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Improving Jelly Nutrient Profile with Bioactive Compounds from Pine (*Pinus sylvestris* L.) Extracts

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Abstract: This study aimed to enhance the nutritional value of jellies by fortification with polyphenol extracts derived from *Pinus sylvestris* L. shoots at various maturation stages. *Pinus sylvestris* L., a coniferous species, is widely used in traditional medicine and functional foods due to its antioxidant, anti-inflammatory, and antimicrobial properties. Its needles, bark, and shoots are commonly used to extract bioactive compounds such as phenolic acids and flavonoids. In the current study, extracts were derived from young shoots collected directly from natural forest environments and processed using a decoction method to preserve bioactive compounds. The novel jelly formulations were prepared using pine shoots harvested at three maturity stages: stage I (4 cm), stage II (8 cm), and stage III (12 cm). All determinations were conducted both on the pure decoction extracts and the jelly samples to ensure a comprehensive analysis. High-performance liquid chromatography coupled with electrospray ionization mass spectrometry (HPLC-ESI-MS) allowed the identification of eight phenolic acids and six flavonoids in the samples. Significant differences were observed between the pine shoot extracts and jellies at different development stages. Notably, stage II exhibited optimal polyphenol content (312.2 mg GAE/100 g), DPPH free radical scavenging activity (94.9%), dry matter content (79.5%), and acidity (0.79% citric acid/g). A similar pattern emerged in the jelly samples (jelly2 (pine decoction stage II) > jelly1 (pine decoction stage I) > jelly3 (pine decoction stage III)). All extracts demonstrated antioxidant potential in DPPH free radical quenching assays. FTIR analysis evaluated structural changes in phenolic compounds during jelly formulation, focusing on key absorption bands at 1600 cm^{-1} (C=C stretching) and 3336 cm^{-1} (-OH stretching) using a Shimadzu IR Prestige-21 spectrophotometer. Compared to extracts, jellies showed diminished band intensities, indicating thermal degradation of phenolic compounds during processing. This aligns with observed reductions in antioxidant capacity and phenolic content, suggesting partial destabilization of these bioactive compounds. However, their integration into the jelly matrix highlights the potential for functional applications. The textural attributes of jellies were also assessed, and differences were attributed to the changes in acidity and moisture content of the pine shoots during maturation. Pine shoot extracts at specific maturation stages are valuable sources of antioxidant and polyphenol

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compounds and were successfully employed in functional applications belonging to the food or nutraceutical industry.

Keywords: *Pinus sylvestris* L.; natural antioxidants; phenolic compounds; HPLC; FTIR

1. Introduction

Plants possess a diverse array of phenolics, playing a vital role in various metabolic and physiological processes, including growth, development, and stress response. Phenolics influence seed germination, cell division, and photosynthetic pigments' synthesis [1]. Their applications extend to bioremediation, allelochemicals, plant growth promotion, and food additives, leveraging their antioxidant potential. Under stress, phenolic accumulation serves as a defensive mechanism, mitigating abiotic challenges [2,3]. These compounds enhance plant tolerance and adaptability under suboptimal conditions [1]. Previous studies identified polyphenols in Pinus genus species, highlighting their benefits. For instance, quercetin, kaempferol, and glycoside derivatives in *Pinus sylvestris* L. needles exhibit antioxidant and cellular protective properties [4,5].

Ferulic and p-coumaric acids in *Pinus sylvestris* L. bark contribute to anti-inflammatory and antimicrobial effects [6,7]. Many secondary metabolites with antioxidant properties belong to this group, enhancing plant performance under stress [5,8]. Phenylpropanoid biosynthesis produces over 8000 secondary metabolites, while flavonoid metabolism yields nearly 6000 molecules [2–4]. Despite their widespread availability, coniferous shoots remain underutilized as food ingredients [9]. However, pine tree parts, including needles, cones, bark, and pollen, are used in traditional medicine and for healthy food production [10]. Pine shoot products, such as syrup, beer, and teas, demonstrate antioxidant, anti-inflammatory, anticancer, antimicrobial, and cytotoxic properties [11]. The bark of *Pinus sylvestris* L. is rich in phenolic compounds, including quercetin, eleutheroside, vanillic acid, myricetin, catechin, taxifolin, and ferulic acid. The use of *Pinus sylvestris L.* shoots has gained commercial interest due to their high phenolic content, which is valuable for their antioxidant, anti-inflammatory, and antimicrobial properties in nutraceuticals, food, and cosmetics [12,13]. Additionally, while the phenolic compounds in *Pinus sylvestris L.* are generally considered safe, further research on their toxicity is needed to ensure their long-term safety in food and medicinal products.

Recently, researchers have explored incorporating pine elements into fortified foods like kefir and beer, resulting in products with enhanced antioxidant capacity, improved storage stability, and superior quality [14]. Pine needles possess antioxidants, anticancer effects, and detoxifying properties, helping eliminate heavy metals, exhibit antibacterial and anti-inflammatory effects, and improve serum lipid metabolism [15]. They also display cytotoxic effects and inhibit free radicals. Due to their antioxidant properties, pine extracts are used in soaps, essential oils, hangover remedies, and health drinks [16–18]. Pine needle extracts have shown potential as effective remedies for arteriosclerosis and diabetes, attributed to their high content of vitamins, iron, high-quality protein, lipids, and dietary fiber. These extracts exhibit antioxidant, anti-aging, antimicrobial, and anticancer properties owing to their rich composition of unsaturated fatty acids (USFAs) and aromatic agents. Aromatic hydrocarbons, such as myrcene, α-pinene, and terpinene, present in pine needle extracts have demonstrated superior efficacy in combating free radicals compared to vitamin E [17].

The growth and development of plants significantly influence their secondary metabolite profiles [18]. For example, young pine needles contain the highest levels of monoter-

penes compared to mature needles [19]. Notable bioactive compounds, including quercetin, catechin, and 3-O-glucoside, have been identified in the needles of *Pinus sylvestris* L. Additionally, two dominant phenolic acids—3-hydroxybenzoic and 3,4-dihydroxybenzoic acids—were found in the needles of *Pinus strobus* L. Research by Ozgen et al. highlighted quercetin as a potent antioxidant with benefits for treating heart diseases, diabetes, and cancer. Similarly, catechin, quercetin, and epicatechin have been recognized for their strong antioxidant properties [20].

Jelly is a popularly consumed product, particularly among individuals under 17 years of age, due to its organic and chewy texture, as well as its diverse variety of fruity flavors [21,22]. This product is typically made by gelatinizing a sugar base consisting of sucrose and corn syrup, followed by the addition of flavors, acids, and colorants. However, the high sugar content and limited nutritional value of conventional jellies pose health risks such as tooth decay, obesity, and hyperglycemia [23]. Transforming jelly into a healthier option can be achieved by reducing or eliminating sugar content or substituting sugars with natural sweeteners like concentrated fruit juice. Additionally, fortifying jelly with specific bioactive compounds can enhance its functionality and potentially provide health benefits [24,25]. Developing products enriched with natural ingredients is vital for the growth of the food industry, particularly in developing regions.

This study aimed to enhance the nutritional value of jellies by fortification with polyphenol extracts derived from *Pinus sylvestris* L. shoots at various maturation stages. The pine shoots were harvested at three maturity stages and were characterized in terms of chemical properties and nutritional value. Decoction extracts were prepared from the shoot and were further employed in manufacturing jelly products. The physicochemical and nutritional aspects of the novel jelly formulations were assessed.

2. Materials and Methods

2.1. Materials

Pine buds (*Pinus sylvestris* L.) at three developmental stages (I, II, and III) served as the biological material for this study (Figure 1). The samples were collected by the "Lotru" Forestry Society from forests in Romania, specifically in Vâlcea County and Brezoi City, across various altitudes. The elevation in the inhabited area ranges from 275 to 350 m, increasing in the surrounding mountainous regions. The highest peaks include Sterpu (2114 m), Pârcalabu (1980 m), and Robu in the Lotrului Mountains. The specific geographic coordinates for these locations are Sterpu (45.4723° N, 24.2034° E), Pârcalabu (45.4521° N, 24.1789° E), and Robu (45.4878° N, 24.2506° E). Brezoi City is situated in a depression area characterized by a temperate continental mountainous climate typical of the Southern Carpathians region. The region experiences average temperatures of 15–16 °C in July, the warmest month, and −3.5 °C in January, with an annual average temperature ranging from 5–6 to 8 °C. The soils, primarily cambisols and spodosols, are low in humus and influenced by various environmental factors. The soil pH values in the collection area are reported to range between 4.5 and 5.5, characteristic of acidic conditions typical for cambisols and spodosols in forested regions. The relative humidity of the region varies seasonally, averaging around 70%–85%, depending on the altitude and vegetation cover. To study the influence of the developmental stage on the accumulation of phenolic compounds and antioxidant capacity, *Pinus sylvestris* L. shoots were harvested at 14-day intervals. Shoot lengths of 4 cm, 8 cm, and 12 cm were selected to represent early, intermediate, and mature growth stages, respectively, based on their association with peak accumulation of bioactive compounds such as polyphenols and organic acids [8,9]. The collection of samples was conducted in accordance with Romanian biodiversity regulations, adhering to

the criteria for the care and proper use of biodiversity to ensure sustainable and responsible harvesting practices.

