SUMMARY OF Ph.D. THESIS

Studies on bioactive compounds in pine buds (*Pinus sylvestris* L.) at different stages of vegetation and their valorization in food products

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Introduction

The growing interest of consumers in healthy eating has highlighted the necessity of developing functional food products with health benefits. Consequently, consumers are increasingly eager to purchase foods that not only meet their basic nutritional needs—such as the provision of carbohydrates, proteins, and fats—but also foods with documented effects on improving well-being, promoting health, and reducing the risk of illness. These types of products are referred to as functional or enriched foods (Piechowiak *et al*., 2023).

The use of raw materials to obtain extracts from coniferous shoots can contribute to the development of a functional food sector. Foods enriched with ingredients containing active phytochemicals enable a reduction in disease incidence, which is an extremely important factor (Dziedziński *et al*., 2020).

An innovative approach in the valorization of plant materials for functional foods consists in the integration of these extracts into products already recognized for their beneficial properties.

Honey is one of the most complex natural products, with over four hundred different components identified in its composition (Lazarieva *et al*., 2021). It is valued for its high nutritional value and medicinal properties. The most commonly reported bioactive compounds in honey are flavonoids and phenolic acids. Due to its therapeutic value, honey is used as an essential ingredient, and its specific phytochemical compositions provide valuable bioactive molecules that can be used in the treatment of various types of cancer and bacterial infections (Hamadou *et al.,* 2022; Waheed *et al*., 2019).

The use of *Pinus sylvestris* L. buds has gained commercial interest due to their high phenolic content, which is valuable for its antioxidant, anti-inflammatory, and antimicrobial properties in nutraceuticals, food products, and cosmetics (Papp *et al*., 2022; Semeniuc *et al*., 2016).

Recently, researchers have explored the incorporation of pine elements into fortified foods such as kefir and beer, resulting in products with enhanced antioxidant capacity, storage stability, and superior quality (Hamad *et al*., 2019). Pine needles contain antioxidants with anticancer effects and detoxifying properties, aiding in the elimination of heavy metals, exhibiting antibacterial and anti-inflammatory effects, and improving serum lipid metabolism (Penkina *et al*., 2017). They also show cytotoxic effects and inhibit free radicals. Due to their antioxidant properties, pine extracts are used in soaps, essential oils, hangover remedies, and health drinks (Graikou *et al*., 2012; Kim *et al*., 2017; Sawant *et al*., 2023).

The aim of this thesis is to evaluate the main biochemical compounds found in the buds of *Pinus sylvestris* L., extracted through maceration at different developmental stages, and to develop functional food products.

To achieve this goal, the doctoral research followed four major directions, which represent the specific objectives of the present thesis:

- Evaluation of the physicochemical parameters of the extracts obtained from *Pinus sylvestris* L. buds at three developmental stages;
- Assessment of the antioxidant properties and polyphenol content of the bud extracts and the resulting products, using spectrophotometric and advanced techniques (HPLC and FT-IR);
- Fortification of food products, such as jelly and honey, with biologically active compounds from *Pinus sylvestris* L. buds at different developmental stages;
- Evaluation of consumer perception regarding the fortified products.

This thesis is structured in two main parts: the first part covers the current state of knowledge through an extensive review of the specialized literature, and the second part presents the author's own research, detailing the methodology, results obtained, and their interpretation across two studies.

Study I: Influence of Developmental Stages on the Accumulation of Phenolic Compounds and Antioxidant Capacity in *Pinus sylvestris* L. Bud Extracts and Derived Jelly

Jelly is a commonly consumed product, especially among individuals under the age of 17, due to its soft and chewable texture and its wide range of fruity flavors (Figueroa & Genovese, 2019; Mazur *et al.*, 2018).

However, the high sugar content and limited nutritional value of conventional jelly pose health risks such as tooth decay, obesity, and hyperglycemia (Ben Rejeb *et al*., 2020). Transforming jelly into a healthier option can be achieved by reducing or eliminating the sugar content or by replacing sugars with natural sweeteners, such as concentrated fruit juice. Additionally, enriching jelly with specific bioactive compounds can enhance its functionality and offer health benefits (Moura *et al*., 2019a; Miranda *et al*., 2020). The development of products enriched with natural ingredients is vital for the growth of the food industry, particularly in developing regions.

The objective of this study was to evaluate the physicochemical indicators, antioxidant properties, and polyphenol content using spectrophotometric and advanced techniques (HPLC and FT-IR) of *Pinus sylvestris* L. bud extracts and the derived products obtained at three developmental stages of the buds, as well as the fortification of biologically active compounds in jelly and the evaluation of consumer perception regarding the resulting jelly.

Total acidity showed a consistent increase across the developmental stages of pine buds, with stage III buds exhibiting the highest acidity (0.22%). This increase in acidity during bud maturation was in line with the metabolic changes occurring during plant growth, likely due to the accumulation of organic acids. This trend was also observed in the jelly samples, where the jelly prepared with stage III buds had the highest acidity (0.79%), followed by the jelly from stage II buds (0.75%) and stage I (0.69%).

