

Comparative quality assessment of the spring and autumn – harvest spruce sprouts

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Abstract. Spruce sprouts of Norway spruce (*Picea Abies* (L.) H. Karst) develop in early spring and late autumn. However, information on quality in these stages remains limited. This study aimed to evaluate the physical and biochemical properties of spruce sprouts harvested in May (FS-S) and in September (FS-A). To assess their quality, moisture, pH, titratable acidity, colour, vitamin C, total chlorophyll, total carotenoids, total phenolic compounds, flavonoids, tannin content, antiradical activity and antioxidant capacity, as well as microbiological parameters and volatile compounds, were evaluated. The FS-A sample exhibited a significantly higher pH (4.25 ± 0.01) compared with the FS-S sample (3.10 ± 0.05). In contrast, most bioactive compounds were present at higher levels in the FS-S than in FS-A. Vitamin C content was almost two times higher in FS-S (915.56 ± 0.05 mg 100 g⁻¹ FW) compared to FS-A (505.55 ± 0.05 mg 100 g⁻¹ FW). Total chlorophyll content was 15 times higher in FS-S (95.14 ± 12.12 mg 100 g⁻¹ DW) compared to FS-A (6.19 ± 0.78 mg 100 g⁻¹ FW); however, chlorophyll was greater in FS-A, likely due to the higher pH value. FS-S showed slightly higher total microbial, yeast, and mould counts, while *Escherichia coli* and *Salmonella spp.* were not detected in any samples. Volatile compound analysis revealed a higher proportion of D-limonene in FS-S (35.56%), contributing to the citrusy aroma, whereas FS-A was characterised by fenchene (14.81%) and D-limonene (14.57%). Overall, spring-harvest spruce sprouts exhibited a higher bioactive compound content, supporting their potential as a functional and sustainable food ingredient.

Key words: development stages, seasonal variation, bioactive compounds, physical parameters.

Introduction

Forests are essential to both people and the environment. They provide a habitat for many plant and animal species and help maintain biodiversity. Forests also provide numerous ecosystem services, including timber production, climate regulation, and air purification (Schulte *et al.*, 2026). In addition to timber, forests provide many non-timber forest products. These resources make up about 80% of all forest resources. Non-timber forest products contribute to food and nutrition security in several ways. Forest plants provide many types of food, including mushrooms, berries, resins, pollen, sap, and young shoots (Asamoah *et al.*, 2025), as well as nuts, seeds, and edible lichens. Because of this, forests are an important source of essential nutrients and vitamins for human diets (Min *et al.*, 2024). One of the most valuable forest materials is the Norway spruce tree (*Picea abies* (L.) H. Karst), which is

essential both ecologically and economically. During the growth stage of the spruce tree, new growth may occur. Spruce sprouts, which are the young shoots of the Norway spruce tree, are widely known and used in food, functional foods, and folk medicine. They can be harvested annually for a relatively short period, one to three weeks early in the spring, April – May, depending on the growing location and climatic conditions (Jyske *et al.*, 2020; Karklina, 2022, 2024; Karklina *et al.*, 2024). The tree's growing conditions also determine whether Lamma shoots will appear. Lamma shoots are spruce shoots that can grow in late summer or early autumn. The shoots often occur in response to favourable growing conditions, such as soil fertility, soil moisture, and nutrient availability (Skrøppa & Fundova, 2025). In recent years, Lamma shoots have been increasingly observed in Norway spruce, especially in Norway and the Baltic countries, possibly due to changing climatic conditions (Katrevis

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et al., 2018). Limited information is available on the compounds and the quality of Lamma shoots. However, the young spruce sprouts that are harvested in the spring are rich in vitamin C, minerals, and powerful antioxidants, including phenolic compounds, flavonoids such as quercetin and kaempferol, as well as terpenoids (Sirgedaitė-Šėžienė *et al.*, 2022). The quality and biochemical composition of spruce sprouts are influenced by several factors, including tree location, environmental conditions, and the tree's developmental stage. Differences in geographical origin or altitude can affect the physiological responses and metabolite composition, including terpene and antioxidant contents of spruce trees (Marešová *et al.*, 2022). Environmental factors, such as drought or cold, can modify the composition of volatile compounds and other secondary metabolites. In addition, the stage of plant development and seasonal timing significantly influence the concentrations of essential oils, vitamins, and other bioactive compounds, ultimately affecting their nutritional, medicinal, and industrial qualities (Esposito *et al.*, 2016). The novelty of this study lies in the evaluation and comparison of the physical and biochemical properties of spring and autumn sprouts of *Picea abies*, while spring sprouts have been relatively well studied (Jyske *et al.*, 2020; Karklina, 2022, 2024; Karklina *et al.*, 2024). There is limited information about the quality of Lamma shoots that develop in the autumn season. This research provides new insights into their composition. This study aimed to evaluate the physical and biochemical properties of spruce sprouts and Lamma shoots.