Previous studies indicate that plant growth stages significantly impact the concentration of flavonoids, phenolic acids, and other antioxidants, as these compounds play a crucial role in protecting plants from abiotic and biotic stress. The selected shoot lengths (4 cm, 8 cm, and 12 cm) reflect these developmental stages, allowing for a detailed analysis of polyphenol content and antioxidant activity at different maturities [26]. This approach provides insight into how the developmental stage influences the bioactive compound profile and the potential effectiveness of shoot extracts for jelly fortification. The experimental design focused on assessing whether the maturation stage of pine shoots impacts the nutritional profile of fortified jelly. By analyzing the antioxidant and polyphenol content of the jelly, the study aims to evaluate how fortification with shoot extracts enhances its nutritional value and functional properties.

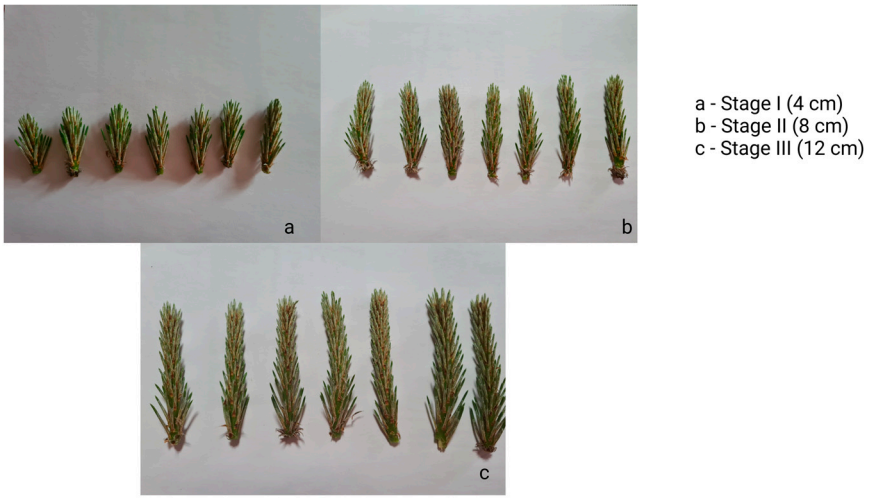

Figure 1. Experimental harvesting protocol.

2.2. Chemicals, Reagents, Measurement Instruments, and Specifications

In the experiment, reagents and instruments procured from reliable suppliers were utilized, and their details are provided below to ensure the reproducibility and accuracy of the study. The reagents used were as follows: gallic acid (Sigma-Aldrich, St. Louis, MO, USA, Catalog No. G7384), employed as a standard for determining the phenolic compound content, and chlorogenic acid (Sigma-Aldrich, Catalog No. 75053), used for HPLC calibration. Rutin (Sigma-Aldrich, Catalog No. R5143) served as the standard for flavonoid analysis, while the Folin–Ciocalteu reagent (Sigma-Aldrich, Catalog No. F9252) was essential for determining the total phenolic content (TPC). Antioxidant activity was assessed using DPPH (2,2-diphenyl-1-picrylhydrazyl, Sigma-Aldrich, Catalog No. D9132), and calibration for antioxidants was conducted with Trolox (Sigma-Aldrich, Catalog No. 238813). Additional reagents included sodium carbonate (Sigma-Aldrich, Catalog No. S7795), used in the phenolic analysis, acetic acid (Sigma-Aldrich, Catalog No. 27225) for preparing the HPLC mobile phase, and HPLC-grade acetonitrile (Sigma-Aldrich, Catalog No. 34998) as a solvent for chromatographic analyses.

The instruments employed in the study were selected for their precision and sensitivity. The HPLC-DAD-ESI-MS system used was the Agilent 1200 model, equipped with a diode-array detector and API-electrospray MS-6110 mass spectrometer. Compound separation was performed using an Agilent Eclipse XDB-C18 column (Agilent Technologies, Santa Clara, CA, USA) (5 µm, 4.5 × 150 mm i.d.), with a flow rate of 0.5 mL/min, and data were collected at wavelengths of 280 nm and 340 nm. For spectrophotometric measurements, a

Shimadzu UV-1700 spectrophotometer was used, calibrated for determinations at 517 nm and 750 nm. Spectral analyses were complemented by a Shimadzu IR Prestige-21 FTIR spectrometer with a diamond ATR accessory, providing a resolution of 4 cm^{-1} and 16 scans per spectrum. The textural studies of the jellies were conducted using a Brookfield (Middleboro, MA, USA)CT3 texture analyzer configured to apply 50% compression using a TA44 probe at a speed of 1 mm/s. Sample centrifugation was performed with an Eppendorf 5810 R centrifuge, capable of a maximum speed of 14,000 rpm and temperature control ranging from −9 °C to 40 °C. Determination of dry substance was carried out using an Atago PAL-1 refractometer, with a measurement range of 0–53°Bx and an accuracy of ±0.1°Bx.2.3.

Preparation of *Pinus* Shoot Extract and Jelly

A comparative longitudinal experimental design was employed to evaluate the effects of *Pinus sylvestris* L. shoot extract supplementation on jelly production. Pine shoots were harvested at three developmental stages to prepare a decoction, which was subsequently used to produce health-enhancing gelled jelly. The shoots were collected at 14-day intervals, representing three growth stages: stage I (4 cm), stage II (8 cm), and stage III (12 cm). After collection, the samples were vacuum-sealed and stored at −18 °C until further processing. A total of 168 g of pine shoots from each phenophase were crushed and hydrated with 200 g of cold water, followed by a 5 min maceration. Next, 300 g of hot water was added, and the mixture was boiled for 20 min. The shoots were then chopped and boiled for an additional 15–30 min, with water periodically added to compensate for evaporation. The resulting solution was filtered to obtain the decoction. The jellies were prepared by combining the pine shoot decoction with sugar and pectin. Glucose syrup, preheated to 60 °C, was added to the solution maintained at 75 °C. The mixture was boiled until it reached a dry substance of 78° Bx, confirmed using the refractometer. Once the desired dry substance content was achieved, a 50% citric acid solution was incorporated. The jelly composition was then poured into starch molds and left to gel at room temperature for 30–40 min.

After gelling and cooling, the jelly was removed from the starch molds, brushed with powdered sugar, and dried at 40–50 °C for 15 min. Finally, the dried jelly was packed, completing the finishing stage of the product [24]. The jellies obtained were jelly1 (pine decoction stage I), jelly2 (pine decoction stage II), and jelly3 (pine decoction stage III).

While the preparation of the decoction extracts was optimized to preserve bioactive compounds, the final yield of the extracts was not measured during this study. Future research will incorporate yield calculations as part of the protocol to provide a more comprehensive understanding of the extraction process and facilitate reproducibility.

2.3. Physicochemical Properties of Pine Shoot Extract and Pine Shoot-Derived Jelly

2.3.1. Determination of Total Acidity

The determination of total acidity involved titration with a standard volumetric solution of 0.1 N NaOH in the presence of phenolphthalein as an indicator. Pine shoot samples were shredded using a Philips HR1614/00 650 W vertical blender. Extractions were conducted in accordance with the international standard (ISO 750:1998) (Fruit and Vegetable Products-Determination of Titratable Acidity, 1998) [27] with some modifications. A 5 g sample of pine shoots was homogenized with 25 mL distilled water to obtain a puree, which was then quantitatively transferred to a 50 mL volumetric flask. After centrifugation and filtration, 25 mL of the filtrate was titrated with 0.1 N NaOH solution in the presence of phenolphthalein until a light pink color appeared [28].

2.3.2. Determination of Dry Matter Content

The dry matter content of pine shoot extract was determined using the standard AOAC method [29]. The refractometric method was employed for the determination of the

dry substances. The samples were diluted with distilled water in a certain proportion, and the refractive index of the solution obtained was determined with the refractometer, while the result took into consideration the dilution proportion.

