Agronomy Research **24**(2), 347–364, 2026
https://doi.org/10.15159/AR.26.055

Evaluation of drying techniques to preserve bioactive compounds in spruce sprouts during storage

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Received: January 30th, 2026; Accepted: July 8th, 2026; Published: July 17th, 2026

Abstract. Spruce sprouts are a rich source of vitamins and antioxidants. Still, there is little information about the stability of compounds in different dried spruce sprouts during storage. This study aims to analyse the optimal drying method for spruce sprouts to ensure higher bioactive compound content for a shelf life of one year. Four types of spruce sprouts were analysed – fresh, dried at 40 °C, at 60 °C, and freeze-dried. Spruce sprouts were analysed for moisture, Vitamin C content, total tannin content, a-, and b- chlorophyll, total chlorophyll and total carotenoids, as well as volatile compounds in different spruce sprouts were characterised. To evaluate the optimal drying method for preserving bioactive compounds for over a year, the technique for order of preference by similarity to an ideal solution, TOPSIS, was used. According to TOPSIS, the most optimal results were observed in fresh samples. However, when analysing drying methods, the most optimal for spruce sprouts were freeze-drying and drying at 40 °C. Characterising volatile compounds in different processed spruce sprouts, regardless of the drying method, D-limonene was present at the highest concentration in all spruce sprout samples, imparting a characteristic citrus aroma. Overall, the results indicate that while fresh spruce sprouts initially contained higher levels of bioactive compounds, freeze-drying proved to be the most effective method for preserving these compounds during a year of storage. This method not only ensures long-term stability but also offers a promising approach for developing stable, nutrient-rich spruce sprout products with extended shelf life and high functional value suitable for industrial applications.

Key words: chlorophyll, freeze drying, vitamin C, volatile compounds, TOPSIS.

INTRODUCTION

Non-timber forest products (NTFPs) are biological resources derived from natural forests that exclude timber and other wood-based products (Qiao et al., 2024). These products are increasingly recognised for their contribution to economic development and rural livelihoods, serving as valuable forestry by-products with both commercial and subsistence value (Nguyen et al., 2020; Bhardwaj et al., 2021; Qiao et al., 2024), including sprouts, buds, mushrooms, berries, honey, resin, cones, and nuts (Asamoah et al., 2025). Among these, spruce sprouts represent a relatively small but increasingly

valued category. Spruce sprouts are the newly developed shoots of the Norway spruce, *Picea abies* (L.) H. Karst., and can be readily identified in early spring by their distinctive light-green colouration. They are typically harvested between late April and mid-June, although the precise harvesting period varies according to climatic conditions and geographical location (Jyske et al., 2020; Karklina, 2022, 2024; Karklina et al., 2024). Spruce sprouts are rich in bioactive compounds, particularly vitamin C, which enhances their nutritional value and provides antioxidant benefits (Sirgedaitė-Šėžienė et al., 2022). Like other conifers, spruce sprouts are also a valuable source of monoterpenes, which serve as a natural defence mechanism for the plant but also exhibit antimicrobial properties, making them useful for food, pharmaceutical, and cosmetic applications (Bhardwaj et al., 2021). Fresh spruce sprouts have a relatively short shelf life, and their bioactive compounds can degrade when exposed to light and oxygen (Jyske et al., 2020). Comparing preservation methods, especially drying, can extend the shelf life of spruce sprouts while keeping their bioactive content and benefits. Since spruce sprouts are only available for a short harvest season, effective preservation is needed to make them available all year and maintain consistent quality. Freezing is an effective preservation method for maintaining the nutritional value and overall quality of spruce sprouts outside the harvesting season (Schudel et al., 2021). However, freezing is a complex process involving heat transfer and physicochemical changes that may affect product quality (Jha & Sit, 2022). Although low temperatures inhibit microbial growth and slow biochemical reactions, freezing and subsequent frozen storage can degrade bioactive compounds, including carotenoids and chlorophyll (Grover & Negi, 2023; Li et al., 2023). Furthermore, the formation of ice crystals may cause cellular damage, potentially affecting the texture and quality of plant materials. An alternative approach for preserving valuable plant constituents is drying. Drying is a widely employed preservation method that reduces moisture content while facilitating the retention of essential oils and other bioactive compounds (Thamkaew et al., 2021). Among the various drying techniques, freeze-drying, also known as lyophilisation, removes water from plant material through the sublimation of ice under reduced pressure. Before the lyophilisation process, the water present within the plant tissue is frozen, thereby inhibiting chemical, biochemical, and microbiological reactions. As a result, lyophilisation effectively preserves product quality, including texture, flavour, aroma, and colour, while extending shelf life and maintaining the stability of valuable bioactive compounds (Nowak & Jakubczyk, 2020). To objectively determine which drying method most effectively preserves the quality and bioactive composition of spruce sprouts, multi-criteria decision-making tools such as the Technique for Order Preference by Similarity to an Ideal Solution (TOPSIS) can be employed. TOPSIS is a widely used multi-criteria decision-making method that ranks alternatives based on their relative proximity to an ideal solution. This method identifies a positive ideal solution, which represents the best achievable performance across all selected criteria, and a negative ideal solution, which represents the worst performance (Rahman et al., 2024). When evaluating the stability of bioactive compounds in spruce sprouts during drying and storage, multiple quality attributes and processing parameters must be considered simultaneously, including the retention of bioactive compounds, antioxidant activity, and drying efficiency. In this context, the Technique for Order Preference by Similarity to an Ideal Solution (TOPSIS) is particularly useful, as it enables the simultaneous

evaluation of multiple performance criteria and facilitates the systematic assessment of trade-offs between competing factors, such as compound preservation and process efficiency. By calculating geometric distances to ideal and negative-ideal solutions, the method provides an objective, quantitative, and reproducible ranking of drying or storage conditions. Its flexibility makes it well-suited for optimising complex processes where multiple quality and functional parameters must be balanced (Mathebula & Mbuli, 2025). Building on this approach, the present study aims to identify the optimal drying method for spruce sprouts that maximises the retention of bioactive compounds over a one-year storage period. TOPSIS is used to evaluate and rank the drying techniques based on their effectiveness in preserving these compounds. The results of this study provide practical guidance for the food industry in developing functional plant-based products with enhanced nutritional quality and prolonged shelf life.