The dry matter content also varied significantly across the different stages. Extracts from stage III pine buds had the highest dry matter content (31.6%). This increase in dry matter content indicated a reduction in water content as the buds matured. Similarly, in the corresponding jelly samples, the dry matter content increased with the bud developmental stage, with jelly from stage III buds showing the highest dry matter content (79.5%). The higher dry matter content in the jelly from stage III suggests improved microbial resistance, as lower moisture typically reduces the potential for microbial growth and spoilage. These findings are consistent with literature regarding the role of moisture content in food preservation.

The ash content, which represents the mineral content of the samples, also showed significant variation across stages. Extracts from stage III pine buds had the highest ash content (0.05%), while stage I had the lowest (0.019%). In the jelly samples, the highest ash content was found in jelly made with stage II buds (0.094%), whereas jelly from stage III buds had the lowest ash content (0.075%). These differences suggest that maturation may have led to the accumulation of certain minerals in the buds, which were then transferred to the jelly.

The study revealed significant variations in polyphenol content and antioxidant potential in pine bud extracts and fortified jelly across the developmental stages. The polyphenol content showed a statistically significant increase from stage I to stage II, reaching a peak value of 312.2 mg GAE/100 g in stage II. However, extracts from stage III showed a sharp decline, registering the lowest polyphenol content. This decline in stage III may be attributed to metabolic changes or the depletion of phenolic compounds during advanced maturation. Stage II buds exhibited the highest antioxidant capacity (94.9%). These results highlight the importance of stage II buds as a superior source of polyphenols and antioxidant potential among the three stages.

In the jelly samples, the polyphenol content was significantly lower than in the raw extracts, likely due to the thermal processing involved in jelly production. Stage II jelly showed the highest polyphenol content (114.4 mg GAE/100 g), followed by jelly from stage I (47.7 mg GAE/100 g), and stage III jelly with the lowest (25.5 mg GAE/100 g). A similar trend was observed in antioxidant potential, with stage II jelly presenting the highest DPPH FRSA (16.4%), while stage III jelly had the lowest (10.9%), slightly

lower than stage I. The substantial reduction in polyphenol content and antioxidant potential in jellies, compared to raw extracts, highlights the impact of thermal processing.

The antioxidant capacity of pine bud extracts decreased as the buds reached maturity, from stage II to stage III, with stage II showing the highest activity (94.9%) and stage III the lowest (76.8%). Stage I extracts showed an intermediate antioxidant potential (82.6%). This trend suggests that stage II buds are the most potent source of antioxidants, possibly due to their maximal accumulation of phenolic compounds and flavonoids.

In contrast, the antioxidant potential of the jelly was significantly lower than that of the corresponding extracts at all stages. Among the jelly samples, stage II jelly showed the highest DPPH FRSA (16.4%), followed by stage III (10.9%) and stage I (10.7%). The drastic reduction in antioxidant potential during jelly preparation may be attributed to the degradation of antioxidant compounds, such as phenolic compounds and flavonoids, due to high temperatures and pH changes during processing.

Pearson correlation analysis revealed statistically significant positive correlations ($p < 0.05$) between various physicochemical properties of pine bud extracts and jelly. In particular, a strong positive correlation was observed between the developmental stages of pine bud extracts and the acidity of the decoction ($r^2 = 0.94$). Furthermore, the polyphenol content in pine bud extracts was strongly linked to antioxidant capacity ($r^2 = 0.94$).

A strong positive correlation ($r^2 = 0.96$) was also observed between the polyphenol content in the pine bud jelly and its antioxidant capacity. This finding is consistent with the study by Ben Rejeb *et al*. (2020), which reported a significant positive association between antioxidant potential and polyphenol content in citrus juice.

This study identified 14 phenolic compounds in *Pinus sylvestris* L. buds using HPLC-DAD-ESI-MS. The phenolic profile varied with developmental stage, with stage II pine buds containing the highest concentrations of most phenolic compounds, such as 4-hydroxybenzoic acid (820.5 ± 5.8 μg/g DW) and chlorogenic acid (512.1 ± 4.5 μg/g DW), followed by significant reductions in stage III.

FT-IR spectra of *Pinus sylvestris* L. buds from stages I, II, and III, as well as the jelly obtained from the extracts, confirmed the presence of significant compounds such as phenolic acids, flavonoids, catechins, epicatechins, procyanidins, organic acids, and carbohydrates. Although all samples exhibited a comparable spectral fingerprint, distinct differences were observed between the developmental stages and the resulting jelly, indicating variations in the accumulation of bioactive compounds during maturation and processing.

All samples subjected to sensory analysis were accepted by the evaluators, with the preferred sample being the jelly fortified with stage II pine bud extract.

Study II: Influence of Antioxidant Capacity and Polyphenol Content on Acacia and Polyfloral Honey Fortified with Pine Bud Extract

Despite the well-documented nutritional and medicinal properties of honey, few studies have explored its enhancement through fortification with plant-derived extracts, particularly pine bud extracts at different developmental stages.

