104$ ) and gender ( $p = 0.931$ ) (Table 16).



**Figure 32** – Favorite’s formulations of consumers

In terms of the desire to purchase the preferred formulation, no significant differences were detected among the four age classes ( $p = 0.802$ ) or by gender ( $p = 0.420$ ), with the positive answer being the most frequent. On the other hand, only two consumers said that they would not purchase the preferred formulation. These results indicated that the tested formulations had the potential to be well accepted in the market in the future.

When considering the possible purchase value, significant differences were detected among the four age classes ( $p = 0.041$ ). The majority of the youngest consumers (18-24 and 25-30 years) mentioned a recommended purchase value between 0.70 and 1.09 euros, while two consumers aged 51-60 years mentioned a price equal to or higher than 1.30 euros, which is likely related to their higher economic income. On the contrary, no significant differences in the purchase value were observed between genders ( $p = 0.202$ ).

## 6. Conclusions and Future perspectives

The present study aimed to develop and characterize energy ball formulations using natural ingredients typical of the Trás-os-Montes region, with an emphasis on the incorporation of bee pollen as a protein source. The physicochemical, microbiological, and sensory analyses demonstrated that the developed formulations met technological feasibility criteria, food safety standards, and were well accepted by consumers.

The data revealed that, although the presence of bee pollen at high concentrations (30%) had a slightly negative impact on sensory acceptance in some attributes, mainly those related to aroma and texture, the formulation with 10% pollen stood out for achieving the highest overall approval ratings. This finding reinforces the potential of pollen as an innovative protein ingredient, as long as it is used in balance with other ingredients that promote smoothness, sweetness, and softness in the product. However, to achieve a product considered as a protein source, it is needed to use at least 30% of pollen.

Furthermore, this research significantly contributes to the valorization of regional ingredients, rescuing traditional food practices and promoting the local bioeconomy through the use of beekeeping and fruit by-products. The selection of raw materials such as dried fig, almond flour, honey, and olive oil reflects a commitment to resource utilization and the creation of value-added foods aligned with current trends in healthy and conscious consumption.

From a methodological standpoint, the integrated application of rigorous statistical analyses (Shapiro-Wilk, Levene, ANOVA, and Tukey's test) stands out, adding relevance to the results obtained and credibility to the interpretation of sensory differences observed among the formulations. The use of real consumers in the sensory evaluation also brings scientific research closer to market reality.

As a perspective for future studies, it is recommended to investigate aroma-masking techniques or alternative textures that allow for greater inclusion of pollen in food formulations, as well as expanding the consumer sample and conducting studies with different population profiles. It is also worth highlighting the potential to explore additional functional properties of pollen, such as antioxidant compounds and bioactive activities, to add distinct nutritional values to the products.

In summary, this work reinforces that it is possible to combine tradition and innovation in the development of natural and sensorially appealing food products,

contributing to the diversification of healthy foods and the appreciation of regional biodiversity.

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## Appendices

# Análise Sensorial de Bolas Energéticas

O presente trabalho insere-se na tese de mestrado a ser desenvolvida pela aluna Ana Gabriela Santana de Brito no âmbito do Mestrado de Engenharia Biotecnológica da Escola Superior Agrária do Instituto Politécnico de Bragança.

Este formulário tem como objetivo recolher a sua opinião sobre três amostras de bolas energéticas desenvolvidas nesse trabalho.

Indica-se que as mesmas podem ter na sua constituição **azeite, mel, amêndoa, figo e pólen. Se tiver alguma alergia ou intolerância a qualquer destes ingredientes, NÃO EFETUE A PROVA SENSORIAL.**

As suas respostas são confidenciais e os dados recolhidos serão utilizados apenas para fins académicos.

Obrigada pela colaboração!

**\* Indica uma pergunta obrigatória**

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*Pular para a pergunta 1*

## INFORMAÇÕES DO AVALIADOR

### 1. Faixa etária \*

*Marcar apenas uma oval.*

- Menor de 18 anos
- 18 a 24 anos
- 25 a 30 anos
- 31 a 50 anos
- 51 a 60 anos
- Acima de 60 anos

**2. Género \***

*Marcar apenas uma oval.*

Feminino

Masculino

Prefiro não informar

Outro: \_\_\_\_\_

**3. Com que frequência consome produtos naturais, energéticos e proteicos? \***

*Marcar apenas uma oval.*

Diariamente

Semanalmente

Mensalmente

Raramente

Nunca

4. **Tem conhecimento do produto alimentar intitulado "bolas energéticas"?** \*



Marcar apenas uma oval.