MATERIALS AND METHODS

The study was carried out in the laboratories of the Food Institute at the Latvia University of Life Sciences and Technologies. Spruce sprouts were harvested in the spruce forest of 8–10-year-old trees near Jelgava, Latvia (latitude – 56° 37'N; longitude – 23°54'E) in May 2024 and frozen in a PE plastic bag at a temperature of -18 ± 2 °C until further use.

Processing techniques of spruce sprouts

Fresh or frozen spruce sprouts were used in this study. Freezing was also considered a storage method for preserving fresh samples; however, it requires considerably more storage space. Therefore, different drying methods were also analysed and compared to identify the most suitable drying technique for long-term storage while maintaining quality. Spruce sprouts were dried following the method described by Karklina (2022). For conventional drying, a convective dryer (Universal Oven UF160, Memmert, Germany) was used at 40 °C for 20 hours and at 60 °C for 5 hours until the samples reached a constant weight. For freeze-drying, samples were first frozen at −20 ± 2 °C for 2 days, then dried in a T33 freeze-dryer (Armfield Ltd, UK) under a vacuum of 6.4 Pa for 48 hours. The dried spruce sprouts were stored in glass jars in the dark until further analysis at room temperature of 18 ± 2 °C (Karklina, 2022). Before the analysis, spruce sprout samples were ground for one minute using the electric coffee grinder CLATRONIC KSW 3306. Dried samples were visually characterised as dry sand; however, the fresh sample had a bigger particle size and had a more pieces consistency. All samples were relatively homogeneous. Grounded spruce sprouts were stored in two separate glass jars for one year. Samples were collected from the same jars at three-month intervals throughout the storage period to assess the stability of bioactive compounds. At each sampling time point, two replicate samples were collected from each jar, resulting in a total of four replicates (two per jar) for analysis.

Determination of the moisture, %

The moisture content of fresh and dried spruce sprouts was determined using an electronic moisture balance (RADWAG 'MA 50/1. R. WH', Poland). Three repetitions were made for each sample.

Determination of vitamin C

Vitamin C in spruce sprouts were determined by the iodometric method Segliņa (2007); Karklina (2022) and Karklina (2024), and Karklina et al. (2024). Each sample had three replicates, and the results were calculated as mg 100 g^{-1} dry weight (DW) of product.

Determination of Total Tannin Content (TTC)

The sample extract for total tannin determination was prepared according to the method of Jyotismita Khasnabis et al. (2015) with minor modifications. The sample weight was reduced to avoid multiple dilutions. The grounded spruce sprout sample was weighed to 1.0 ± 0.05 g, then mixed with 25.0 mL of distilled water and heated at 100 °C for 10 min. The extract was left to cool down to room temperature for 20 minutes. Then filtered through filter paper, Whatman Nr. 6. Total tannin content was determined by the Ojha et al (2018) method with minor modifications. In the tubes, 0.5 mL of extract, 1.0 mL of $FeCl_3$, 1.0 mL of 1% $K_3[Fe(CN)_6]$, and 7.5 mL of distilled water were weighed. Mixed using a centrifuge (CM-6MT Elmi Ltd., Riga, Latvia). Absorbance was measured with a Libra S70 PC spectrophotometer (Biochrom Ltd., USA) at 720 nm; three replicates were performed for each extract. Results expressed as mg TA 100 g^{1} DW.

Determination of Chlorophyll a, Chlorophyll b and total carotenoid

Chlorophyll a, chlorophyll b and total chlorophyll and total carotenoid content were determined by the spectrophotometric method according to Tomsone & Kruma (2019). The method was applied with minor modifications, using acetone instead of the four solvents originally described. A homogenised sample (0.5 ± 0.1 g) was weighed into a 50 mL beaker, and 10 mL of acetone was added. The mixture was stirred with a magnetic stirrer at 700 rpm for 15 minutes at room temperature (20 ± 2 °C) using a 4.0×0.5 cm magnetic bar. After extraction, the supernatant was separated by decanting. The residue was then re-extracted using the same procedure. Both supernatants were combined and filtered. The extraction was carried out in triplicate. Chlorophyll a (Ch a), chlorophyll b (Ch b), and total carotenoids (Ca) were determined using a Libra S70 PC spectrophotometer (Biochrom Ltd., USA) at wavelengths of 470, 645, and 662 nm. The concentrations of Ch a, Ch b, and Ca were calculated using Eqs 1, 2, 3, and 4. Results were calculated and expressed as mg 100 g^{-1} DW of plant material. All determinations were performed in triplicate. Additionally, total extractable chlorophyll content (Ch t) was calculated.

$$\text{Ch a} = 12.25 \cdot \text{A662} - 2.79 \cdot \text{A645} \quad (1)$$

$$\text{Ch b} = 21.5 \cdot \text{A645} - 5.1 \cdot \text{A662} \quad (2)$$

$$\text{Ch t} = \text{Ch a} + \text{Ch b} \quad (3)$$

$$\text{Ca} = (1{,}000 \cdot \text{A470} - 1.82 \cdot \text{Ch a} - 85.02 \cdot \text{Ch b})/198 \quad (4)$$

where A is the absorbance of the direct wavelength.