2.3.3. Determination of Total Phenolic Content

The total polyphenol content (TPC) was determined using a Shimadzu (Shimadzu Corporation, Kyoto, Japan) spectrophotometer following a modified Folin–Ciocalteu method [30]. The calibration curve for total phenolic content (TPC) was constructed using gallic acid as the reference standard, which was dissolved in methanol to prepare stock solutions. A series of dilutions were made to achieve concentrations ranging from 0.25 mg/mL to 1.00 mg/mL. The absorbance of each concentration was measured at 750 nm, and a straight-line calibration curve was obtained. The equation of the resulting straight line was $y = 0.999x + 0.012$, with an R^2 value of 0.998, indicating excellent linearity and reliability of the calibration method. For the analysis, 1 g of sample was ground and homogenized in a mortar with a pestle. This homogenized sample was combined with methanol, then centrifuged at 6000 rpm for 5 min, and subjected to successive extractions. The resulting extracts were filtered and concentrated at 35 °C under reduced pressure using a Heidolph Rotavapor. The concentrated samples were reconstituted in 6 mL of methanol and stored at −20 °C until analysis. The phenolic compounds were identified by comparing their retention times, UV spectra, and mass spectra with those of authentic standards, including gallic acid, chlorogenic acid, rutin, catechin, ferulic acid, protocatechuic acid, quercetin, and kaempferol. These standards were used for calibration and quantification of the compounds. For quantification, the diode-array detector (DAD) was utilized to determine total hydroxybenzoic acid and hydroxycinnamic acid contents, with gallic acid and chlorogenic acid serving as calibration standards, respectively. Flavonoids were quantified using rutin as the standard. Results are expressed as micrograms of equivalents per gram of sample (e.g., μg gallic acid equivalents (GAE)/g, μg chlorogenic acid equivalents (ChAE)/g, μg rutin equivalents (RE)/g). Mass spectrometry (MS) was employed to identify individual compounds through their fragmentation patterns and m/z values using the positive ion mode. Compounds that lacked direct standards were identified based on fragmentation data and quantified using structurally similar standards, reported as equivalents of the most closely related standard compound. For example, p-coumaric acid was quantified as chlorogenic acid equivalents. This methodology follows established protocols as described in Dulf et al. [31] and related studies. The results section reports these findings consistently, with quantified values expressed in the equivalent terms specified above, enabling clarity and reproducibility.

For the TPC assay, 25 μL of the sample extract was mixed with 1.8 mL of distilled water and 120 μL of Folin–Ciocalteu reagent in a glass vial. After allowing the mixture to react for 5 min, 340 μL of a 7.5% sodium carbonate solution was added to adjust the pH to approximately 10, facilitating the redox reaction between the phenolic compounds and the Folin–Ciocalteu reagent. The mixture was then kept at room temperature for 90 min. to allow complete development of the color.

The TPC was quantified and expressed as gallic acid equivalents (GAEs), reported as mg GAE per 100 g of sample. This method provided an accurate measurement of the polyphenol content in the samples.

2.3.4. DPPH Free Radical Scavenging Assay

The determination of DPPH radical scavenging activity [32] was conducted using a modified method from the literature, optimized for *Pinus sylvestris* L. extracts. A DPPH solution (0.1 mM) was prepared in methanol and kept in the dark. For each sample, 100 μL

of extract at a concentration of 1 mg/mL was added to 2.9 mL of the DPPH solution, creating a total volume of 3 mL. The mixture was kept in dark conditions for 30 min., and the absorbance was measured at 517 nm using a spectrophotometer. Methanol, used for preparing the DPPH solution and as a solvent for the extracts, was also used as the reagent blank to correct baseline absorbance.

Antioxidant activity was calculated using the following formula:

$$\text{DPPH scavenging effect } (\%) = \frac{(A_0 - A_s) \times 100}{A_0}$$

where A_0 is absorbance of the blank, and A_s is absorbance of the samples.

2.3.5. Determination of Phenolic Compounds by the HPLC-DAD-ESI-MS Method

The phenolic compounds in pine shoot extracts were identified and quantified using an advanced HPLC-DAD-ESI-MS system (Agilent Technologies, Santa Clara, CA, USA) comprising an HP-1200 liquid chromatograph with a quaternary pump, autosampler, diode-array detector (DAD), and an MS-6110 single-quadrupole API-electrospray detector. Compound separation was performed using an Agilent Eclipse XDB-C18 column (5 µm; 4.5 × 150 mm i.d.) maintained at 250 °C. The analysis followed a multistep linear gradient elution program described by Dulf et al. [31], employing distilled water acidified with 0.1% acetic acid as solvent A and acetonitrile acidified with 0.1% acetic acid as solvent B. The flow rate was set to 0.5 mL/min, with a gradient schedule beginning at 5% solvent B for the initial 2 min, gradually increasing to 90% over the next 20 min, holding at 90% for 4 min, and returning to 5% over 6 min, culminating in a total analysis time of 30 min. Chromatograms were recorded at wavelengths of 280 nm and 340 nm, and data processing was carried out using Agilent ChemStation Software (Versiunea C.01.10, Agilent Technologies, Santa Clara, CA, USA). Mass spectrometric detection was conducted in positive ion mode with a capillary voltage of 3.5 kV, fragmentor voltage set at 100 V, and a drying gas temperature of 350 °C. Nebulizer pressure was maintained at 40 psi with a drying gas flow rate of 10 L/min. Scans were performed over a range of 120–1200 m/z, with a scan rate of 1.5 scans/s, ensuring high sensitivity for phenolic compound detection. Mass spectrometric detection in the positive ion mode was conducted with a scan range of 120–1200 m/z. This provided additional confirmation of the phenolic compounds based on their fragmentation patterns and mass spectra, ensuring precise identification. Operational parameters for the MS were optimized for reliable detection.

Phenolic compounds were identified by comparing their retention times, UV spectra, and mass spectra with authentic standards and published references. Quantification was achieved using calibration curves with high correlation coefficients. Hydroxybenzoic acids were quantified using a gallic acid calibration curve (10–100 µg/mL; r^2 = 0.9978) and expressed as gallic acid equivalents (µg gallic/g). Hydroxycinnamic acids were quantified using a chlorogenic acid calibration curve (10–50 µg/mL; r^2 = 0.9937) and expressed as chlorogenic acid equivalents (µg chlorogenic/g). The concentrations of individual hydroxycinnamic acids identified through mass spectrometry were estimated using the chlorogenic acid calibration curve as a reference standard. Similarly, hydroxybenzoic acids were quantified using a gallic acid calibration curve (10–100 µg/mL; r^2 = 0.9978) and expressed as gallic acid equivalents (µg gallic/g). Flavonoids were quantified using a rutin calibration curve (10–100 µg/mL; r^2 = 0.9981) and expressed as rutin equivalents (µg rutin/g). This approach allowed for the estimation of the concentration of each identified compound based on equivalent standards rather than total content alone, ensuring detailed and compound-specific quantification. This method ensured reliable and consistent reporting of phenolic compounds, leveraging both DAD and MS for precise identification and quantification.

The HPLC-DAD-ESI-MS method enabled the identification and quantification of 14 phenolic compounds, including hydroxybenzoic acids and other phenolic subclasses. MS data further validated the identification through unique fragmentation patterns and mass spectra. The database used for compound identification included spectral data from the published literature and authentic standards, ensuring the reliability of the assignments. Fragmentation patterns were compared against entries in recognized databases such as METLIN and PubChem to corroborate the identification of compounds without direct standards. A detailed table documenting the compounds, their m/z values, and corresponding adducts was compiled to support the findings (Table S1 in Supplementary Materials). This table includes the retention times, molecular weights, m/z values, and specific adducts identified for each compound.

Additionally, the mass spectra of the 14 identified compounds have been included as Supplementary Materials (Supplementary Table S1), providing a visual confirmation of the MS data. These spectra demonstrate the fragmentation patterns and key ion peaks used for compound validation. This comprehensive analytical approach ensured accurate and reliable profiling of the phenolic compounds in pine shoot extracts 2.5. FTIR spectra.

The analyzed samples (pine buds (*Pinus sylvestris* L.) at different stages (pine shoot jellies at stages I, II, and III) were centrifuged for 15 min at 3000 rpm. Infrared absorption spectra were recorded directly using a Shimadzu IR Prestige-21 spectrophotometer with a single-reflection diamond ATR (Attenuated Total Reflectance) accessory from PIKE. Spectra were recorded in the wavelength range of 600–4000 cm^{-1}, with a resolution of 4 cm^{-1} and 16 scans per spectrum. A total of 10 µL of extract was placed on the ATR accessory. The absorption bands characteristic of bond types and functional groups (expressed in cm^{-1}) were identified. These bands were determined in the ranges 650–1800 cm^{-1} and 2750–3500 cm^{-1}. The primary data obtained were processed using Irsolution Software Overview (Shimadzu Kyoto, Japan) and OriginR 7SR1 Software (OriginLab Corporation, Northampton, MA, USA) [33].

2.4. Texture Analysis

The texture of pine shoot jelly was determined following Bourne's methodology [34]. For this purpose, a load of 50 kg was applied using a Brookfield CT3 texture analyzer. Within the TPA test, aTA44 probe was used to compress the jelly cubes for 50% in two cycles at a rate of 1 mm/s. The test was conducted at room temperature. Various textural parameters such as consistency cycle 1 (g), total mechanical work cycle 1 (g), stickiness (g), cohesiveness, elasticity index (mm), and gumminess (g) were determined.

