

Influence of storage conditions and preparation methods on changes in ascorbic acid, phenolic compound content, and antioxidant activity of bee pollen

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Bee pollen (BP) is widely recognized in modern phytomedicine for its rich phytochemical composition. However, its application faces challenges due to bioactive compound variability influenced by botanical and geographical origin, preparation methods, and storage conditions. Drying at high temperatures causes significant losses of biologically active compounds, particularly vitamins, while storage alters BP's phytochemical composition, emphasizing the need for strict quality control.

This study aimed to evaluate changes in ascorbic acid, polyphenols, and antioxidant activity of BP under various preparation and storage conditions. BP samples were collected from an apiary in Talkoniai, Pasvalio district, Lithuania, and prepared via drying at +28°C (first day) and +35°C (second day), or freezing at –20°C and –80°C. Ascorbic acid, total phenolic compounds (TPC), and total flavonoids were quantified using titrimetric and spectrophotometric methods, while phenolic profiles were analyzed by UPLC–MS over a 15-month storage period.

Results demonstrated significant storage-related declines in TPC. Frozen BP stored at –20°C and –80°C showed decreases after 6 months ($p < 0.05$), while dried BP exhibited reductions after 3 months ($p < 0.05$), with continuous decline thereafter. Total flavonoid content remained stable for 6 months in all samples, then declined gradually. Rutin was the predominant phenolic compound, comprising 29.4% of TPC. Ascorbic acid retention was highest in BP stored at –80°C, with significant loss only after 9 months ($p < 0.05$), compared to 6 months storage at –20°C and 3 months in dried BP.

Antioxidant activity, assessed via FRAP and ABTS methods, declined after 6–9 months in frozen BP and after 3–6 months in dried BP. Frozen BP (–80°C) retained the highest antiradical and reducing activity, 1.8–3.4 and 2.6–3.1 times higher, respectively, than dried BP extracts during 6–15 months of storage.

Melissopalynological analysis confirmed a polyfloral pollen mixture. Pollen of *Brassica napus* L. and *Salix* spp. dominated, comprising 36.8% and 34.3%, respectively. *Acer platanoides* L., *Malus domestica* Borkh., and *Taraxacum officinale* L. accounted for 12.8%, 9.0%, and 5.9%, respectively, as important minor pollens.

In conclusion, freezing at –80°C ensures superior preservation of phenolic compounds, ascorbic acid, and antioxidant activity in BP compared to drying, highlighting the value of fresh frozen pollen in maintaining phytochemical quality.



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2025



Bee pollen

- Widely consumed as a **functional food** and natural supplement
- Rich in **vitamins, phenolic compounds, flavonoids, and amino acids**
- Attributed health benefits:
 - Antioxidant protection
 - Immune system support
 - Anti-inflammatory effects
- **Challenge:**
- Its **bioactive composition is unstable** and may degrade depending on storage and processing.

Problem Statement



- Composition of bee pollen is **highly sensitive** to:
 - Storage conditions (temperature, humidity, light, oxygen)
 - Preparation methods (drying, freezing, fermentation, grinding)
- Consequences:
 - Degradation of bioactive compounds
 - Reduced antioxidant potential
- **Scientific gap:** Optimal preservation methods are still debated

Bee pollen drying – benefits and limitations

Benefits:

- Facilitates consumption in situations where refrigeration is not available (e.g., during transportation or travel)
- Ensures convenient handling, packaging, and long-term storage
- Reduces microbial contamination and extends shelf-life

Limitations:

- Exposure to elevated temperature leads to **degradation of enzymes**, vitamins, and other **thermolabile compounds**
- Potential decrease in the concentration of vitamins, phenolic compounds and overall **antioxidant capacity**

Bee pollen freezing – benefits and limitations

Benefits:

- Preserves almost all nutritional and biological properties
 - Better retention of **bioactive compounds** (e.g., vitamins, phenolics, flavonoids)
 - Maintains **higher antioxidant activity** during storage
- Superior option for consumption in a “fresh-like” form
 - Improved **sensory qualities**: taste, color, and aroma remain closer to fresh pollen

Limitations:

- Requires **continuous refrigeration and stable freezing conditions**
- More difficult to distribute and market in larger quantities due to **cold chain dependence**
- Potential risk of **microbiological contamination** if handling and storage are inadequate

The aim of this study

- To compare the changes in **phenolic compounds, ascorbic acid, and antioxidant activity** *in vitro* of **fresh-frozen** (-20°C and -80°C) and **dried** bee pollen during storage.