Determination of volatile compounds

Volatile compounds in spruce sprouts were determined according to the methods of Karklina (2024), Karklina & Ozola (2023) and Zhu et al. (2022). Solid phase microextraction was used to isolate compounds from spruce shoots, and a gas

chromatograph coupled to a mass spectrometer was used to determine volatile compounds. 3.00 ± 0.01 g of crushed spruce shoots were weighed into a 25 mL transparent vial, then the vial was closed with a stopper. The fibre used was coated with divinylbenzene, carboxene and polydimethylsiloxane (DVB/CAR/PDMS). The vial was placed on a hot plate at 40 °C for 30 minutes to equilibrate the volatile compounds. The previously prepared SPME fibre DVB/CAR/PDMS was inserted into the vial through the stopper for 15 minutes at 40 °C. A Perkin Elmer Clarus 500 chromatograph with an Elite-Wax ETR mass spectrometer (60 m × 0.25 mm inner diameter; DF 0.25 column) was used for the determination of volatile compounds. The desorption time was 15 minutes at +250 °C. The oven was maintained at 40 °C for 7 minutes, then the temperature was increased to 160 °C at a rate of 6 °C min^{-1}, followed by a further increase to 210 °C at a rate of 10 °C min^{-1} for 15 minutes. Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. Compounds were identified by comparing their mass spectra with the NIST98 spectral library. Two replicates were performed for each sample.

Statistical analysis

Statistical analyses were performed using Microsoft Excel 365 (2024 version). Due to the utilisation of a single open container over time, data were analysed descriptively to avoid temporal pseudoreplication. Parameters are presented as the mean ± standard deviation (SD) of technical replicates at each time point. Temporal trends and stability were evaluated by monitoring changes in these mean values across the study duration.

Tukey's test was used to determine differences among the samples. Differences were considered significant at ($p \leq .05$). Data visualisation was conducted using R software (Posit Software, 2022). To evaluate the best processing method of spruce sprouts, a multi-criteria method for determining the order by similarity to the ideal solution (TOPSIS) was used. The data were normalised to eliminate scale differences and multiplied by the assigned weights (Table 1) to obtain a weighted normalised matrix.

Table 1. Weights of the used parameters

Criterion	Weight
Moisture	0.05
Vitamin C	0.3
Total chlorophyll	0.15
Chlorophyll a	0.05
Chlorophyll b	0.05
Total carotenoids	0.1
Total tannins	0.3

Table 2. Ideal and non-ideal solutions for different dried spruce sprouts according to TOPSIS

Criteria	Ideal solutions	Non-ideal solutions
Moisture	0.003724	0.04912
Vitamin C	0.276822	0.061171
Total chlorophyll	0.145485	0.017224
Chlorophyll a	0.046795	0.007342
Chlorophyll b	0.049332	0.003469
Total carotenoids	0.070252	0.025906
Total tannins	0.055555	0.283284

In TOPSIS, the criteria were selected based on the importance of the parameters and the concentration of compounds in spruce sprouts, as reported in previous studies. Positive and negative ideal solutions were then defined, with higher values considered better for all criteria except TTC, for which lower values were preferred due to its negative nutritional and sensory effects (Cosme et al., 2025) (Table 2).

The distances of each method from the ideal and anti-ideal solutions were calculated, and a proximity coefficient was determined for each extraction method, with higher values indicating better overall extraction performance (Karabašević et al., 2020).

RESULTS AND DISCUSSION

For spruce sprout samples, moisture, vitamin C, total tannin, chlorophyll (a and b), total chlorophyll, and total carotenoid contents were analysed during storage, and volatile compounds were characterised. Moisture is an important factor for plants, particularly during storage, as it affects spruce sprout appearance and the stability of bioactive compounds (Rumicha et al., 2025). In this study, moisture content was analysed (Table 3) for fresh (S_F), dried at 40 °C (S_40), dried at 60 °C (S_60) and freeze-dried (S_Fd) samples. In fresh spruce sprouts, moisture content ranged from 82.42 ± 0.85% at the beginning of storage to 83.15 ± 0.85% at the end of storage.

Table 3. Moisture % content in spruce sprouts during storage

Samples	October (2024)	January (2025)	April (2025)	July (2025)
S_F	82.42 ± 0.85^{a}	82.5 ± 1.17^{a}	82.92 ± 1.17^{a}	83.15 ± 0.85^{a}
S_40	5.83 ± 0.24^{b}	7.09 ± 2.36^{a}	8.46 ± 2.36^{a}	9.01 ± 0.24^{a}
S_60	11.96 ± 0.43^{b}	12.43 ± 0.52^{b}	13.12 ± 0.52^{a}	13.61 ± 0.43^{a}
S_Fd	6.22 ± 0.12^{c}	6.50 ± 0.71^{b}	6.7 ± 0.19^{b}	7.48 ± 0.71^{a}

*Data were analysed descriptively due to the use of a single open container over time, to avoid temporal pseudoreplication. Values are presented as mean ± SD of technical replicates at each time point. Temporal trends and stability were assessed from changes in these mean values across the study period. Data in the same row with different letters are significantly different ($p \leq 0.05$). S_F – fresh spruce sprouts, S_40 – spruce sprouts dried at 40 °C, S_60 – spruce sprouts dried at 60 °C, S_Fd – freeze-dried spruce sprouts.