Materials and Methods

A comparative study on the quality of spring sprouts and Lamma shoots was conducted in the laboratories of the Food Institute, Latvia University of

Life Sciences and Technologies. Two types of spruce sprouts were used (Fig. 1):

- Spruce sprouts harvested in late spring – May (FS-S);
- Spruce shoots harvested in early autumn – beginning of September, also known as Lamma shoots (FS-A).

Spruce sprouts and Lamma shoots were harvested from the same location near Jelgava, Latvia, with a latitude of 56° 37' N; longitude – 23° 54' E. The harvested spruce sprouts and Lamma shoots were stored in polyethene (PE) at -20 ± 2 °C. The samples were frozen for an initial period of two days and subsequently maintained under frozen storage until further analyses were performed.

Determination of Physicochemical Parameters (moisture, pH, titratable acidity (TA) and colour analysis)

Moisture content was determined using a moisture analyser (RADWAG MA50/1, R.WH, Poland), according to the method described by Esposito *et al.*, 2016. Approximately 2 g of each sample was placed on a moisture analyser, and measurements were performed in triplicate (Esposito *et al.*, 2016).

Sample pH was determined following the method described by Amboulou Badinga *et al.*, 2023, with minor modifications. 5 g of the sample was homogenised with 50 mL of distilled water. The mixture was centrifuged at 1000 rpm for 1 min. (CM-6MT, Elmi Skyline Ltd, Latvia) and subsequently filtered through Whatman No. 2 filter paper (150 mm). Filtration after centrifugation was used as an additional step to purify the sample and remove smaller resin particles that don't dissolve in water. This can help to obtain a clearer extract for more accurate pH determination (Buyel, 2016). The pH of the filtrate was measured using a pH meter (Seven Compact

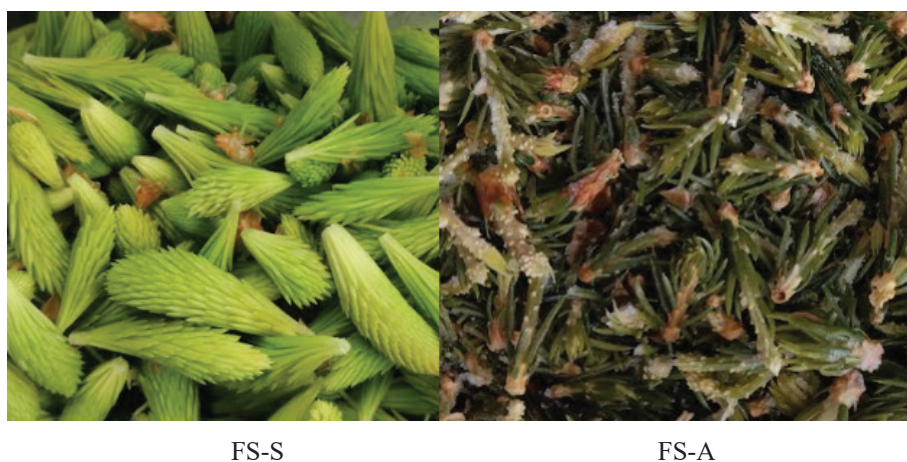


Figure 1. Spring sprouts and Lamma shoots were used for the study.

FS-S – spruce shoots harvested in May, FS-A – Lamma shoots harvested in September

S220, Switzerland); measurements were performed in triplicate (Amboulou Badinga *et al.*, 2023).

Titrateable acidity (TA) was determined following the method described by Karklina *et al.* (2024). Results were expressed as mg citric acid 100 g⁻¹ of sample. Measurements were performed in duplicate.

Colour analysis (L*, a*, b*) was measured according to Karklina *et al.* (2024), with minor modifications using a COLORFlex EZ colour analyser (HunterLab, USA). Five measurements were performed on each sample. Hue angle (h°) and chroma (C*) were not calculated.

Determination of Bioactive Compounds and Antioxidant Activity

Vitamin C content was determined using the iodometric titration T-138-15-01:2002 method according to Segliņa (2007) and Karklina (2024). Samples were titrated with iodine solution until a persistent pale blue colour (30 s) was observed, indicating the endpoint. The volume of iodine solution consumed was recorded, and results were expressed as mg 100 g⁻¹ fresh weight (FW). Analyses were performed in triplicate (Segliņa, 2007; Karklina, 2024).