Study design

- **Sample origin:** Bee pollen collected in May 2022, Talkoniai, Pasvalys district (Lithuania).
- **Sample preparation:**
 - Dried in a laboratory dryer (first 24 h at $+28^{\circ}\text{C}$, followed by 24 h at $+35^{\circ}\text{C}$)
 - Frozen at -20°C and -80°C



Methods



- **Analytical methods:**
 - Spectrophotometry (total phenolics (Folin –Ciocalteu) and flavonoids)
 - HPLC (profile of phenolic acids and flavonoids)
 - Titrimetric (Vitamin C)
 - ABTS and FRAP (antioxidant activity)
- **Study duration:** 15 months, with analyses performed every 3 months.
- Statistical analysis



antioxidants



Article

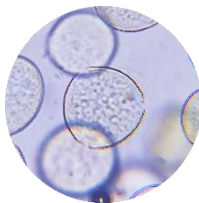
Changes in Ascorbic Acid, Phenolic Compound Content, and Antioxidant Activity In Vitro in Bee Pollen Depending on Storage Conditions: Impact of Drying and Freezing

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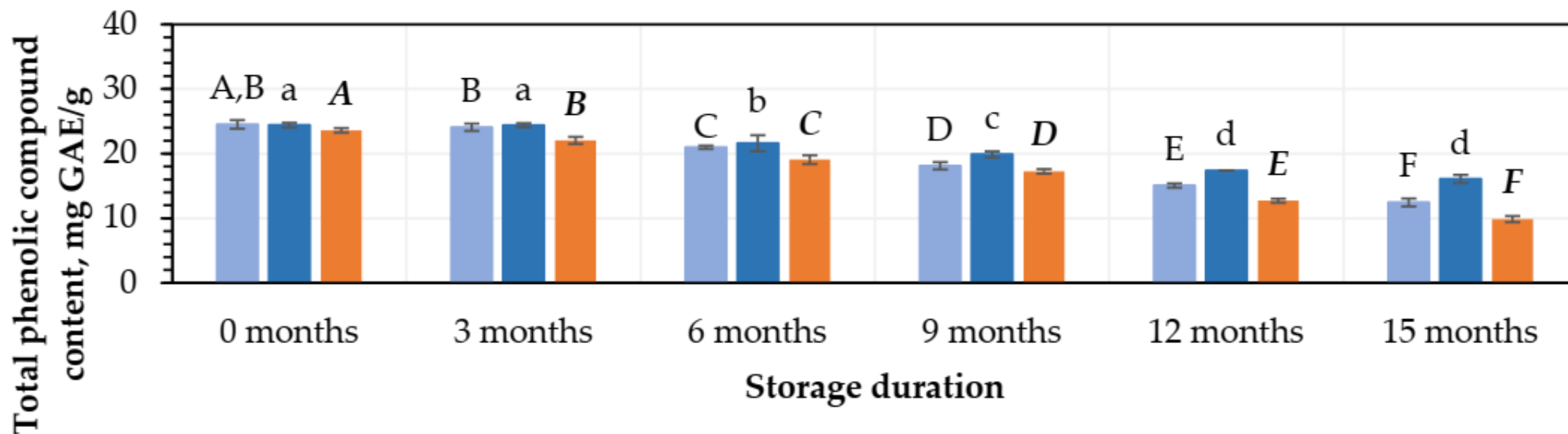


The **botanical** composition of bee pollen

Plant Pollen	Average, %
<i>Brassica napus</i> L.	35.37
<i>Salix</i> spp.	36.87
<i>Acer platanoides</i> L.	12.83
<i>Malus domestica</i> Borkh.	9.03
<i>Taraxacum officinale</i> L.	5.93



Changes in total phenolic content during storage



	3 months	6 months	9 months	12 months	15 months
Dried pollen	-7* %	-10* %	-27* %	-46* %	-58* %
Frozen -20 °C	-2 %	-14* %	-26* %	-39* %	-49* %
Frozen - 80 °C	0 %	-12* %	-19* %	-29* %	-34* %