A slight increase at the end of storage was observed. There were no significant differences in moisture in F_S during storage ($p \geq 0.05$). The moisture content of fresh spruce sprouts is similar to that reported by Jyske et al. (2020), who found 83-84%. Slightly different moisture content in plant samples can be explained by tree growth conditions, such as soil moisture and air humidity during the growth stage (Ievinsh, 2023). Among dried samples, the lowest moisture content was in samples dried at 40 °C, 5.83 ± 0.24% at the beginning of storage and 9.01 ± 0.24% at the end of storage. There was a significant difference in moisture in sample S_40 during storage ($p \leq 0.05$). A relatively low moisture content was also observed in sample S_Fd at the beginning of storage (6.22 ± 0.12%) and at the end of storage (7.48 ± 0.71%); a significant difference in moisture content in S_60 during storage ($p \leq 0.05$). Among the dried samples, sample S_60 had the highest moisture content during storage, at 11.96 ± 0.43% at the beginning and 13.61 ± 0.43% at the end. This phenomenon may be attributed to the drying temperature. As reported by Shaukat et al. (2024), higher drying temperatures can cause the plant surface to dry rapidly, leading to the formation of a hardened outer layer. This layer can hinder the diffusion of internal moisture, thereby limiting moisture dissipation from the material's interior. A similar behaviour was observed in sample S_40, suggesting that elevated drying temperatures played a key role in this outcome. There was a significant difference in moisture content in S_Fd during storage ($p \leq 0.05$). The moisture content of samples S_40 and S_FD were lower than that of the other spruce sprout samples. Although S_40 exhibited a lower initial moisture content than S_FD, the

increase in moisture during storage was higher in S_40, whereas S_FD showed a smaller change. This difference could be attributed to the drying technique used. Conventional drying primarily removes free water from the surface and near-surface regions of plant tissues. This process may lead to partial structural collapse and the exposure of hydrophilic cell wall components, thereby enhancing the material's ability to bind water vapour during storage. In contrast, freeze-drying removes water by sublimation, resulting in a highly porous and less structurally altered matrix with a lower overall sorption capacity. Consequently, freeze-dried samples reach lower moisture content under identical storage conditions (Dalgıç et al., 2012). Across all spruce sprout samples, a slight increase in moisture content was observed during storage. This behaviour is consistent with the intrinsic composition of spruce sprout tissues, which are rich in cellulose, hemicellulose, and other hydrophilic polymers that readily absorb and retain moisture from the surrounding air (Caprai et al., 2018).

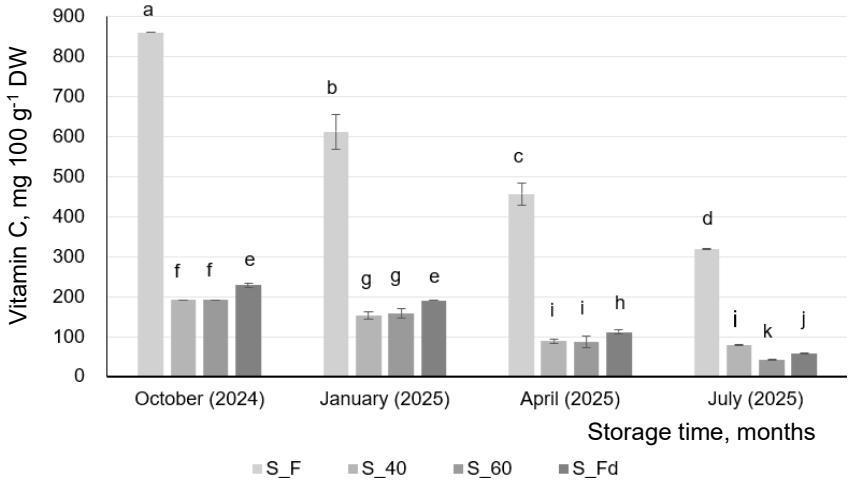


Figure 1. Vitamin C content of spruce sprouts during storage. Data were analysed descriptively due to the use of a single open container over time, to avoid temporal pseudoreplication. Values are presented as mean ± SD of technical replicates at each time point. Temporal trends and stability were assessed from changes in these mean values across the study period. Different letters indicate significant differences ($p \leq 0.05$).

S_F – fresh spruce sprouts, S_40 – spruce sprouts dried at 40 °C, S_60 – spruce sprouts dried at 60 °C, S_Fd – freeze-dried spruce sprouts.

Vitamin C is important for plant metabolism to protect plants from oxidative stress and growth processes, and it's also an important micronutrient in human nutrition (Paciolla et al., 2019). In this study, vitamin C (Fig. 1) was analysed in all spruce sprout samples. In fresh spruce sprouts, vitamin C content decreased during storage. At the beginning of storage, the vitamin C content was 858.72 ± 0.005 mg 100 g^{-1} DW and it was 319.46 ± 0.30 mg 100 g^{-1} DW at the end of storage. There was a significant difference in vitamin C content observed in samples F_S during storage ($p \leq 0.05$). At the beginning of storage, the vitamin C content measured in this study was approximately twice Jyske et al. (2020) that reported by Jyske et al. (2020), at 406.7 mg 100 g^{-1} DW. Differences in vitamin C concentration among fresh spruce

sprouts may be partly explained by variations in the trees' growth conditions. Several studies have shown that conifers growing in northern climates tend to accumulate higher levels of vitamin C, likely due to its protective role against environmental stressors such as low temperatures, intense light, and heat (Raal, 2018). However, this trend was not clearly observed in the present study, suggesting that additional factors may have influenced the vitamin C content. Another factor that may contribute to variability in vitamin C content is tree age. Various studies indicate that older trees often exhibit higher photosynthetic activity, which can lead to increased production of reactive oxygen species. In response, plants may enhance vitamin C synthesis as part of their antioxidant defence system, resulting in higher vitamin C concentrations in sprouts (Paciolla et al., 2019). Comparing vitamin C content among dried spruce sprout samples, the S_Fd sample had a higher vitamin C content during storage, decreasing from 228.29 ± 5.42 mg 100 g^{-1} DW at the beginning of storage to 58.42 ± 0.59 mg 100 g^{-1} DW at the end of storage. A significant difference in vitamin C content was observed in sample S_FD during storage ($p \leq 0.05$). As demonstrated in this study, freeze-drying was more effective at preserving vitamin C in plant tissues compared to conventional drying methods. This can be attributed to the freeze-drying process, which removes water under vacuum at low temperatures, thereby minimising exposure to heat and reducing thermal degradation of vitamin C (Nowak & Jakubczyk, 2020). During storage, samples S_40 and S_60 showed comparable initial vitamin C contents; at the beginning of storage, sample S_40 contained 192.40 ± 0.005 mg 100 g^{-1} DW, while sample S_60 contained 191.64 ± 0.005 mg 100 g^{-1} DW. However, by the end of storage, vitamin C levels declined to 78.27 ± 1.02 mg 100 g^{-1} DW in S_40 and to 41.63 ± 1.10 mg 100 g^{-1} DW in S_60. A statistically significant decrease in vitamin C content was observed in both samples (S_40 and S_60) during storage ($p \leq 0.05$), indicating substantial losses over time. Overall, vitamin C content decreased in all spruce sprout samples throughout storage. In the fresh sample (F_S), vitamin C losses were significantly greater than in the dried samples, possibly due to differences in moisture content. Higher moisture levels promote chemical reactions and enzymatic activity within plant tissues, accelerating vitamin C degradation (Dalgıç et al., 2012). In contrast, the dried samples exhibited a more gradual decline in vitamin C during storage. Although drying removes most of the water, residual moisture can still support slow chemical reactions, including oxidative degradation of vitamin C, which explains the observed losses during storage (Nowak & Jakubczyk, 2020).