The objective of this study was to analyze how fortification affects key honey parameters, including moisture content, antioxidant activity, total polyphenol content, and color, as well as to assess consumer perception of the fortified honey. The novelty of this research lies in establishing stage-specific correlations between fortification and honey quality improvement, offering valuable insights into optimizing functional food products.

Acacia honey had a moisture content ranging from 5.80% to 17.5%, with the highest level observed in acacia honey fortified with pine bud extract at developmental stage III. Polyfloral honey had a higher dry matter content (%) compared to acacia honey.

In the case of acacia honey fortification, the highest polyphenol content was observed in AHS1, at 247 ± 0.04 mg GAE/100 g, while polyfloral honey fortified with pine extract at developmental stage II showed a high polyphenol content of 232.9 ± 0.9 mg GAE/100 g. The DPPH value of acacia honey was highest when it was fortified with pine bud extract at developmental stage II.

The color of the honey samples was quantified during the study. The results showed that throughout the fortification process, acacia honey maintained a light color (L*) ranging from 40.03 ± 0.32^{e} to 41.16 ± 0.33^{f}. Polyfloral honey exhibited some variation in L* values and was observed to darken during fortification. It also showed higher a* values, especially in samples PHS2 and PHS3, indicating a reddish hue after fortification.

FT-IR analysis revealed absorption patterns in both acacia and polyfloral honey, indicating the presence of water, carbohydrates, and typical organic honey compounds. In polyfloral honey, a strong positive correlation was found between dry matter and moisture content ($r^2 = 0.98$). DPPH absorption and total phenolic content showed a positive correlation, highlighting the importance of phenolic content in enhancing antioxidant properties. The developmental stages of the honey correlated negatively with color attributes, especially brightness (L*). In acacia honey, moisture content showed a strong positive correlation with total dry matter ($r^2 = 0.997$) and DPPH ($r^2 = 0.995$). DPPH also showed strong positive correlations with total phenolic content (0.99064) and with the color attributes L* and a* ($r^2 = 0.985$; $r^2 = 0.98$).

Conclusions

The two studies highlight the potential of *Pinus sylvestris* L. bud extracts in the fortification of functional foods. The findings demonstrated that the use of pine extracts in jelly and honey can significantly enhance the nutritional profile of these products by increasing their polyphenol content and antioxidant capacity.

The fortification of jellies with pine bud extracts showed that polyphenol levels and antioxidant activity vary according to the developmental stage of the buds used. The best results were obtained from extracts derived from buds at stage II of development, which recorded the highest content of bioactive compounds (312.2 mg GAE/100 g) and the strongest antioxidant capacity (94.9% DPPH).

Fortifying both acacia and polyfloral honey with pine bud extracts significantly improved the antioxidant properties of the honey. Honey fortified with extracts from buds at stage II had the highest polyphenol content (247 mg GAE/100 g) and the greatest antioxidant activity (55% DPPH).

HPLC analyses confirmed the presence of key phenolic compounds such as kaempferol, quercetin, and stilbene derivatives with high antioxidant potential.

FT-IR analyses revealed chemical changes in the composition of the fortified honey and jellies, indicating the integration of bioactive compounds from pine extracts into the food matrix. However, partial degradation of polyphenols was observed during thermal processing, suggesting the need to optimize production technologies.

Sensory evaluation showed that the addition of the extracts did not significantly affect the taste and texture acceptability of the products, confirming their potential in the development of functional foods.

The research confirms that *Pinus sylvestris* L. buds are a valuable source of bioactive compounds with potential applications in functional food products.

Following the research conducted on the bioactive compounds in pine buds (*Pinus sylvestris* L.) and their integration into functional food products such as jelly and honey, it is recommended to use pine buds harvested at the second stage of development, as they exhibit the highest content of phenolic compounds and antioxidant activity. Additionally, the implementation of low-temperature processing technologies is proposed, in order to reduce the degradation of phenolic compounds during processing.

Originality and Innovative Contributions of the Thesis

This thesis introduces the following innovative elements that reflect the originality of the research:

- Identification and quantification of bioactive compounds in *Pinus sylvestris* L. bud extracts at three developmental stages;
- Development of a functional jelly enriched with pine buds harvested at three developmental stages;

- Production of honey fortified with pine bud extract at various stages of development;
- Analysis of consumer perception and acceptability of innovative products based on acacia and polyfloral honey enriched with pine buds;
- Establishment of specific correlations between the developmental stages of *Pinus sylvestris* L. buds and the improvement of honey quality, providing perspectives for optimizing functional food products.

The results obtained in this thesis highlight the scientific and practical value of using *Pinus sylvestris* L. bud extracts in the development of functional foods. Their integration into products such as jelly and honey has shown a significant improvement in nutritional value and antioxidant activity without compromising sensory acceptability. Thus, this research opens promising avenues for the valorization of forest biomass in the food and nutraceutical sectors, offering innovative solutions for health-conscious consumers. Future studies could focus on expanding the range of fortified products, optimizing technological processes, and assessing the stability of bioactive compounds under real storage conditions.

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