2.5. Statistical Analysis

Each experiment was conducted in triplicate, presenting the results as mean ± standard deviation. Statistical analyses, including Tukey's post hoc test (at a significance level of $p < 0.05$), t-test, and analysis of variance (ANOVA) were performed using IBM SPSS Statistics 2021 (SPSS v.22; IBM, Armonk, New York, NY, USA). Pearson correlation analysis was conducted to examine the interactions between different physicochemical properties and their changes across maturation stages using OriginPro 2021 software. A heatmap was generated to visualize the variations in phenolic compounds across maturation stages using a hierarchical clustering model in OriginPro 2022. This software directly compares compound concentrations and clusters them across samples in the data matrix.

3. Results and Discussion

3.1. Physicochemical Properties of Pine Shoots and Pine Shoot Jelly During Different Stages of Maturation

The study revealed significant variations in the physicochemical properties of pine shoots and their corresponding biofortified jelly because of the sample's collection at different maturation stages. Analysis of variance indicated that maturation of the pine shoots had a significant effect on several key parameters, including acidity, dry matter content, and ash content, which varied significantly between stages I, II, and III ($p < 0.05$), as presented in Tables 1–3.

Table 1. Physiochemical properties of pine shoots and pine shoot jelly during different stages of maturation.

Sample	Total Acidity (%)	Dry Matter (%)	Ash (%)
Shoot Stage I (Decoction extract from 4 cm shoots)	0.126 ± 0.005^{a}	26 ± 0.28^{a}	0.019 ± 0.001^{b}
Shoot Stage II (Decoction extract from 8 cm shoots)	0.16 ± 0.01^{b}	26.3 ± 0.28^{a}	0.014 ± 0.002^{c}
Shoot Stage III (Decoction extract from 12 cm shoots)	0.22 ± 0.01^{c}	31.6 ± 0.50^{b}	0.05 ± 0.0005^{a}
Jelly Stage I (Prepared from Stage I shoots)	0.69 ± 0.01^{a}	77.5 ± 0.40^{a}	0.091 ± 0.001^{b}
Jelly Stage II (Prepared from Stage II shoots)	0.75 ± 0.01^{b}	78.3 ± 0.50^{b}	0.094 ± 0.002^{c}
Jelly Stage III (Prepared from Stage III shoots)	0.79 ± 0.005^{c}	79.5 ± 0.40^{c}	0.075 ± 0.0005^{a}
Mean	2.73	317	0.343
CV%	11.6	8.71	10.2
F-ratio	0.945	2.451	21.94
p-value	<0.01	<0.01	<0.01

Note: Superscripts with the same letters indicate no significant difference among mean values (n = 3), while different letters indicate a significant difference among mean values at $p < 0.05$. F-ratio: value for variance analysis; CV%: coefficient of variation.

Table 2. Antioxidant profile of pine shoot jelly at different developmental stages.

Sample	Polyphenols (mg GAE/100 g)	DPPH FRSA (%)
Stage I	252.84 ± 0.20^{b}	82.6 ± 0.50^{b}
Stage II	312.2 ± 1.10^{c}	94.9 ± 0.02^{c}
Stage III	164.6 ± 0.50^{a}	76.8 ± 0.30^{a}
Jelly Stage I	47.7 ± 0.17^{b}	10.7 ± 0.18^{a}
Jelly Stage II	114.4 ± 0.40^{c}	16.4 ± 0.20^{c}
Jelly Stage III	25.5 ± 0.35^{c}	10.9 ± 0.05^{b}
Mean	917.24	917.24
CV%	12.30	12.30
F-ratio	2.89	0.16
p-value	<0.01	<0.01

Note: Superscripts with same letters indicate no significant difference among mean values (n = 3), while different letters indicate a significant difference among mean values at $p < 0.05$. DPPH FRSA: 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity; GAE: gallic acid equivalent; F-ratio: value for variance analysis; CV%: coefficient of variation.

Total acidity showed a steady increase across the maturation stages of pine shoots, with shoot stage III (0.22%) exhibiting the highest acidity, followed by stage II (0.16%) and stage I (0.13%). This increase in acidity as the shoots matured was consistent with metabolic changes that occurred during plant growth, likely due to the accumulation of organic acids. This trend continued in the jelly samples, where jelly formulated with stage III shoots exhibited the highest acidity (0.79%), followed by jelly made from stage II (0.75%) and stage I (0.69%). These findings suggested that the maturation stage of pine

shoots contributed significantly to the acidity of the final jelly, which could enhance its preservative qualities. The increase in acidity was particularly noteworthy as it served a dual function: it contributed to flavor enhancement and improved the jelly's shelf life by acting as a natural preservative.

Table 3. Phenolic compound content in pine shoot extracts and pine shoot-derived jelly.

Phenolic Compounds	Pine Shoots			Pine Shoot Jelly		
	Stage I	Stage II	Stage III	Stage I	Stage II	Stage III
	Concentrations (μg/g DW)					
2-Hydroxybenzoic acid	221.3 ± 8.7 ^{f}	305.9 ± 2.3 ^{e}	131.9± 9.3 ^{d}	21.6 ± 3.7 ^{b}	39.14 ± 7.1 ^{c}	12.73 ± 1.81 ^{a}
2,4-Dihydroxybenzoic acid	90.7± 3.9 ^{f}	45.4 ± 4.4 ^{e}	27 ± 2.31 ^{d}	9.16 ± 1.5 ^{c}	7.02 ± 1.2 ^{b}	5.23 ± 1.7 ^{a}
Protocatecuic acid	157.8 ± 3.9 ^{b}	241.8 ± 4.1 ^{c}	108.9 ± 1.9 ^{a}	0 ± 0.0	0 ± 0.0	0 ± 0.0
4-Hydroxybenzoic acid	774.1± 4.2 ^{e}	820.5 ± 5.8 ^{f}	450.09 ± 14.3 ^{d}	10.76 ± 1.54 ^{b}	26.8 ± 2.3 ^{c}	5.23 ± 0.50 ^{a}
Chlorogenic acid	78 ± 2.2 ^{d}	512.1 ± 4.5 ^{e}	65.5 ± 1.7 ^{c}	14.53 ± 1.3 a	23.6 ± 2.7 ^{b}	14 ± 0.7 ^{a}
Catechin	14.8 ± 6.5 ^{a}	77.7 ± 4.6 ^{c}	48.1 ± 1.3 b	0 ± 0.0	0 ± 0.0	0 ± 0.0
Vanilic acid	35.7 ± 1.17 ^{c}	20 ± 2.03 ^{b}	15.9 ± 0.4 ^{a}	0 ± 0.0	0 ± 0.0	0 ± 0.0
Myricetin-glucoside	39.1 ± 1.48 ^{a}	71.1 ± 0.9 ^{c}	62.95 ± 3.01 b	0 ± 0.0	0 ± 0.0	0 ± 0.0
p-Coumaric acid	76.94 ± 1.7 ^{e}	76.14 ± 2.5 ^{e}	70.83 ± 1.8 ^{d}	18.78 ± 0.6 ^{a}	21.2 ±1.1 ^{c}	19.58 ± 1.2 ^{b}
Quercetin-glucoside	109.58 ±0.9 ^{d}	274.7 ± 7.6 ^{f}	175.31 ± 1.4 ^{e}	9.5± 0.3 ^{a}	11.7 ± 1.8 ^{c}	10.6 ± 0.2 ^{b}
Ferulic acid	9.75 ± 0.98 ^{a}	165.1 ± 7.1 ^{c}	106.41 ± 5.4 ^{b}	0 ± 0.0	0 ± 0.0	0 ± 0.0
Quercetin-arabinoside	98.6 ± 2.5 ^{d}	129.4 ± 1.4 ^{f}	106.2 ± 10.7 ^{e}	6.5 ± 1.6 ^{c}	6.3 ± 0.7 ^{b}	5.64 ± 0.8 ^{a}
Kaempferol-glucoside	101.2 ±5.79 ^{d}	285.8 ± 6.5 ^{e}	312.7 ± 3.9 ^{f}	12.2± 0.8 ^{a}	14.1 ± 0.1 ^{b}	15.87 ±0.2 ^{c}
Kaempferol-acetyl-glucoside	15.8 ± 0.2 ^{d}	150.7± 6.4 ^{f}	126.41 ± 6.1 ^{e}	6.1 ± 0.5 ^{a}	8.4 ± 0.7 ^{b}	6.7 ±0.4 ^{c}
Mean	162.40	168.71	118	7.795	11.3	0
CV %	111.6	95.5	91	10.3	15.9	1360
F ratio	75.05	2.7	22.7	217	209	9.8
p value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.04

Note: Superscripts with same letters indicate no significant difference among mean values (n = 3), while different letters indicate a significant difference among mean values at $p < 0.05$. F-ratio: value for variance analysis; CV%: coefficient of variation. F-ratio represents the ratio of variance between groups to variance within groups, and CV% (coefficient of variation) indicates the relative variability of the data. These metrics provide insights into the consistency and statistical robustness of the results.