Tannins are important for plants for their antioxidant protection and growth regulation, and for humans for their health-promoting antioxidant effects and functional food benefits (Szczurek, 2021; Iqbal & Poór, 2025). In this study, total tannin content (Fig. 2) was analysed in all spruce sprout samples. In F_S, total tannin content was higher than in dried samples. At the beginning of storage, total tannin content was 5.23 ± 0.12 mg TA 100 g^{-1} DW, and at the end of storage, it was 5.27 ± 0.48 mg TA 100 g^{-1} DW. There was no significant difference in total tannin content in sample F_S during storage ($p \geq 0.05$), indicating that total tannin content remained stable throughout storage. This stability can be attributed primarily to the intrinsic chemical structure of tannins, a large group of phenolic compounds characterised by multiple aromatic rings and strong intramolecular bonds, which confer considerable resistance to chemical degradation. Such inherent structural stability allows tannins to be preserved over extended storage periods, particularly under cold conditions

where reaction rates are markedly reduced (Zayed et al., 2025), other studies have reported similar observations that storage at very low temperatures effectively maintains tannin and other phenolic compounds, with only minor reductions observed during long storage (Ben Abda et al., 2025). Some studies indicate that total tannin content varies among different parts of a tree. In a study on spruce bark, the total tannin content was reported to be 10.7 mg TA per 100 g^{-1} DW (Kemppainen et al., 2014), which is approximately twice the value observed in this study, in which spruce sprouts were analysed. When comparing fresh and dried spruce sprout samples, the total tannin content in the dried samples was approximately four times lower than that in the fresh sample (F_S). No significant differences in total tannin content were observed among the dried spruce sprout samples during storage ($p \geq 0.05$). The significant difference between fresh and dried samples can be attributed to the drying process itself, which may affect the extractability and stability of tannins (Ben Abda et al., 2025). Prolonged drying at moderate temperatures can promote heat- and oxidation-induced degradation of tannin molecules or their transformation into less soluble forms, thereby reducing the measured total tannin content. A similar decreasing trend in tannin levels in dried samples has been reported for wood tissues (Jylhä et al., 2021). In the dried spruce sprout samples, total tannin content remained relatively stable throughout storage. This stability can be explained by the low moisture content of the dried plant material. Under such conditions, the biochemical reactions responsible for tannin degradation are greatly limited, as these processes require water, oxygen, and active enzymes (Bechtold & Mussak, 2009).

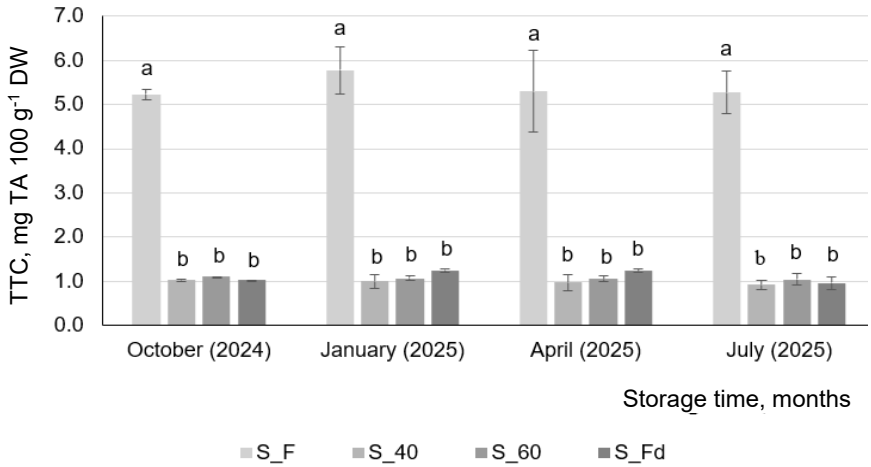


Figure 2. Total tannin content in spruce sprouts during storage. Data were analysed descriptively due to the use of a single open container over time, to avoid temporal pseudoreplication. Values are presented as mean ± SD of technical replicates at each time point. Temporal trends and stability were assessed from changes in these mean values across the study period. Different letters indicate significant differences ($p \leq 0.05$).

S_F – fresh spruce sprouts, S_40 – spruce sprouts dried at 40 °C, S_60 – spruce sprouts dried at 60 °C, S_Fd – freeze-dried spruce sprouts.