First-order degradation kinetics of phenolic compounds

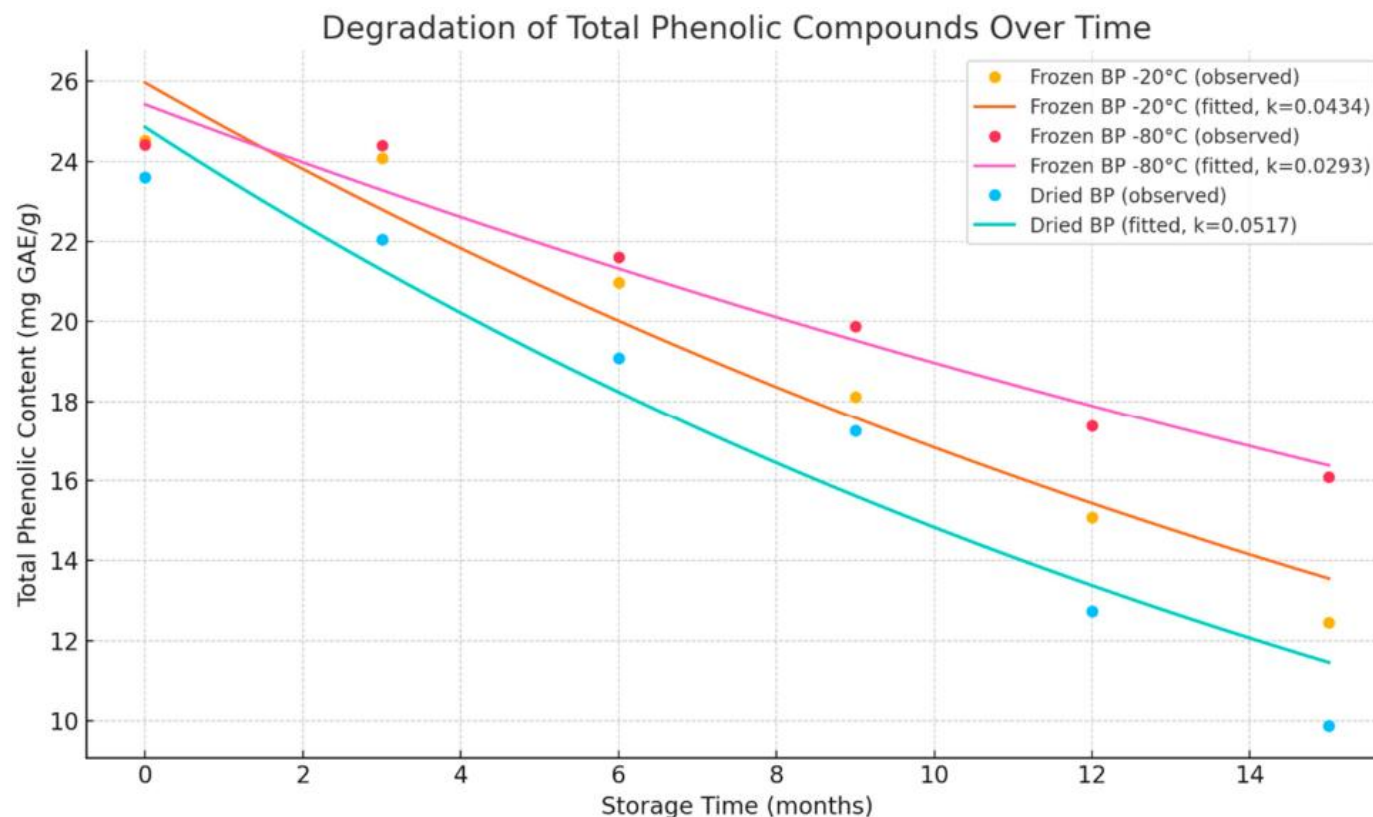
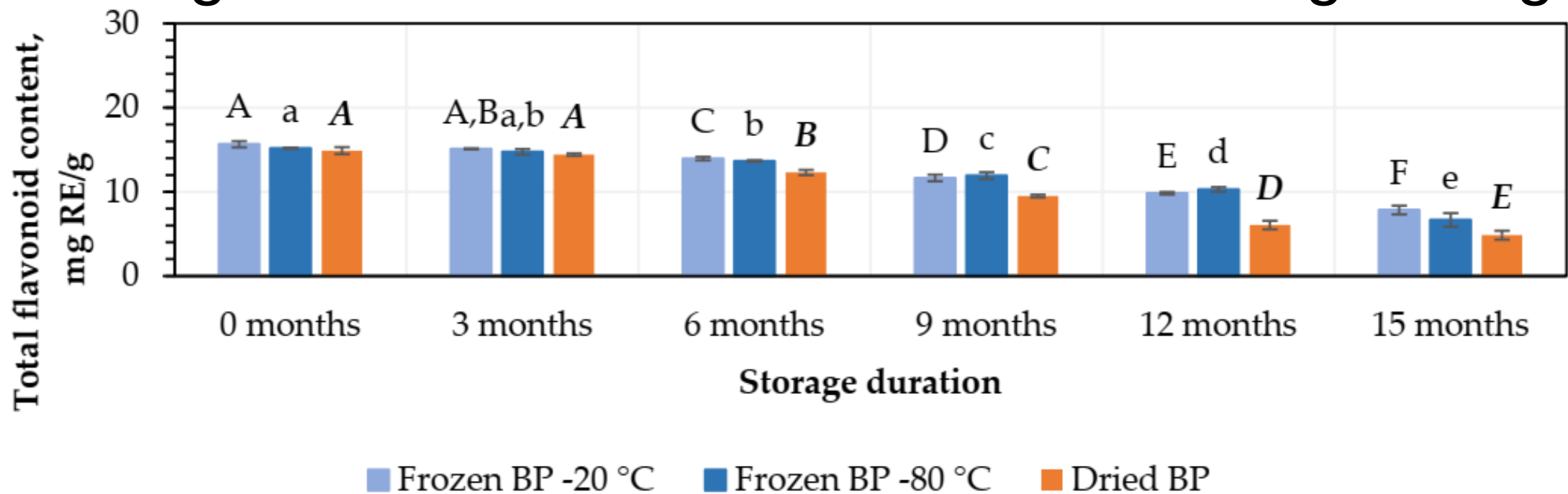