Sim

Não

5. **Já consumiu bolas energéticas?** \*

Marcar apenas uma oval.

Sim *Pular para a pergunta 6*

Não *Pular para a pergunta 7*

Seção sem título

**6. Com que frequência consome bolas energéticas? \***

Marcar apenas uma oval.

- Diariamente
- Semanalmente
- Mensalmente
- Raramente
- Nunca

*Pular para a pergunta 7*

**AValiação DAS AMOSTRAS**

Por favor, avalie as amostras 137, 459 e 623 fornecidas em relação a cada atributo indicado, utilizando a escala de 1 a 7, em que:

**1 = Desgostei muito**

**7 = Gostei muito**

**7. Amostra 137 \***

Marcar apenas uma oval por linha.

	1	2	3	4	5	6	7
<b>Aparência geral</b>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<b>Aroma / Cheiro</b>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<b>Cor</b>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<b>Textura na boca</b>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<b>Sabor</b>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<b>Apreciação Global</b>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

8. Em relação ao aroma, avalie o mesmo em termos de intensidade utilizando a escala de 1 a 7, em que: \*

*Marcar apenas uma oval.*

1 2 3 4 5 6 7

Nad        Muito Intenso

9. Que aroma detecta em maior intensidade? \*

\_\_\_\_\_

10. Em relação ao sabor, avalie o mesmo em termos de intensidade utilizando a escala de 1 a 7, em que: \*

*Marcar apenas uma oval.*

1 2 3 4 5 6 7

Nad        Muito Intenso

11. Que sabor detecta em maior intensidade? \*

\_\_\_\_\_

12. **Amostra 459 \***

Marcar apenas uma oval por linha.

	1	2	3	4	5	6	7
<b>Aparência geral</b>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<b>Aroma / Cheiro</b>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<b>Cor</b>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<b>Textura na boca</b>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<b>Sabor</b>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<b>Apreciação Global</b>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

13. Em relação ao aroma, avalie o mesmo em termos de intensidade, utilizando a escala de 1 a 7, em que: \*

Marcar apenas uma oval.

	1	2	3	4	5	6	7	
Nad	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	Muito Intenso

14. Que aroma deteta em maior intensidade? \*

\_\_\_\_\_

15. Em relação ao sabor, avalie o mesmo em termos de intensidade, utilizando a escala de 1 a 7, em que: \*

*Marcar apenas uma oval.*

	1	2	3	4	5	6	7	
Nad	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	Muito Intenso

16. Que sabor deteta em maior intensidade? \*

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17. **Amostra 623** \*

*Marcar apenas uma oval por linha.*

	1	2	3	4	5	6	7
<b>Aparência geral</b>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<b>Aroma / Cheiro</b>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<b>Cor</b>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<b>Textura na boca</b>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<b>Sabor</b>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<b>Apreciação Global</b>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

18. Em relação ao aroma, avalie o mesmo em termos de intensidade, utilizando a \*  
escala de 1 a 7, em que:

*Marcar apenas uma oval.*

1 2 3 4 5 6 7

Nad        Muito Intenso

19. Que aroma deteta em maior intensidade? \*

\_\_\_\_\_

20. Em relação ao sabor, avalie o mesmo em termos de intensidade, utilizando a \*  
escala de 1 a 7, em que:

*Marcar apenas uma oval.*

1 2 3 4 5 6 7

Nad        Muito Intenso

21. Que sabor deteta em maior intensidade? \*

\_\_\_\_\_

22. **Entre as três amostras avaliadas, qual foi a sua preferida? \***

*Marcar apenas uma oval.*

- Amostra 137 *Pular para a pergunta 23*
- Amostra 459 *Pular para a pergunta 23*
- Amostra 623 *Pular para a pergunta 23*
- Nenhuma *Pular para a pergunta 25*

Seção sem título

23. Estaria disposto/a a comprar a sua amostra preferida? \*

*Marcar apenas uma oval.*

- Sim *Pular para a pergunta 24*
- Não *Pular para a pergunta 25*
- Talvez *Pular para a pergunta 24*

### Seção sem título

24. No caso de ter respondido “Sim” ou “Talvez”, quanto estaria disposto/a a pagar \* por uma embalagem de 34 g, com duas bolas energéticas?

*Marcar apenas uma oval.*

- Entre 0,70 a 0,89 Euros
- Entre 0,90 a 1,09 Euros
- Entre 1,10 a 1,29 Euros
- Igual ou superior a 1,30 Euros

*Pular para a pergunta 25*

### **AGRADECEMOS A SUA COLABORAÇÃO**

25. Gostaria de deixar algum comentário ou sugestão sobre as amostras?

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