Total chlorophyll and total carotenoid content were determined spectrophotometrically following Tomsone & Kruma (2019), with minor modifications. Briefly, 0.5 ± 0.1 g of homogenised sample was extracted with 10 mL of acetone under magnetic stirring (700 rpm) for 15 min. at 20 ± 2 °C. Absorbance was measured at 470, 645, and 662 nm using a spectrophotometer (Libra S70 PC, Biochrom Ltd, USA). Pigment contents were calculated using standard equations. Analyses were performed in triplicate (Tomsone & Kruma, 2019).

Preparation of extracts for TPC, TFC, TTC, DPPH, and ABTS⁺. Extracts were prepared according to the Aqueous decoction method described by Adouni *et al.* (2009), with minor modifications. Briefly, 1 g of the sample was mixed with 25 mL of distilled water and heated at 100 °C for 5 min. The mixture was filtered and diluted to 50 mL in a volumetric flask. Extracts were stored at 3 ± 2 °C until analysis. Two independent extracts were prepared per sample (Adouni *et al.*, 2009).

All of the spectrophotometric methods mentioned below, used for extract evaluation, were analysed using a Libra S70 PC spectrophotometer (Biochrom Ltd, USA).

Total phenol content (TPC) was determined using the Folin-Ciocalteu spectrophotometric method (Karklina *et al.*, 2024; Siddiqui *et al.*, 2017). Absorbance was measured at 765 nm, and the results were expressed as mg gallic acid equivalents (GAE) 100 g⁻¹ fresh weight (FW). Measurements were

performed in triplicate (Karklina *et al.*, 2024; Siddiqui *et al.*, 2017).

Total flavonoid content (TFC) was determined according to Karklina (2024) and Puoci *et al.* (2011). Absorbance was measured at 415 nm, and the results were expressed as mg catechin equivalents (CAE) 100 g⁻¹ fresh weight (FW). Measurements were performed in triplicate (Karklina, 2024; Puoci *et al.*, 2011).

Total tannin content (TTC) was determined according to Nisca *et al.* (2021), Wilhelmy *et al.* (2021), and Zeppetbauer *et al.* (2023). Absorbance was measured at 760 nm, and the results were expressed as mg tannic acid equivalents (TAE) 100 g⁻¹ fresh weight (FW). Measurements were performed in triplicate (Nisca *et al.*, 2021; Wilhelmy *et al.*, 2021; Zeppetbauer *et al.*, 2023).

Antiradical activity (DPPH assay) was determined according to Karklina *et al.* (2024) and Semeniuć *et al.* (2016). Absorbance was measured at 517 nm, and the results were expressed as mg Trolox equivalents (TE) 100 g⁻¹ fresh weight (FW). Measurements were performed in triplicate (Karklina *et al.*, 2024; Semeniuć *et al.*, 2016).

ABTS⁺ radical scavenging activity was measured according to Dobravalskytė *et al.* (2012). Absorbance was measured at 734 nm, and the results were expressed as mg Trolox equivalents (TE) 100 g⁻¹ fresh weight (FW). Measurements were performed in triplicate (Dobravalskytė *et al.*, 2012).

Analysis of Volatile Compounds

Volatile compounds (Karklina & Ozola, 2023; Zhu *et al.*, 2022) were determined using solid-phase microextraction (SPME) and the gas chromatography-mass spectrometry (GC-MS) method by Karklina & Ozola (2023) and Zhu *et al.* (2022). About 5.00 g of spruce sprouts and Lamma shoots was weighed on a Precisa XB 1200 C balance and placed in a 25 mL vial, sealed with a stopper. An SPME fibre coated with DVB/CAR/PDMS was used for extraction. The vial was heated at 40 °C for 40 minutes for headspace equilibration. The fibre was then exposed to the headspace for 15 minutes. Analysis was performed on a PerkinElmer Clarus 500 GC with an Elite-Wax ETR column, coupled to a mass spectrometer. Compounds were identified via the NIST98 library, and flavour characteristics were analysed using the Flavornet database. Measurements were in duplicate (Karklina & Ozola, 2023).

Microbiological Evaluation

To evaluate the microbiological quality of spruce sprouts, several microbiological parameters were analysed. The total viable count (mesophilic aerobic and facultative anaerobic microorganisms, MAFAM) was determined using Plate Count Agar (PCA)

according to ISO 4833-2:2013, with incubation at 30 °C for 72 h.

Lactic acid bacteria (LAB) were enumerated on De Man-Rogosa-Sharpe agar (MRS) following ISO 15214:1998, with incubation at 37 °C for 72 h.

Yeasts and moulds were determined using Malt Extract Agar (MEA) according to ISO 21527 (please verify the standard number), with incubation at 27 °C for 5–7 days.