Dry matter content also varied significantly across the different stages. Pine shoot extracts from stage III had the highest dry matter content (31.6%), while stage I had the lowest (26%). This increase in dry matter content indicated a reduction in water content as the pine shoots matured. Similarly, in the corresponding jellies, dry matter content increased as maturation advanced, with jelly from stage III shoots having the highest dry matter (79.5%) compared to jelly from stage I (77.5%) and stage II (78.3%). The higher dry matter content in stage III jelly suggested better microbial resistance, as lower moisture content typically reduced the potential for microbial growth and spoilage. These findings were consistent with the previous literature on the role of moisture content in the preservation of food products. Ash content, representing the mineral content of the samples, also showed significant variation between stages. Pine shoot extracts from stage III 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 prepared from stage II shoots (0.094%), while stage III jelly had the lowest (0.075%). These differences suggested that maturation may have led to the accumulation of certain minerals in the shoots, which were then transferred into the jelly. The ash content in jelly was essential, as it could influence the overall nutritional value and stability of the product. The production of secondary metabolites in plants mainly depends on plant growth stages, seasons, and organs [18]. The flavor profile, particularly acidity, sourness, and sweetness, plays a crucial role in determining the overall

taste of fruits and vegetables [35,36]. The proximate analysis of pine shoot extracts and biofortified jelly is presented in Table 1. The dry matter content of pine shoot extracts increased significantly with maturation, indicating a decrease in water content [37]. This reduction in moisture content lowers the likelihood of microbial contamination in jelly [38]. Compared to previous studies, where guava leaf extract jelly showed a moisture content range of 44.25%–45.05% [39], biofortified jelly exhibited higher dry matter content.

3.2. The Polyphenol Content of Pine Shoot Extracts and Jelly

The study revealed significant variations in the polyphenol content and antioxidant potential of pine shoot extracts and their biofortified jelly across the different maturation stages (Table 2). The chemical structures of the major phenolic compounds identified in *Pinus sylvestris* L. shoots are shown in Figure 2. Polyphenol content exhibited a statistically significant increase as the shoots matured from stage I to stage II, peaking at 312.2 mg GAE/100 g in stage II. However, stage III extracts demonstrated a marked decline, recording the lowest polyphenol content (164.6 mg GAE/100 g). This decline in stage III may be attributed to metabolic shifts or the depletion of phenolic compounds during advanced maturation. In comparison, stage I shoots exhibited intermediate polyphenol levels (252.84 mg GAE/100 g).

Similarly, the antioxidant potential, measured as DPPH free radical scavenging activity (FRSA), followed a comparable pattern. Stage II shoots showed the highest antioxidant capacity (94.9%), significantly outperforming stage I (82.6%) and stage III (76.8%). These findings underscore the importance of stage II shoots as a superior source of polyphenols and antioxidant potential among the three stages.

For the jelly samples, polyphenol content was notably lower than what was detected in the raw extracts, likely due to the thermal processing involved in jelly production. Stage II jelly exhibited the highest polyphenol content (114.4 mg GAE/100 g), followed by stage I jelly (47.7 mg GAE/100 g) and stage III jelly, which had the lowest content (25.5 mg GAE/100 g). A similar trend was observed in antioxidant potential, with stage II jelly demonstrating the highest DPPH FRSA (16.4%), while stage III jelly showed the lowest (10.9%), marginally lower than stage I jelly (10.7%).

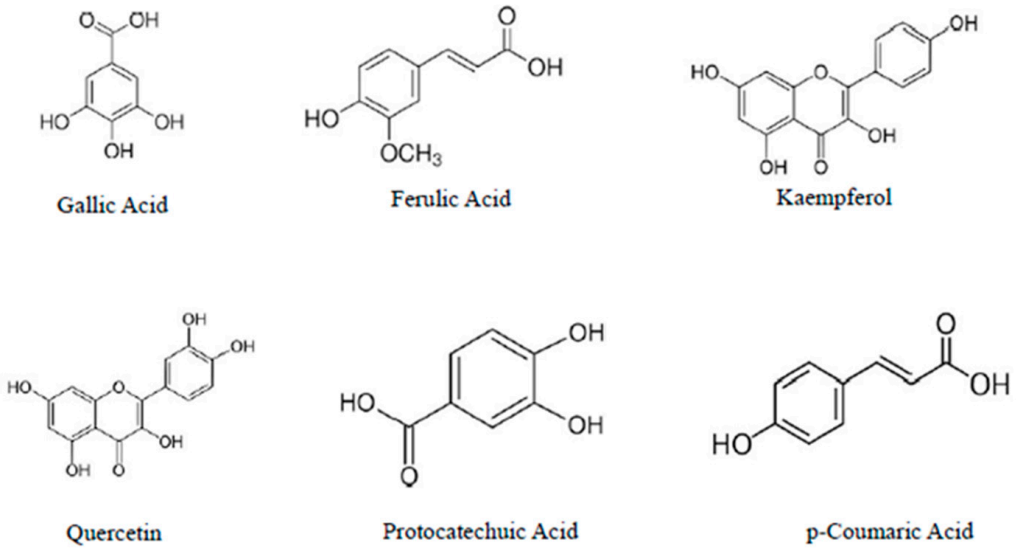

Figure 2. Chemical structures of major phenolic compounds identified in *Pinus sylvestris* L. shoots.

The substantial reduction in polyphenol content and antioxidant potential in jellies, compared to the raw extracts, highlighted the impact of thermal processing. Previous studies have similarly reported that high-temperature processing can lead to significant losses in phenolic compounds and antioxidant activity. However, the higher polyphenol retention and antioxidant potential in stage II jelly suggest that optimal harvesting at this stage could enhance the nutritional quality of the final product.

These findings indicate that stage II pine shoots are optimal for both polyphenol content and antioxidant potential, offering the best balance for producing high-quality biofortified jelly. In contrast, stage III extracts and jellies exhibited the lowest values, emphasizing the impact of maturation and processing on the nutritional properties of pine-based products.

Polyphenols play a crucial role in plant defense against pathogens and ultraviolet radiation [5]. Similarly, biofortified jelly containing pine shoot extract exhibited a comparable trend, albeit at lower concentrations. However, the heat processing required for jelly production likely contributed to the reduced polyphenol content. Food processing involves complex interactions between internal and external factors, such as heat, solvents, and raw materials, which can significantly alter the nutritional profile of plants [40–42]. These interactions can lead to the loss of organoleptic properties and secondary metabolites. Thermal processing, in particular, has been shown to negatively impact the antioxidant profile and secondary metabolite concentrations. For instance, drying figs thermally compromises their antioxidant profile [43]. Similarly, Jeong et al. [44] reported that increasing temperature significantly reduces antioxidant potential and total phenolic content in orange peels. However, optimal temperature control can minimize losses. El Gamal et al. [45] found that maintaining a drying temperature of 55–60 °C preserves phenolic compound concentrations.

3.3. DPPH Free Radical Scavenging (%) of Pine Shoot Extracts and Jelly

The study revealed significant differences in the antioxidant potential of pine shoot extracts and their corresponding jelly across different maturation stages, as evaluated by the DPPH free radical scavenging assay (DPPH FRSA). The antioxidant capacity of pine shoot extracts decreased as the shoots matured from stage II to stage III, with stage II extracts showing the highest activity (94.9%) and stage III extracts the lowest (76.8%). Stage I extracts exhibited an intermediate antioxidant potential (82.6%). This trend suggests that stage II shoots are the most potent source of antioxidants, potentially due to their peak accumulation of phenolic and flavonoid compounds.

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

The variation between the highest and lowest antioxidant activities was notable. For extracts, the antioxidant potential ranged from 94.9% (stage II) to 76.8% (stage III), a difference of 18.1%. For jellies, the activity ranged from 16.4% (stage II) to 10.7% (stage I), a difference of 5.7%. This highlights that while the processing impact is universal, the extent of antioxidant loss varies among different maturation stages.

Stage II emerges as the most favorable stage for antioxidant capacity, both in extracts and jellies, indicating that shoots harvested at this stage have the highest potential for producing nutritionally enriched jelly. Conversely, stage III shoots, despite their higher maturity, demonstrated the lowest antioxidant potential in both forms, suggesting a decline in the availability or stability of antioxidant compounds at advanced maturation stages.