Figure S1. First-order degradation model of phenolic compounds in bee pollen samples.

Changes in total flavonoid content during storage



	3 months	6 months	9 months	12 months	15 months
Dried pollen	-3 %	-17* %	-36* %	-59* %	-67* %
Frozen -20 °C	-5 %	-11* %	-26* %	-37* %	-50* %
Frozen - 80 °C	-3 %	-10* %	-21* %	-32* %	-56* %

First-order degradation kinetics of flavonoids

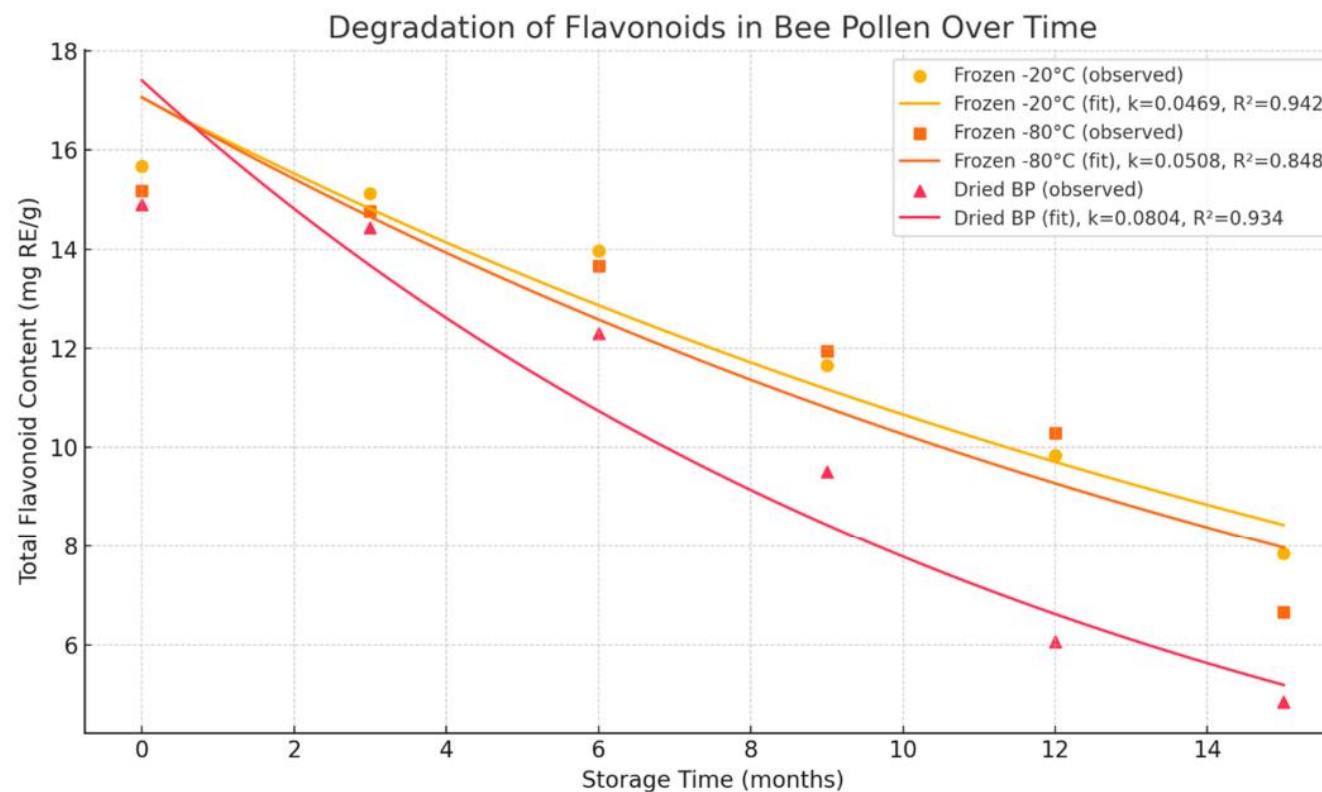
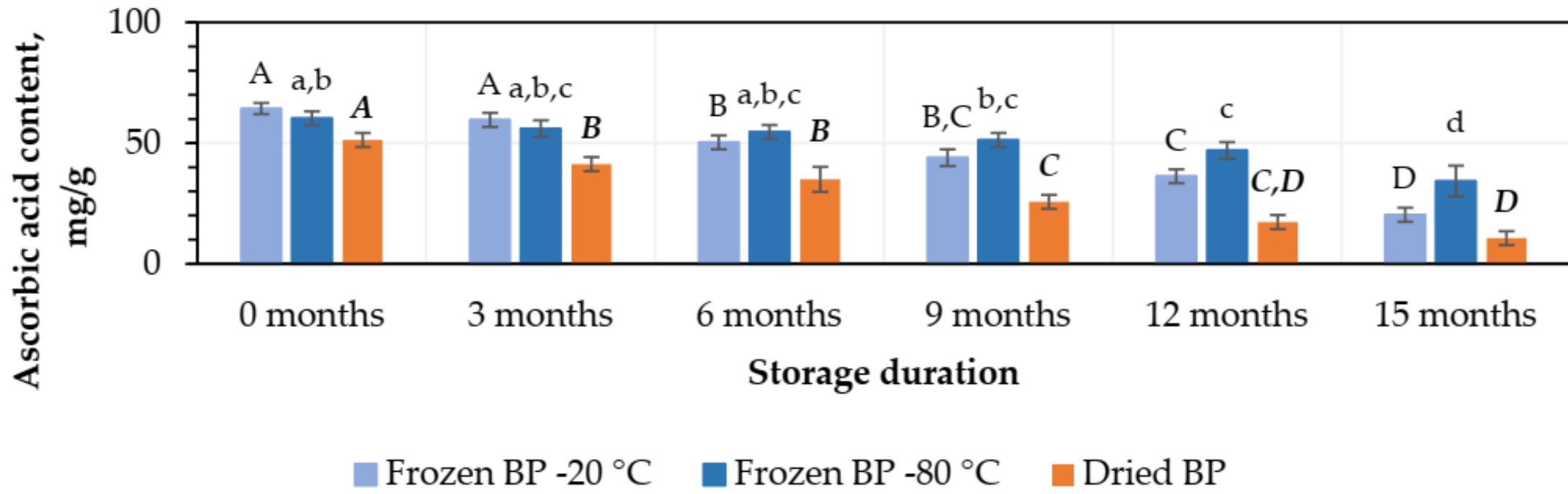


Figure S2. First-order degradation model of flavonoids in bee pollen samples.