Escherichia coli was enumerated on Tryptone Bile X-Glucuronate (TBX) agar according to ISO 16649-1, with incubation at 37 °C for 24 h.

Statistical Analysis

All data are presented as mean $\pm$ standard deviation (SD). Statistical analyses were performed using JASP (version 0.95.4.0). Differences between spring sprouts (FS-S) and Lamma (FS-A) shoots were evaluated using independent samples t-tests for normally distributed data. Spearman's correlation analysis was performed to evaluate relationships among bioactive compounds and antioxidant activity. Principal Component Analysis (PCA) was applied to assess the combined variability of physical and biochemical parameters and to identify patterns of co-variation among variables. Statistical significance was set at $p < 0.05$.

Results and Discussion

Physical and biochemical parameters were analysed for both spruce sprouts harvested in spring sprouts and Lamma shoots to assess their quality. Table 1 summarises physical parameters for spring and Lamma shoots. Higher moisture content was observed in FS-S ($87.82 \pm 1.32\%$), as well as titratable acidity, which was higher (1.84 ± 0.06 mg 100g⁻¹ DW), but pH was higher in FS-A (4.26 ± 0.01). The higher moisture content in spring sprouts can be explained by the plant's structure. Spring spruce sprouts are characterised by low lignin and cellulose content, as well as a small amount of structural carbohydrates. In general, the literature on plant physiology indicates that young sprout tissue contains more water than mature tissues because dry matter accumulation occurs later in the growth stage, during cell wall thickening or needle

maturation (Sutinen et al., 2015; Zlobin et al., 2024). However, the lower moisture content in Lamma shoots can be explained by the stage of shoot maturation, as their growth slows or stops. During this time, a second thickening of the cell walls (lignification) occurs, as well as the accumulation of carbohydrates. In the autumn period, plant development shifts from intensive growth to the accumulation of substances and the strengthening of tissues (Katrevis et al., 2018; Luoranen, 2018; Zlobin et al., 2024). Parameters such as pH and titratable acidity can be combined; titratable acidity measures the total amount of acids in plant tissues, while pH represents the concentration of free hydrogen ions in the water phase (Caenn et al., 2011). Both indicators change during the development phase of spruce sprouts. Titratable acidity is usually higher during the active growth stage of a plant and decreases during the maturation stage (Ilek et al., 2024; Schoss et al., 2023). In comparison, the pH in spruce can vary depending on soil acidity, spruce species, harvest time, and age of needles: younger needles may have a lower pH, while older needles may have a higher pH, ranging from 3.0 to 5.5, respectively (Pramsohler et al., 2022). Compared to this study, the measured pH values were within the typical range reported for spruce tissues. The slightly higher pH observed in Lamma shoots may be related to their more lignified and woody character compared with young spring sprouts, which generally exhibit more acidic metabolism and higher concentrations of organic acids (Fober, 1987), as also observed with the titratable acidity results.

Colour changes between developmental phases of spruce sprouts are very closely related to physical and bioactive parameters (Table 2). The CIE L*, a* and b* colour system describes colour according to three parameters: L* represents lightness, ranging from black (low values) to white (high values); a* describes the green-red axis, where negative values indicate greenness and positive values indicate redness; and b* represents the blue-yellow axis, where negative values correspond to blueness and positive values to yellowness (Karklina et al., 2024; A. Wang et al., 2019).

Spring sprouts had a higher L* value (41.04 ± 0.88) compared to Lamma shoots (37.17 ± 0.81), indicating

Table 1

Physical parameters of spring sprouts and Lamma shoots

Sample	Moisture, %	pH	TA, mg 100g ⁻¹ DW
FS-S	87.82 ± 1.32^a	3.10 ± 0.01^b	1.84 ± 0.06^a
FS-A	65.13 ± 1.5^b	4.26 ± 0.01^a	1.52 ± 0.023^b

*FS-S is Spruce sprouts harvested in spring; FS-A is Lamma shoots harvested in autumn. TA – titratable acidity. The t-test was used for statistical analysis. Data in the same column with different letters are significantly different ($p \leq 0.05$).