The findings align with previous studies emphasizing the degradation of temperature-sensitive antioxidant compounds during processing. Future studies focusing on optimizing processing conditions could help retain the antioxidant potential of biofortified jellies while leveraging the nutritional advantages of stage II pine shoots [46,47]. The scientific literature supports that high temperatures and food processing reduce antioxidant potential by breaking down flavonoids and phenolic acids [48]. Studies have shown that thermal processing affects antioxidants like chlorogenic acid and quercetin [49]. Skrypnik et al. [50] corroborate that thermal processing significantly reduces the antioxidant profiles of plant-derived products. Fortifying food products with plants enhances shelf life and sensory properties [51]. Young shoots of *Pinus sylvestris* L. are rich in antioxidant compounds [5]. Our findings align with Piechowiak et al. [9]'s study, which reported that increasing young pine shoot concentration improves jelly's polyphenolic content, vitamin C, and antioxidant profiles.

3.4. Correlation Matrix Showing Pearson Coefficient Values for the Physicochemical Properties of Pine Shoot and Pine Shoot Jelly at Various Maturation Stages

The Pearson correlation analysis revealed significant positive correlations ($p < 0.05$) among various physiochemical properties of pine shoot extracts and jelly, as presented in Figure 3. Notably, a strong positive correlation was observed between the maturation of pine shoot extract and dry matter content ($r^2 = 0.85$). Furthermore, the polyphenol content of pine shoot extract was closely linked to antioxidant capacity ($r^2 = 0.85$), as illustrated in Figure 3. Additionally, the mineral content of pine shoot jelly positively correlated with polyphenol levels ($r^2 = 0.7$). Thermal processing and additives used in jelly preparation contributed to changes in total acidity and dry matter content from extracts to jelly. These changes play a critical role in enhancing the quality and stability of the jelly formulation. Increased acidity, resulting from both the thermal degradation of acidic compounds and the addition of citric acid, contributes to improved flavor by balancing sweetness and adding a tangy profile. Moreover, higher acidity acts as a natural preservative, reducing microbial growth and extending shelf life. Changes in dry matter content, primarily due to water evaporation during processing, enhance the texture and firmness of the jelly, which are desirable attributes for consumer acceptance. Thus, these transformations not only reflect the impact of processing but also benefit the functional and sensory properties of the final product. Specifically, water evaporation during processing temporarily concentrated certain compounds while added ingredients diluted and redistributed dry matter, altering the jelly's final structure and physicochemical properties. Moreover, heat-induced degradation of acidic compounds increased acidity. These findings highlight distinct dynamics between extract and final product physicochemical properties, underscoring the impact of processing on the nutritional and chemical composition of pine shoot jelly.

A strong positive correlation ($r^2 = 0.99$) was observed between the polyphenol content of pine shoot jelly and its antioxidant capacity. This finding is consistent with Ben-Rejeb et al. [52] study, which reported a significant positive association between DPPH free radical scavenging potential and polyphenol content in citrus fruit juice. Polyphenols play a crucial role in antioxidant potential by activating the production of various antioxidant enzymes, including superoxide dismutase, glutathione peroxidase, and catalase [53]. These enzymes neutralize free radicals, thereby protecting against oxidative stress and cellular damage.

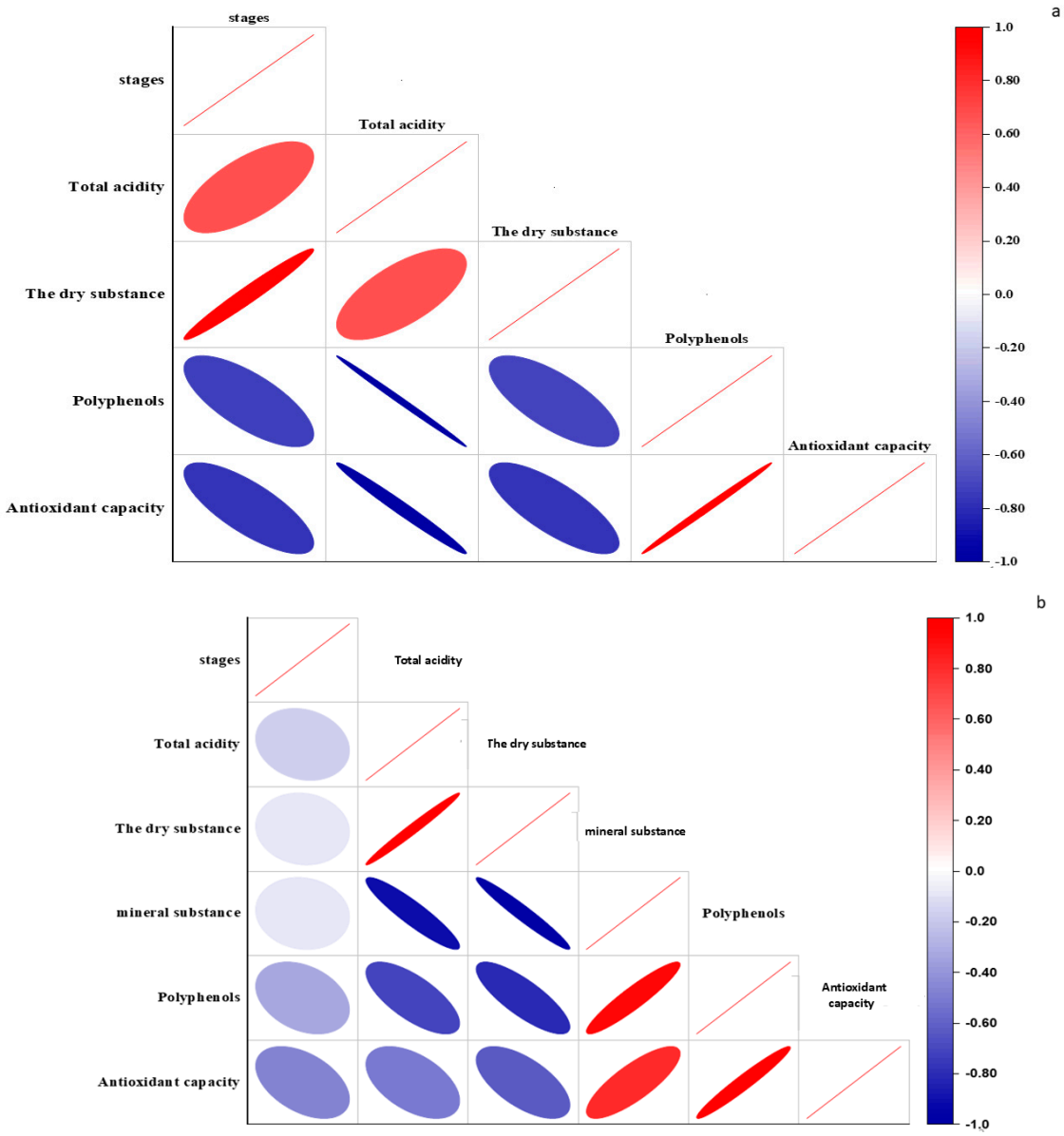

Figure 3. Correlation analysis using Pearson coefficient values examines the relationship between the physicochemical properties of both pine shoot (**a**) and pine shoot jelly (**b**) at different maturation stages.

3.5. Identification and Quantification of Phenolic Compounds Through HPLC-DAD-ESI-MS

Polyphenols, essential secondary metabolites in plants, play critical roles in growth, development, and defense against biotic and abiotic stressors [4,54]. This study identified 14 phenolic compounds in *Pinus sylvestris* L. shoots using HPLC-DAD-ESI-MS, including five phenolic acids (e.g., gallic acid, protocatechuic acid), three hydroxycinnamic acids (e.g., ferulic acid, p-coumaric acid), and six flavonoids (e.g., quercetin, kaempferol), consistent with previous reports for *Pinus* species [49,55] (Table S1). The phenolic profile showed stage-dependent variations, with stage II pine shoots 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. The data presented in Table 3 include statistical analysis results, where different superscript letters indicate significant differences ($p < 0.05$). These differences are interpreted within the same column, indicating variations between shoot stages for the respective parameter (e.g., total acidity, dry matter, or ash content). For example, values within the same

column that share the same superscript letter do not differ significantly, while those with different letters represent statistically significant differences. The statistical analysis was conducted using Tukey's post hoc test following one-way ANOVA, ensuring a robust comparison of means across the developmental stages of pine shoots and corresponding jellies. The stage-dependent decrease in phenolic compounds corroborates earlier findings that developmental stages influence secondary metabolite synthesis and storage, as seen in Fernandez et al. [17], who observed high monoterpene and caffeic acid concentrations in young pine needles.

Comparisons between pine shoot extracts and derived jelly revealed lower phenolic concentrations in jelly across all maturation stages, attributed to thermal degradation and structural interactions with macromolecules [56]. For instance, 4-hydroxybenzoic acid, abundant in stage II pine shoots (820.5 ± 5.8 μg/g DW), significantly decreased to 26.8 ± 2.3 μg/g DW in stage II jelly. This trend was consistent across other phenolic acids and flavonoids, emphasizing the impact of processing on compound retention [57,58]. Flavonoids such as kaempferol-glucoside showed stage III dominance (312.7 ± 3.9 μg/g DW in shoots) yet retained minimal levels in jelly, reflecting the susceptibility of these compounds to heat and pH changes during processing [59,60].