Chlorophyll a and chlorophyll b are important photosynthetic pigments in plants, where chlorophyll a promotes charge separation at reaction centres while chlorophyll b broadens the light absorption to improve photosynthetic efficiency and plant growth

(Yang & Lin, 2025). In this study, the contents of chlorophyll a, b, and t, and Ca (Table 4) in spruce sprouts during storage were analysed. Chlorophyll a and b, as well as total chlorophyll and total carotenoids, were higher in fresh spruce sprouts compared to the dried ones. This can be explained by the general properties of chlorophyll. Chlorophyll as a compound is chemically unstable to heat, pH changes and oxygen (Östbring et al., 2020). Carotenoids tend to be more stable than chlorophyll, but still, after drying, carotenoid content decreases due to the oxidative process (Thamkaew et al., 2021). In addition to chemical degradation, drying, as with total tannins, can reduce extraction efficiency because the structural changes in plant tissue limit solvent access to the pigments (Sarkar et al., 2020). Among dried spruce sprout freeze-dried (S_Fd) samples were higher in all the parameters of chlorophyll and carotenoids, which can also be explained by the mild drying process of avoiding heat (Östbring et al., 2020). While chlorophyll is important for plant photosynthesis, carotenoids are important because they serve as antioxidants to protect chlorophyll membranes from damage and help to absorb the light (Tuzhilkina, 2017).

Table 4. Chlorophyll a, b, t and Ca content mg 100 g^{-1} DW in spruce sprouts during storage

	Ch a			
Samples	October (2024)	January (2025)	April (2025)	July (2025)
S_F	76.58 ± 0.45^{a}	18.30 ± 0.57^{b}	13.21 ± 0.35^{c}	10.40 ± 0.24^{d}
S_40	14.02 ± 0.06^{a}	5.86 ± 0.56^{b}	4.95 ± 1.19^{c}	4.81 ± 0.13^{c}
S_60	11.21 ± 0.68^{a}	3.43 ± 0.49^{b}	3.15 ± 0.46^{b}	2.14 ± 0.30^{c}
S_Fd	19.99 ± 0.73^{a}	3.95 ± 0.75^{b}	2.54 ± 0.69^{c}	2.48 ± 0.05^{c}
	Ch b			
Samples	October (2024)	January (2025)	April (2025)	July (2025)
S_F	109.77 ± 68.98^{a}	4.55 ± 0.46^{b}	4.09 ± 0.18^{b}	2.65 ± 0.85^{c}
S_40	7.12 ± 1.79^{a}	0.53 ± 0.14^{b}	$0.31 \pm 0.\ 13^{bc}$	0.19 ± 0.05^{d}
S_60	9.37 ± 3.12^{a}	0.54 ± 0.36^{b}	0.44 ± 0.06^{b}	0.39 ± 0.08^{b}
S_Fd	12.15 ± 2.37^{a}	04.23 ± 1.93^{b}	0.57 ± 0.02^{c}	0.32 ± 0.03^{d}
	Ch t			
Samples	October (2024)	January (2025)	April (2025)	July (2025)
S_F	186.34 ± 0.13^{a}	22.86 ± 0.79^{b}	17.30 ± 0.53^{c}	13.06 ± 3.05^{d}
S_40	21.14 ± 4.74^{a}	6.39 ± 0.63^{b}	5.26 ± 1.32^{bc}	4.50 ± 0.08^{c}
S_60	20.59 ± 5.74^{a}	3.83 ± 0.57^{b}	3.55 ± 0.52^{b}	2.68 ± 0.63^{c}
S_Fd	32.15 ± 6.73^{a}	4.27 ± 1.00^{b}	3.05 ± 0.21^{c}	2.77 ± 1.33^{c}
	Ca			
Samples	October (2024)	January (2025)	April (2025)	July (2025)
S_F	9.16 ± 0.95^{a}	6.74 ± 0.20^{b}	6.75 ± 0.45^{b}	3.82 ± 0.81^{c}
S_40 °C	2.36 ± 0.73^{a}	0.79 ± 0.05^{b}	0.55 ± 0.23^{bc}	0.49 ± 0.12^{c}
S_60 °C	0.54 ± 0.04^{a}	0.20 ± 0.07^{b}	0.16 ± 0.08^{b}	0.13 ± 0.01^{c}
S_Fd	3.42 ± 1.51^{a}	0.74 ± 0.21^{b}	0.63 ± 0.04^{b}	0.47 ± 0.08^{c}

*Data were analysed descriptively due to the use of a single open container over time, to avoid temporal pseudoreplication. Values are presented as mean ± SD of technical replicates at each time point. Temporal trends and stability were assessed from changes in these mean values across the study period. Data in the same row with different letters are significantly different ($p \leq 0.05$). S_F – fresh spruce sprouts, S_40 – spruce sprouts dried at 40 °C, S_60 – spruce sprouts dried at 60 °C, S_Fd – freeze-dried spruce sprouts.

In the present study, the chlorophyll t content was higher than the Ca content in all spruce sprout samples. This observation contrasts with the findings of Lepeduš et al.

(2008), who reported higher carotenoid concentrations than chlorophyll. Differences between studies can be partly explained by variations in the developmental stage and size of the plant material analysed, as pigment composition is known to change during early plant growth (Ložienė & Chochlovaitė, 2025). Very young spruce sprouts, typically measuring approximately 0.5–1 cm in length, require greater protection against environmental stressors and therefore tend to accumulate higher levels of carotenoids relative to chlorophyll. As sprouts increase in size and photosynthetic activity becomes more pronounced, carotenoid concentrations generally decrease, while chlorophyll content increases (Lepeduš et al., 2008), a trend consistent with the results observed in this study. Both chlorophyll and carotenoid contents vary with the type and maturity of the plant material used. For example, Lepeduš et al. (2008) reported a total chlorophyll content of 1.12 ± 0.12 mg 100 g^{-1} DW and a total carotenoid content of 0.44 ± 0.05 mg 100 g^{-1} DW in young shoots. Although these values are lower than those observed in the fresh samples analysed in this study, the same overall trend – higher chlorophyll content compared to carotenoids—was also evident. This suggests that sprout maturity plays a key role in determining the relative abundance of these pigments.