Table 2

Colour analysis of spring sprouts and Lamma shoots

Sample	L	a*	b*	ΔE
FS-S	41.04 ± 0.88 ^a	-9.85 ± 0.40 ^b	31.47 ± 0.74 ^a	1.29 ± 0.43 ^a
FS-A	37.17 ± 0.81 ^b	-2.88 ± 0.29 ^a	19.01 ± 0.54 ^b	0.97 ± 0.17 ^b

*FS-S is Spruce sprouts harvested in spring; FS-A is Lamma shoots harvested in autumn. The t-test was used for statistical analysis. Data in the same column with different letters are significantly different ($p \leq 0.05$).

a lighter appearance. Both samples showed negative a* values, confirming a dominant green colour, with spring sprouts displaying greater greenness. Positive b* values in both samples indicated the presence of yellow tones. These colour differences can be explained by structural and compositional variations. Spring sprouts are younger tissues characterised by higher moisture content and less lignified cell walls, which lead to increased light reflection and a brighter appearance (Lybeer *et al.*, 2006; Resente & Crivellaro, 2025). In contrast, Lamma shoots contain lower moisture and more lignin, which leads to a darker colour, leading to reduced greenness and yellowness (Sims & Gamon, 2002).

Pigment composition also plays a key role. Chlorophylls are primary pigments responsible for green colouration (Sudhakar *et al.*, 2016). The high amount of chlorophyll dominates over other pigments, which can create an intense green colour, while carotenoids give the sprouts a slightly warm, yellowish tone due to their contribution to yellow tones (Li *et al.*, 2019; Urbanovich *et al.*, 2021).

Biochemical parameters of spruce sprouts are influenced by environmental conditions, tree vitality, and adaptive responses to factors such as climate variability, pollution, and biotic stress (Ditmarová *et al.*, 2008; Tienaho *et al.*, 2026). The composition of bioactive compounds is summarised in Table 3. Overall, spring sprouts (FS-S) exhibited higher levels of most bioactive compounds compared to Lamma shoots (FS-A), with the exception of total tannin content (TTC).

TTC in spring sprouts was approximately tenfold lower than in Lamma shoots, with values of 5.42 ± 1.20 mg TAE 100 g⁻¹ FW and 56.42 ± 0.53 mg TAE 100 g⁻¹ FW, respectively. This difference can be explained by seasonal and developmental dynamics. During spring, rapidly growing tissues primarily allocate carbon to primary metabolism, supporting cell division and elongation, which limits the accumulation of secondary metabolites such as tannins and other phenolic compounds. In contrast, during autumn, growth slows, and carbon is increasingly directed towards secondary metabolism, resulting in higher tannin accumulation. These compounds

contribute to structural reinforcement and a chemical defence against pathogens and herbivores (Haring *et al.*, 2007; Salminen *et al.*, 2004; Zhang *et al.*, 2009, 2020). In contrast to TTC, other bioactive compounds were generally higher in spring sprouts. Vitamin C content in FS-S was nearly twice that observed in FS-A: 914.57 ± 0.61 mg 100 g⁻¹ FW in FS-S compared to 505.55 ± 0.98 mg 100 g⁻¹ FW. This can be attributed to higher metabolic and photosynthetic activity in young tissues, where ascorbic acid plays a key role in protecting against oxidative stress and supporting cellular metabolism. In Lamma shoots, reduced metabolic activity and photosynthesis are associated with lower vitamin C levels (Paciolla *et al.*, 2019; Pastori *et al.*, 2003). In one study by Jyskes *et al.* (2020), the amount of vitamin C in fresh spruce shoots was analysed and compared to mature needles, which concluded that the amount of vitamin C in fresh spruce shoots was 406.7 mg/100 g DW and in spruce needles 182.5 mg 100 g⁻¹ DW. Comparatively, in this study, fresh spring spruce shoots had almost twice the amount of vitamin C. Changes in vitamin C could be explained by harvesting location, tree age, and climate change. Differences in pigment composition were also observed. In spring sprouts, total carotenoid content exceeded total chlorophylls, whereas the opposite trend was found in Lamma shoots. However, when comparing between seasons, overall pigment levels were higher in spring samples. These variations reflect seasonal changes in pigment metabolism and photoprotective mechanisms (Michelberger *et al.*, 2025; Wong *et al.*, 2019). Elevated carotenoid levels in spring sprouts likely indicate active synthesis of photoprotective pigments during early developmental stages, when exposure to light stress is relatively high (Juvany *et al.*, 2013).