Heatmap clustering illustrated phenolic compound dynamics across maturation stages in extracts and jellies (Figure 4). The clustering patterns indicate that phenolic compounds such as chlorogenic acid and *p*-coumaric acid, which were abundant in stage II extracts, exhibited reduced intensity in stage II jelly due to degradation and potential interactions. KEGG pathway analysis confirmed phenylpropanoid biosynthesis (map00940) and flavonoid biosynthesis (map00941) as key pathways influencing the synthesis of these compounds. The elevated phenylpropanoid biosynthesis activity at stage II aligns with the higher phenolic concentrations observed during this stage, followed by a reduced metabolic rate as tissues matured from stage II to III.

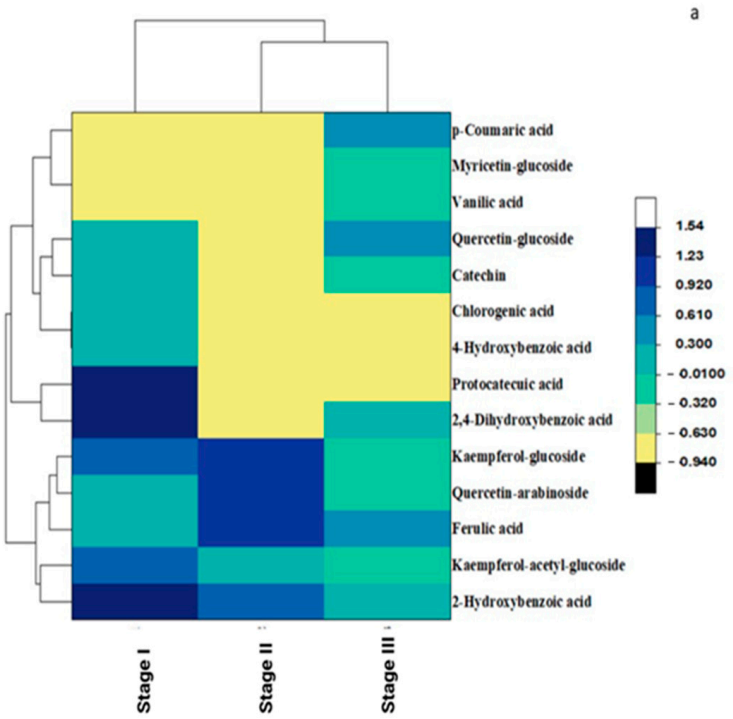

Figure 4. *Cont.*

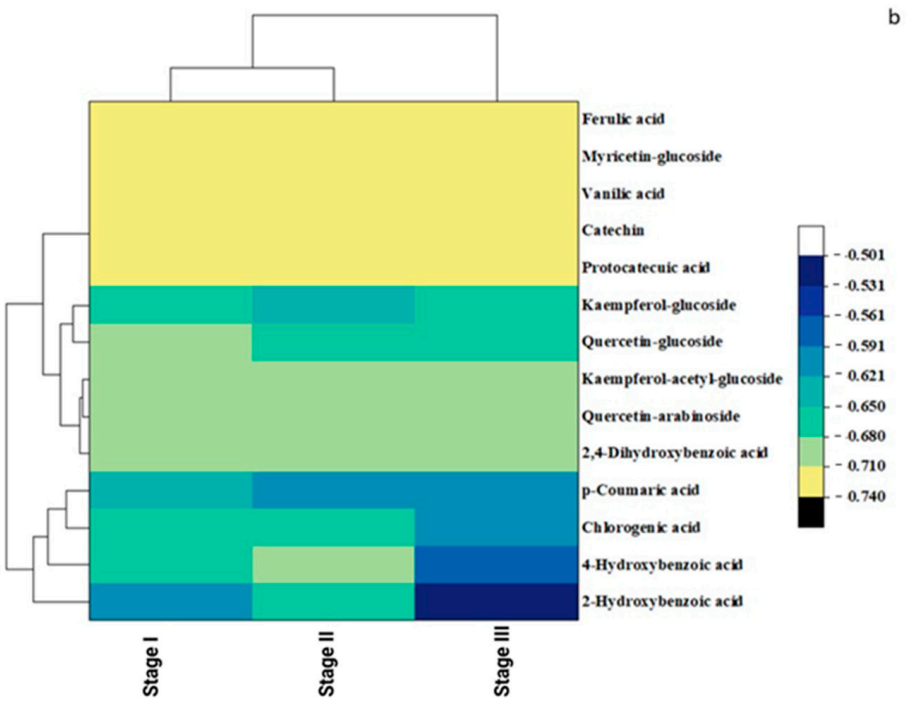

Figure 4. The heatmap with dendrogram illustrates the clustering of phenolic compounds of pine shoot extracts and jelly at different maturation stages. (**a**) Clustering of phenolic compounds in pine shoot extracts; (**b**) Clustering of phenolic compound jelly derived from pine shoots. Note: The phenolic compounds are clustered based on their similarity index across the maturation stage. The scale bar on the right represents the intensity of the compounds in pine shoot extracts and pine shoot-derived jelly. The row z score value of each compound is plotted in yellow color (positive or higher concentration) to dark blue color (negative or lower concentration).

Polyphenols such as phenylpropanoids scavenge reactive oxygen species, inhibit oxidizing enzymes, and catalyze oxygenation reactions via metallic complex formation [61]. These antioxidant properties are critical for mitigating oxidative stress, further underscoring the potential nutritional and therapeutic applications of *Pinus sylvestris* L. phenolic compounds. However, the substantial loss of phenolics during jelly preparation calls for the optimization of processing parameters to enhance the retention of bioactive compounds. By refining these conditions, the nutritional profile of pine shoot-derived jellies can be improved, leveraging the potent antioxidant properties of its phenolic constituents.

3.6. FTIR Spectra of Pine Shoot Extracts and Jellies

The FT-IR spectra of *Pinus sylvestris* L. shoots from maturity stages I, II, and III, as well as that of the jelly obtained from the extracts, confirmed the presence of significant compounds such as phenolic acids, flavonoids, catechins, epicatechins, procyanidins, organic acids, and carbohydrates (Figure 5). While all samples exhibited a comparable spectral fingerprint, distinct differences were observed between the developmental stages and the derived jelly, indicating variations in the accumulation of bioactive compounds during maturation and processing. The consistent detection of these compounds aligns with the existing literature, which underscores the role of phenolics as primary antioxidants in woody plants, essential for mitigating oxidative stress [49].