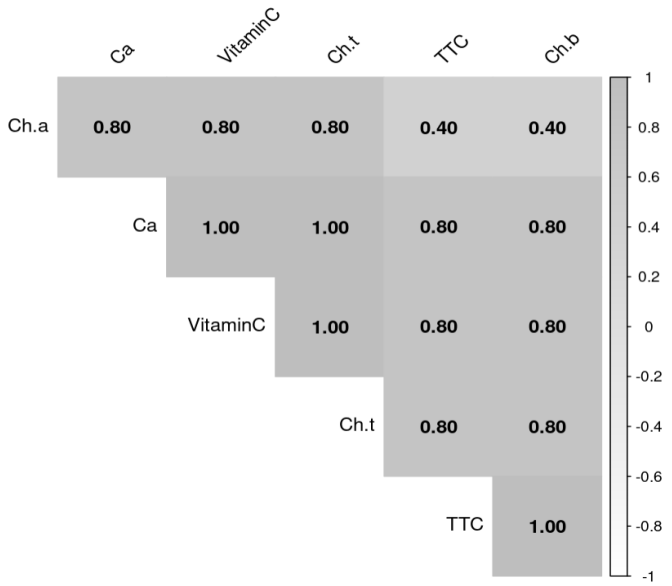


Figure 3. Correlation analysis between bioactive compounds of different processed spruce sprouts.

To explore potential relationships among the quantified bioactive compounds, correlation analyses (Fig. 3) were performed between vitamin C, total tannins, chlorophyll a, b, and t and total carotenoids. According to (Mukaka, 2012; Schober et al., 2018), correlation coefficients can be interpreted with the following established conventions, where values closer to ±1 indicate stronger linear associations and descriptors such as 'moderate' and 'strong' are applied based on magnitude (0.40 as moderate and more than ≥ 0.70 as strong). Correlation analysis was conducted using mean values derived from spruce sprouts subjected to different drying techniques and storage times, enabling evaluation of stable relationships among bioactive compounds

across drying methods. The observed correlations likely reflect coordinated metabolic regulation rather than drying effects. In this study, strong positive correlations were observed among total carotenoids and vitamin C, total carotenoids and total chlorophyll, vitamin C and total chlorophyll, and total tannin content and chlorophyll b. The strong correlation between total carotenoids and vitamin C can be attributed to their complementary roles in antioxidant defence. Both compounds protect the photosynthetic apparatus against oxidative stress by scavenging reactive oxygen species generated during high photosynthetic activity. Their coordinated accumulation suggests that antioxidant capacity increases in parallel with metabolic and photosynthetic activity in developing spruce sprouts (Sarker & Oba, 2018; Rao et al., 2025). The strong correlation between total carotenoids and total chlorophyll likely reflects their joint integration within the photosynthetic apparatus. Carotenoids are structurally and functionally linked to chlorophyll in light-harvesting complexes, where they support light absorption and photoprotection (Balevičius et al., 2017). The strong correlation between vitamin C and total chlorophyll suggests that increased photosynthetic capacity is accompanied by enhanced antioxidant protection. As chlorophyll content rises and photosynthetic electron transport intensifies, reactive oxygen species production increases, requiring higher vitamin C levels to maintain redox balance and preserve chloroplast integrity (Venkatesh & Park, 2014). The observed correlation between total tannin content and chlorophyll *b* may reflect to their shared association with tissue development and secondary metabolism. Chlorophyll *b* is mainly found in light-harvesting antenna complexes, which increase as needles develop. At the same time, tannin production rises as part of secondary metabolic processes involved in defence and tissue strengthening. This pattern suggests coordinated regulation of pigment accumulation and secondary metabolite synthesis during spruce sprout growth (Alugoju & Tencomnao, 2023).

Following the evaluation of bioactive compounds and their interrelationships, the volatile compound profiles of spruce sprouts subjected to different drying techniques and storage durations were characterised, Table 5. A total of 24 volatile compounds were identified across all samples. The number and composition of volatile compounds varied among drying treatments. The sample (F_S) showed a higher number of detected volatile compounds, while S_40, S_60 and S-Fd samples exhibited distinct qualitative differences in volatile composition. α-pinene, β-pinene, and D-limonene were detected in all samples, regardless of drying method, indicating their stability across processing conditions. These compounds are characteristic conifer volatiles and are commonly associated with pine-like, resinous, and citrus-mint aroma notes (Karklina & Ozola, 2023; Bakó et al., 2024). Fresh samples were characterised by the presence of β-carene, ocimene, β-phellandrene, and p-mentha-6,8-dien-2-ol, compounds associated with citrus, herbal, and fresh mint-like aroma notes. In contrast, dried samples (S_40 and S_60) contained compounds such as acetic acid, camphene, calamenene, limonene oxide, and α-cadinol, reflecting qualitative changes in volatile composition at higher drying temperatures. The observed differences between fresh and dried spruce sprouts (S_40 and S_60) are consistent with well-documented effects of drying on volatile compounds in plant materials. Drying exposes plant tissues to elevated temperatures and rapid moisture loss, thereby promoting the evaporation of volatile compounds and accelerating degradation reactions (Chen et al., 2025). Numerous studies have demonstrated that fresh plant materials typically retain a broader spectrum of aroma-active compounds, whereas drying reduces the abundance of compounds

associated with fresh, citrus, and herbal notes and potentially increases the relative proportion of compounds formed or released during thermal processing (Xie et al., 2022).