The total flavonoid content in spring sprouts was almost 12 times higher than in Lamma shoots: 766.34 ± 31.41 mg CAE 100 g⁻¹ FW in spring sprouts compared to 63.80 ± 4.98 mg GAE 100 g⁻¹ FW in Lamma shoots. The antiradical activity (DPPH) and antioxidant capacity (ABTS) were about two times higher in spring sprouts than in Lamma shoots. Similarly, total phenolic content was about 9 times higher in spring sprouts, with 6318.93 ± 141.32 mg

Table 3

Bioactive compound content and antioxidant activity comparison of spring sprouts and Lamma shoots

Sample	FS - S	FS - A
Vitamin C, mg 100g ⁻¹ FW	914.57 ± 0.61 ^a	505.55 ± 0.98 ^b
Total tannin content, mg TA 100g ⁻¹ FW	5.42 ± 1.20 ^b	5.42 ± 0.53 ^a
Total chlorophyll, mg 100g ⁻¹ FW	95.14 ± 12.12 ^a	6.20 ± 0.79 ^b
Total carotenoids, mg 100g ⁻¹ FW	133.80 ± 15.34 ^a	0.92 ± 0.23 ^b
Total phenolic compounds, mg GAE 100g ⁻¹ FW	6318.93 ± 141.32 ^a	730.31 ± 46.57 ^b
Total flavonoids, mg CAE 100g ⁻¹ FW	766.34 ± 31.41 ^a	63.80 ± 4.98 ^b
DPPH, mg TE 100g ⁻¹ FW	1233.58 ± 19.12 ^a	529.63 ± 19.26 ^b
ABTS, mg TE 100g ⁻¹ FW	986.66 ± 11.92 ^a	414.44 ± 16.21 ^b

* FS-S is Spruce sprouts harvested in spring; FS-A is Lamma shoots harvested in autumn. The colour intensity represents the relative magnitude of the values across all samples and parameters. Darker green indicates higher values; lighter green indicates lower values. The t-test was used for statistical analysis. Data in the same row with different letters are significantly different ($p \leq 0.05$).

GAE 100 g⁻¹ FW compared to 730.31 ± 46.57 mg GAE 100 g⁻¹ FW in Lamma shoots.

The elevated levels of these compounds in spring sprouts can be attributed to their structural and physiological characteristics (Camargo *et al.*, 2025). Young tissues are less lignified, have higher moisture content, and are more susceptible to environmental stress, which stimulates the synthesis of protective secondary metabolites such as phenolics and flavonoids. In contrast, Lamma shoots are more mature, with increased lignification and reduced metabolic activity. During early developmental stages, plant tissues typically exhibit higher concentrations of secondary metabolites associated with active growth (Korolyova & Turčáni, 2024) and defence responses (Linkosalo *et al.*, 2014; Morais *et al.*, 2022; Vanhakylä & Salminen, 2023).

Spearman's correlation analysis showed statistically significant positive correlations among a few bioactive compounds (Figure 2). This suggests that these parameters can influence each other. A very strong correlation was observed between total chlorophyll content and total phenolic content ($r = 0.973$), as well as between total carotenoid content and total phenolic content ($r = 0.973$). This could be related to the role of phenolic acids in protection against oxidative stress and their simultaneous accumulation with photosynthetic pigments during active biosynthesis (Sharma *et al.*, 2019). A strong correlation ($r=0.866$) was observed between vitamin C and the antiradical activity of DPPH. This could be because both vitamin C and antioxidant compounds can donate electrons or hydrogen atoms to neutralise free radicals (Pozzo *et al.*, 2025), so higher vitamin C

content enhances the antiradical activity DPPH in plant tissues (Pozzo *et al.*, 2025; Muflihah *et al.*, 2021). A strong correlation was also observed between vitamin C and total phenolic compounds ($r=0.866$), as well as between vitamin C and total flavonoids. A strong correlation was also observed between the antioxidant capacity of ABTS and the antiradical activity of DPPH ($r=0.818$), as well as between the antioxidant capacity of ABTS and vitamin C ($r=0.866$), and ABTS and total flavonoids ($r=0.800$). Both DPPH and ABTS assays measure electron transfer (or hydrogen atom transfer) mechanisms of antioxidant action (Xie & Schaich, 2014), so compounds effective in one assay (e.g., ascorbate, phenolics, flavonoids) tend to show consistent activity across assays (Rumpf *et al.*, 2023). A negative correlation was observed between total tannin content and other bioactive compounds. This can be explained by the fact that tannins are large, structurally diverse phenolic compounds that can form complexes with proteins and other molecules, and their contribution to simple radical-scavenging assays can be limited or inconsistent compared with low-molecular-weight antioxidants; this may result in apparent negative associations when tannin levels are high, but they contribute less to the measured antiradical activity (Gulcin, 2025; Teterovska *et al.*, 2023).