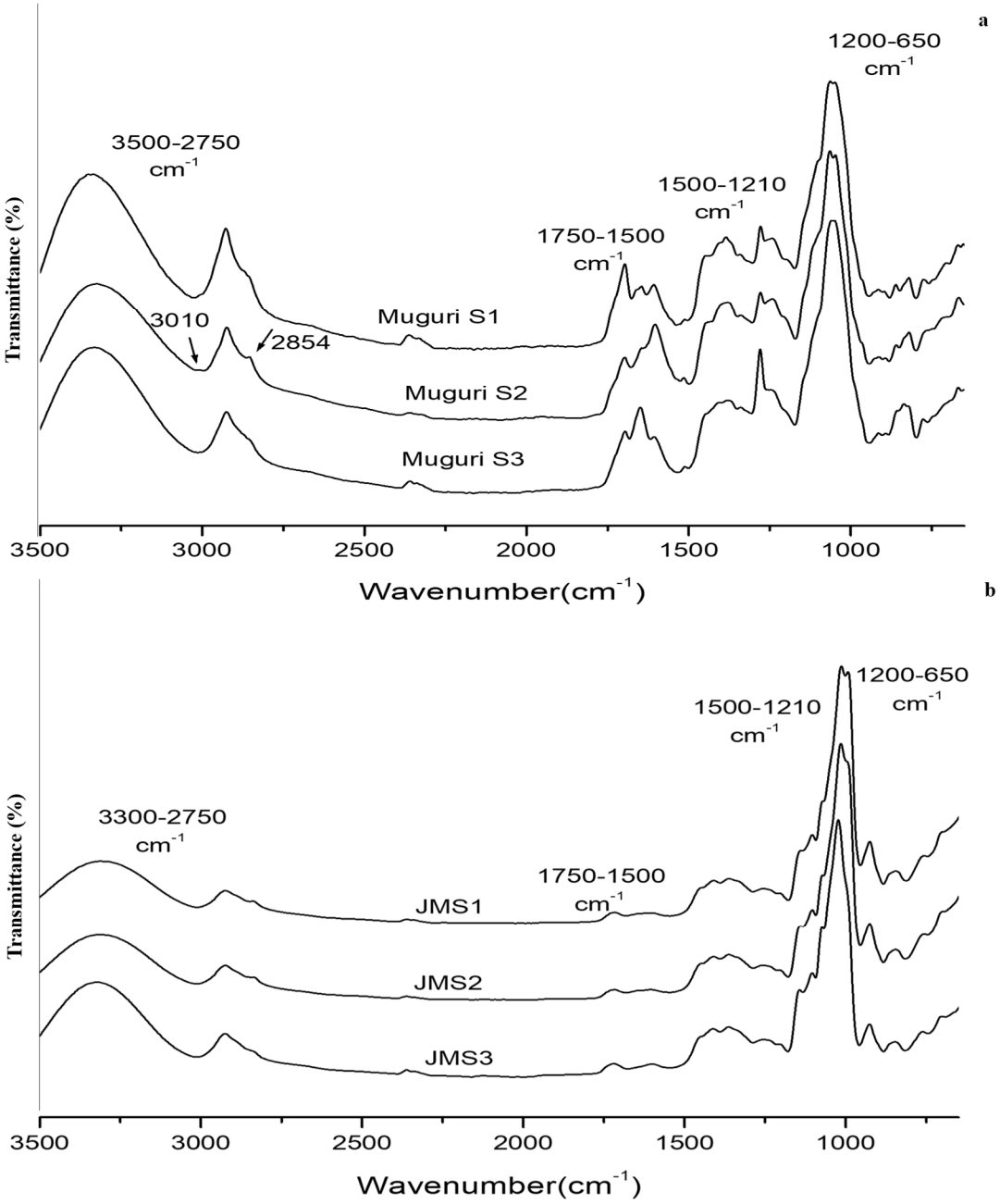

Figure 5. FTIR spectrum of pine shoot extracts (**a**) and jelly (**b**). Note: Muguri S1 = stage I, Muguri S2 = stage II, and Muguri 3 = stage III. JMS1 = jelly stage I, JMS2 = jelly stage II, and JMS3 = jelly stage III.

The absorption bands detected in the 750–900 cm^{-1} region, associated with phenols, esters, and acetals, were more intense in stage III, indicating an increase in these compounds' concentration as the shoot matures, as previously observed by Kholiddinov and Gribov [60]. Absorption at 1200–1250 cm^{-1}, which corresponds to aromatic *OH* stretching, was visible in all stages, confirming the consistent presence of phenolic compounds. This suggests the progressive accumulation of phenolic compounds as *Pinus* shoots mature, corroborated by other research that revealed the accumulation of flavonoids and phenolic acids in species within the *Pinus* genus [4]. In the 1030–1100 cm^{-1} range, corresponding to C-O stretching vibrations associated with carbohydrates (glucose and fructose), increased intensity was observed in early maturity stages, while this band diminished in the final jelly product. This indicates partial utilization of sugars during gelling, as seen in other processed plant-based

products [41]. An additional band, present only in the jelly at 1716–1743 cm^{-1}, corresponds to free organic acids such as citric acid, indicating thermal degradation of certain phenolic compounds and transformation of carbohydrates during the gelling process. The bands at 2854, 2924–2927, and 3010 cm^{-1} correspond to C-H stretching vibrations in CH_3 and CH_2 groups, specific to carbohydrates or lipid precursors, and exhibit increased intensity in stage III [61,62].

These findings suggest that the thermal processing of jelly impacts the stability of bioactive compounds, particularly phenolics and carbohydrates, leading to a reduction in the final product's antioxidant efficacy. To address this, future research could focus on alternative preservation techniques, such as low-temperature processing or the incorporation of stabilizing agents, to enhance the retention of phenolic compounds and optimize the antioxidant potential and nutritional value of pine shoot jellies. Similar approaches have been highlighted in the literature, where lower temperature processing has been shown to better preserve phenolic compounds and maintain antioxidant potential in processed plant-based products [22,41].

3.7. Textural Properties of Pine Shoot-Derived Jelly at Various Maturation Stages

Table 4 presents the textural properties of pine shoot-derived jelly prepared from *Pinus sylvestris* L. shoots at stages I, II, and III. The results show that jelly from stage I had the highest consistency (88 g) and mechanical work (2.85 mJ), indicating a firmer texture. This variation is likely due to increased acidity at later stages, which accelerates gelation but reduces overall cohesion. These findings align with prior studies showing that acidity influences gel network formation, with lower pH typically leading to less robust gels in polysaccharide-based systems. In contrast, stage II and III jellies had lower consistency (41.5 g and 45.5 g) and mechanical work (0.9 mJ and 1 mJ), reflecting a softer texture. This is likely due to the increased acidity at later stages, which accelerates gelation but results in a less cohesive texture.

Table 4. Textural properties of pine-shoot derived jelly.

Sample	Stage I	Stage II	Stage III
Consistency cycle 1 (g)	88	41.5	45.5
Total mechanical work cycle 1 (mJ)	2.85	0.90	1.00
Stickiness (g)	0.20	0.15	0.2
Cohesiveness	0.27	0.37	0.43
Elasticity index (mm)	0.70	0.65	0.63
Gummines (g)	23.50	15.50	19.50

The stickiness was similar across all stages, with stage I and III showing 0.2 g and stage II at 0.15 g. Cohesiveness increased from 0.27 (stage I) to 0.43 (stage III) 0.43, indicating a better ability of the structure to hold itself together. This trend is likely linked to greater dry matter content and moisture reduction in advanced maturation stages, which enhance structural integrity. Elasticity showed minimal variation, with stage I at 0.7 mm, stage II at 0.65 mm, and stage III at 0.63 mm, suggesting that elasticity was not significantly impacted by maturation. Gumminess was highest in stage I (23.5 g), lower in stage II (15.5 g), and intermediate in stage III (19.5 g), correlating with the observed firmness and gelation speed.

The textural differences between stages I, II, and III are linked to changes in acidity and moisture content during maturation. Stage I exhibited the firmest texture due to slower gelation, while stages II and III were softer due to faster gelation at higher acidity. Despite the softer texture, stage III maintained better cohesiveness than stage II. These findings highlight the importance of chemical and physical factors in shaping the texture

of plant-based jellies and suggest that future processing techniques could help optimize both texture and bioactive content [63,64].

Comparing these results to fruit-based jellies or similar systems, the pine shoot jellies exhibited higher consistency during cycle 1 of compression (88 g vs. typical fruit jelly range of 30–60 g) and gumminess, which can be attributed to the unique composition of phenolic compounds and dry matter in the pine shoot extracts. These textural attributes suggest that pine shoot-derived jellies have potential applications in premium or functional food markets, catering to preferences for firmer or denser products.

4. Future Prospects

While thermal processing and the use of additives are effective in achieving the desired jelly texture and acidity, their impact on the degradation of phenolic compounds and antioxidant capacity highlights potential limitations. Future research could explore alternative methods to minimize these losses while maintaining product quality. For instance, low-temperature vacuum evaporation or freeze-drying could replace high-temperature processing to better preserve phenolic compounds and other heat-sensitive bioactives. Additionally, natural pH modifiers, such as citrus or berry extracts, could be used as alternatives to synthetic additives like citric acid, providing similar preservative effects while potentially enhancing the nutritional value. Another promising approach involves the incorporation of stabilizing agents, such as plant-based gums or encapsulation techniques, to protect phenolic compounds during processing. These strategies could offer improved retention of bioactive compounds and align with clean-label consumer preferences.

5. Conclusions

This study explored the impact of the developmental stages of *Pinus sylvestris* L. shoots on their phenolic profiles, antioxidant capacity, and their incorporation into jelly formulations. Extracts from stage II shoots demonstrated the highest polyphenol content (312.2 mg GAE/100 g) and antioxidant potential (94.9% DPPH scavenging activity), underlining the nutritional value of this maturity stage. HPLC-DAD-ESI-MS analysis identified 14 phenolic compounds, with 4-hydroxybenzoic acid being most abundant in stage II extracts (820.5 µg/g DW). Despite this, the phenolic compound content in jellies was significantly reduced due to thermal processing, emphasizing the need for improved preservation techniques. The results highlight the potential of pine shoot extracts as natural antioxidants for fortifying functional foods, contributing both to nutritional enhancement and sustainable ingredient sourcing. To address the reduction of bioactive compounds during jelly preparation, future research should explore alternative processing methods, such as low-temperature or non-thermal technologies, and investigate the application of stabilizing agents like encapsulation to protect phenolic compounds. Additionally, further evaluation of consumer preferences and sensory properties of pine shoot-enriched jellies could support their market integration. These advancements are crucial for developing innovative, health-oriented food products that leverage the unique properties of pine shoots while ensuring bioactive retention.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f16010011/s1, Table S1: DAD and MS data obtained after positive ionization of the samples.

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& editing, R.A.M. and C.C.M.; Visualization, C.C.M. and S.M.; Supervision, C.C.M. and S.M.; Project administration, S.M. All authors have read and agreed to the published version of the manuscript.

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