Table 5. Characteristics of Volatile compounds and their percentage in different dried spruce sprouts

Compound	Flavour	Samples			
		F_S	S_40	S_60	S_Fd
Acetic Acid	Sour	ND	ND	4	ND
Ethanol	Sweet	1	ND	ND	ND
Tricyclene	Cannot be determined	ND	ND	ND	1
α-pinene	Pine	2	3	4	6
β-carene	Orange peel	12	*	*	*
Camphene	Camphor	*	4	4	16
β-pinene	Pine, resin	8	1	1	4
3-carene	Lemon	5	*	2	*
Ocimene	herbs	5	ND	ND	*
D-limonene	Citrus, mint	35	31	36	40
β-phellandrene	Mint, turpentine	2	ND	ND	ND
2-hexenal	Fat, rancid	ND	ND	ND	2
Nonal	Cannot be determined	ND	ND	ND	2
α-longipinene	Wood	1	*	18	*
1-hexanol-2-ethyl	Cannot be determined	ND	ND	ND	2
Bornyl acetate	Cannot be determined	13	*	16	20
Butanoic acid	Cannot be determined	ND	ND	8	3
Isoledene	Cannot be determined	*	17	3	*
y- cadinene	Wood	1	*	ND	2
p-mentha-6,8-dien-2-ol	Fresh, mint	0.3	ND	ND	ND
Calamenene	Fruit	*	15	1	*
Benzyl alcohol	Sweet, flower	ND	ND	ND	0.6
Limonene oxide	Fruit	*	1	0.4	0.3
α-cadinol	Herb, wood	*	11	0.6	0.3

*'ND' indicates not detected, while an asterisk (*) means the compound is present at a very low concentration, below 0.2%. S_F – fresh spruce sprouts, S_40 – spruce sprouts dried at 40 °C, S_60 – spruce sprouts dried at 60 °C, S_Fd – freeze-dried spruce sprouts.

In addition, drying can enhance oxidative and thermal transformations of naturally occurring compounds, leading to the appearance of volatile compounds that are less prominent or absent in fresh samples (Chen et al., 2025). Similar drying-induced qualitative shifts in volatile composition have been widely reported for herbs, fruits, and other plant matrices, supporting the interpretation that the changes observed in dried spruce sprouts arise primarily from temperature-driven volatilisation, degradation, and transformation processes (Chen et al., 2025; Ping et al., 2025). Freeze-dried samples (S-Fd) were characterised by the presence of several volatile compounds that were not detected in either fresh or dried samples, including tricyclene, 2-hexenal, nonanal, benzyl alcohol, and 1-hexanol-2-ethyl. Their occurrence may be attributed to the specific conditions of freeze-drying, which can promote the release and detection of volatile constituents present at low concentrations in plant tissues, while avoiding the thermal losses associated with conventional drying. As a result, freeze-drying may reveal volatile compounds that remain below detection limits in fresh samples or are lost during thermal drying (Abouelenein et al., 2021; Chen et al., 2025; Qiu et al., 2020; Xie et al., 2022).

To integrate the multiple quality attributes evaluated in this study and to identify the most suitable drying method for spruce sprouts, the TOPSIS method was applied. The TOPSIS analysis revealed clear differences in the overall performance of the drying methods (Fig. 4). Sample S_F had the highest closeness coefficient (Ci), indicating the closest proximity to the ideal solution among all samples. Among the dried samples, sample S_Fd showed a higher Ci value than the dried sprouts S_40 and S_60, which exhibited lower closeness coefficients. The lowest Ci value was observed for the samples dried at 60 °C, indicating the least favourable overall performance according to the evaluated criteria. In the study of Jyske et al. (2020), it was also observed that freeze-drying is the most effective drying method for preserving vitamin C in spruce sprouts and needles. The higher overall quality ranking of freeze-dried spruce sprouts observed in the TOPSIS analysis is consistent with general findings reported for plant-based foods. According to a comprehensive review by Bhatta et al. (2020), freeze-drying typically outperforms conventional drying techniques in preserving phytochemicals, antioxidants, and other quality-related components, largely because it does not involve elevated temperatures during processing.

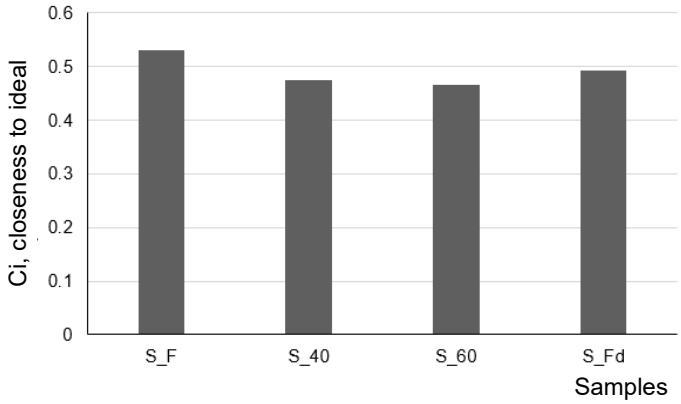


Figure 4. TOPSIS-based evaluation of fresh and dried spruce sprouts.
S_F – fresh spruce sprouts, S_40 – spruce sprouts dried at 40 °C, S_60 – spruce sprouts dried at 60 °C, S_Fd – freeze-dried spruce sprouts.

CONCLUSIONS

In conclusion, the results indicate that fresh spruce sprouts contained the highest initial levels of bioactive compounds, whereas freeze-drying proved to be the most effective method for preserving these compounds during long-term storage. Compared with drying, freeze-drying better maintained overall quality attributes, including bioactive compound content and volatile composition, as reflected in the TOPSIS evaluation. The minimal thermal stress associated with freeze-drying likely contributed to improved retention of quality-related characteristics over the one-year storage period. Consequently, freeze-drying represents a promising processing approach for producing stable, nutrient-rich spruce sprout products with extended shelf life and high functional value, thereby supporting their potential use in industrial and food applications. Future studies should investigate the effects of different packaging materials and storage

temperatures on product quality during storage. In addition, further research should examine extraction processes for both fresh and dried spruce shoots to obtain higher levels of bioactive compounds and explore their additional properties and potential applications.

RESEARCH LIMITATIONS. While this study effectively characterises the longitudinal stability of the sample within a single container, future multi-batch studies are required to confirm these trends across independent lots.

ACKNOWLEDGEMENTS. The authors would like to express sincere gratitude to the LBTU Doctoral School for the support, guidance, and research environment provided throughout this work.

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