Both physical and bioactive parameters were analysed using PCA (Principal Component Analysis; Table 4). The first principal component (PC1) was characterised by strong positive loadings of bioactive compounds and antioxidant activity indicators (DPPH and ABTS), whereas pH and total tannin content (TTC) exhibited negative loadings on the same

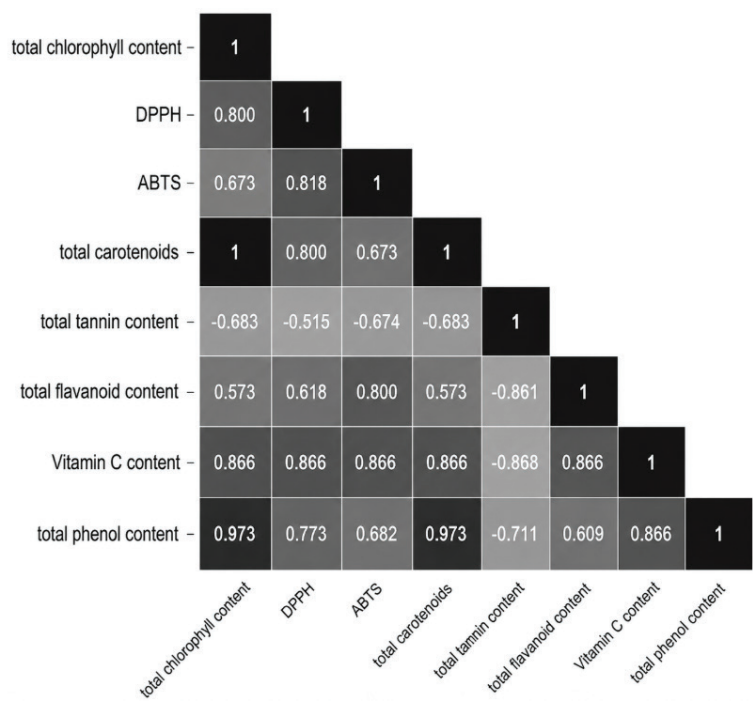


Figure 2. Spearman's correlation between bioactive compounds of spring sprouts and Lamma shoots

component. This indicates contrasting functional roles and regulatory behaviour among these variables.

PCA groups variables that covary together; high positive loadings for antioxidants (such as phenolics, flavonoids, vitamin C, and radical scavenging activity) suggest that these variables increase concurrently across samples. This pattern reflects shared biosynthetic pathways and shared responses to environmental and developmental factors, such as the upregulation of phenolic and flavonoid

synthesis under stress conditions. Similar clustering of antioxidant compounds has been reported in plant metabolomic studies, where PC1 often represents overall antioxidant capacity (Chociejska *et al.*, 2024).

In contrast, pH and TTC vary inversely: lower pH values are often associated with higher antioxidant accumulation, and tannins, as high-molecular-weight phenolic compounds, contribute less to soluble radical-scavenging activity, resulting in negative loadings. This pattern reflects biochemical trade-offs

Table 4

Principal component analysis of spruce sprouts and Lamma shoots

Component Loadings		
Parameters	RC1	Uniqueness
Total phenol content	1.000	0.000
Vitamin C content	1.000	0.000
pH	-1.000	0.000
Antiradical activity of DPPH	0.999	0.002
Antioxidant capacity of ABTS	0.999	0.002
Total tannin content	-0.999	0.002
Titrateable acidity	0.998	0.003
Total flavonoid content	0.998	0.005
Total chlorophyll content	0.995	0.009
Total carotenoids content	0.994	0.012

and differential roles of antioxidants in spruce sprouts and shoots (Dikamu *et al.*, 2025; Safari-Khozani *et al.*, 2025).

Volatile organic compounds (VOCs) are secondary metabolites that evaporate easily and play an important role in plant defence, communication, and interaction with the environment (Montejano-Ramírez *et al.*, 2024; Ueda *et al.*, 2012). In spruce sprouts, these compounds are mainly terpenes (e.g., monoterpenes and sesquiterpenes), and their composition and abundance vary significantly with season and physiological activity, with higher levels often observed during periods of active growth, such as spring (Furnell *et al.*, 2024; Schoss *et al.*, 2023). The volatile profile of spruce sprouts and Lamma shoots (Table 5) is dominated by terpenoid compounds, especially monoterpenes and sesquiterpenes. In spring samples, monoterpenes such as limonene (35.56%), camphene (15.59%), ocimene (13.87%) and bornyl

acetate (15.51%) are dominant, which can be explained by active metabolism, growth, and protection against herbivores (Bakó *et al.*, 2024; Qasim *et al.*, 2024).

In contrast, in Lamma shoots, a decrease in several monoterpenes and an increase in sesquiterpenes such as cadinene (10.09%) and γ -cadinene (5.08%) are observed, as well as the appearance of additional compounds such as fenchene (14.81%). This shift indicates a transition from active metabolic processes to more stable and structurally complex compounds involved in long-term protection and environmental adaptation (Holzke *et al.*, 2006). Overall, the observed seasonal variation reflects the influence of the plant developmental stage on the composition of volatile compounds, with spring sprouts characterised by a higher proportion of lighter, more volatile compounds, while Lamma shoots have a higher proportion of heavier, less volatile compounds. Spring sprouts have higher VOC levels due to increased metabolic activity

Table 5

Comparison of volatile compound profiles (%) in spring sprouts and Lamma shoots

Compound	Descriptive aroma	Samples	
		FS-S	FS-A
Acetone	Acetone	1.3	1
Tricyclene	Woody, pine	2.5	0.99
α - pinene	Pine	2.49	1.59
β - pinene	Pine, resin	0.56	1.89
Ocimene	Citrus herb flavour	13.87	10.86
Camphene	Camphor	15.59	7.02
Fenchene	Herbal, sweet	-	14.81
Limonene	Citrus	35.56	14.57
β - myrcene	Balsamic, must, spice	4.49	-
2 - nonanone	Hot milk, soap, green	-	4.27
α - longipinene	Pine	-	2.35
Terpinolene	Turpentine	0.90	1.35
Alcanfor	Woody, refreshing	-	0.42
Bornyl acetate	Fresh, sweet, balsamic	15.51	13.95
Carvone	-	-	3.85
Isolatedene	Pine	-	1.20
Isoborneols	Strong herbal, woody	-	0.17
Caryophyllene	Spicy, woody, herb	3.11	-
Cadinene	Herb, wood	2.43	10.09
γ - cadinene	Wood	0.87	5.08
Limonene-6ol	Wood, spice	0.51	0.25
α - cadinol	Thyme, wood	0.31	2.94
Humulene	Wood	-	1.35

*FS-S is Spruce sprouts harvested in spring; FS-A is Lamma shoots harvested in autumn. "ND" indicates not detected, while a dash (-) means the compound is present at a very low concentration, below 0.2%.

associated with the resumption of photosynthesis and early stages of development (Y. Wang *et al.*, 2024). Studies have shown that monoterpene emissions peak in spring, and bursts of emissions are associated with the onset of photosynthesis (Aalto *et al.*, 2015). Furthermore, VOC emissions are highest during early growth stages, such as bud development, and decrease as tissues mature (Pei *et al.*, 2025). These compounds play an important role in plant defence (Montejano-Ramírez *et al.*, 2024), especially in interactions with herbivorous insects, protecting vulnerable early growth stages (Effah *et al.*, 2022; Karlsson *et al.*, 2021).

Microbiological evaluation revealed that spring spruce sprouts exhibited higher counts of yeast and mould (1.50×10^3 CFU mL⁻¹) and total plate count (5.60×10^2 CFU mL⁻¹) compared to Lamma shoots (1.12×10^2 and 6.70×10^1 CFU mL⁻¹, respectively).

These differences can be attributed to the developmental stage and tissue structure: spring sprouts are young, with a higher water content, thinner and less lignified cell walls, as well as richer in nutrients, which provide favourable conditions for microbial colonisation (Sá *et al.*, 2025; Zhai *et al.*, 2025). In contrast, Lamma shoots are more mature, with a lower moisture level, thicker lignified cell walls, and an accumulation of secondary metabolites, which together inhibit microbial growth (Bhuiyan *et al.*, 2009).

Pathogenic bacteria, such as *E. coli* and *Salmonella* spp., were not detected in any of the analysed samples, indicating good microbiological quality and safety of the sprouts. For comparison, *Salmonella* spp. was also analysed in the study by Jyske *et al.* (2020) and was likewise not detected. However, *E. coli* was detected in the study by Jyske *et al.* (2020), which may be explained by the harvesting height of the spruce sprouts. In the Jyske *et al.* (2020) study, the spruce sprouts were collected closer to the ground, whereas in the present study, following the recommendations of the same article, the sprouts were collected 0.5 m above ground level.

Conclusions

Seasonal differences significantly affected spruce sprout quality. Spring sprouts showed higher moisture, titratable acidity, a lighter and greener colour, and elevated levels of vitamin C, total phenolic, flavonoid content, total carotenoids and total chlorophyll content, and antioxidant activity, reflecting their active metabolism and young tissues. Lamma shoots had higher pH, increased total tannin content, and a darker colour. Volatile compound profiles differed between seasons: spring sprouts were dominated by monoterpenes, while Lamma shoots contained more

sesquiterpenes. Spring sprouts also showed higher microbial counts due to their higher moisture content, although pathogenic bacteria were not detected in either season. Overall, spring spruce sprouts demonstrated superior nutritional and functional value, whereas Lamma shoots exhibited greater structural stability, highlighting the harvest season as a key factor influencing sprout quality and their use in future studies.

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