Changes in Vitamin C content during storage



	3 months	6 months	9 months	12 months	15 months
Dried	-20* %	-32* %	-50* %	-66* %	-80* %
Frozen -20 °C	-7 %	-22* %	-32* %	-44* %	-68* %
Frozen - 80 °C	-7 %	-9 %	-15* %	-22* %	-43* %

First-order degradation kinetics of **Vitamin C**

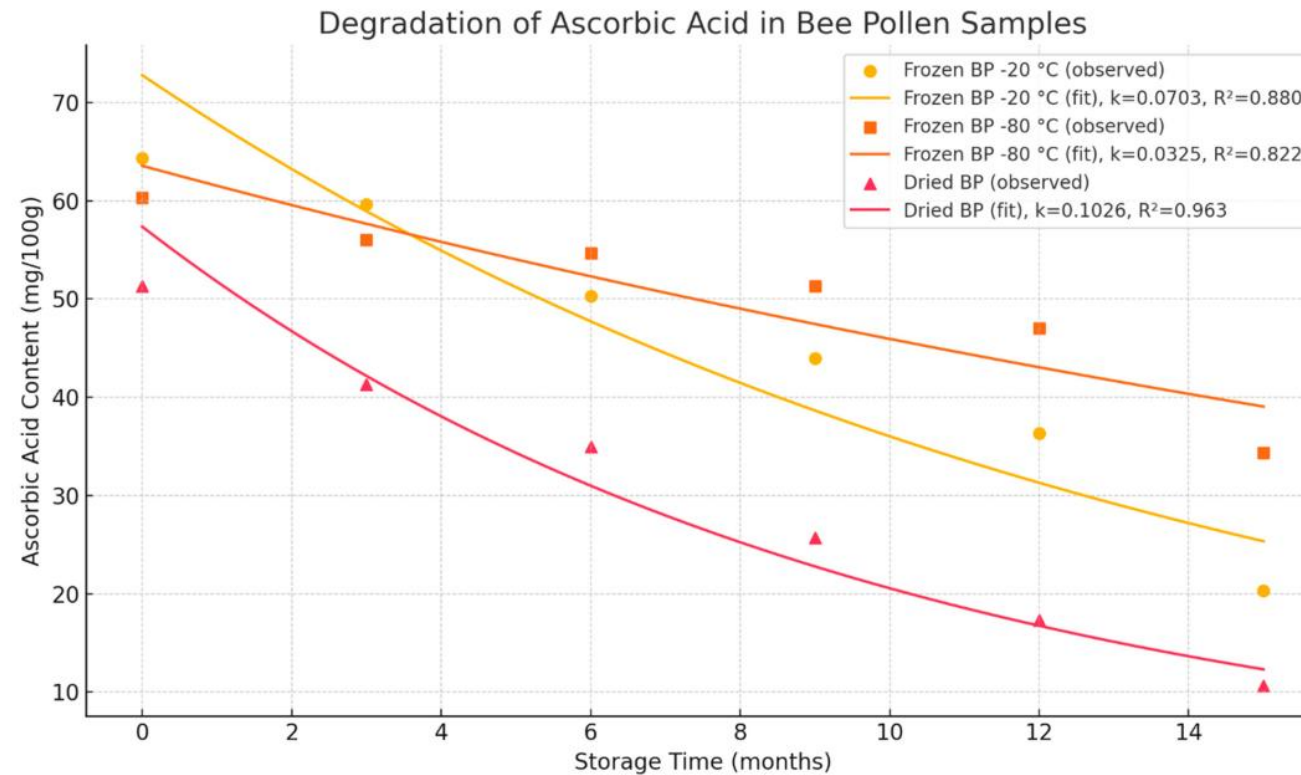
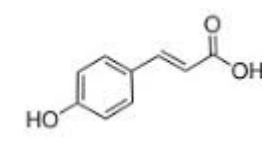
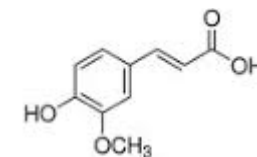
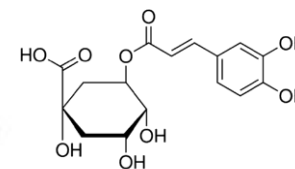
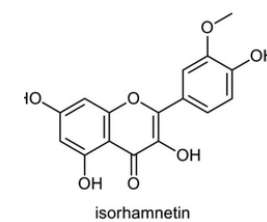
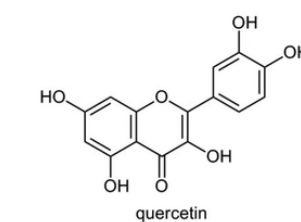
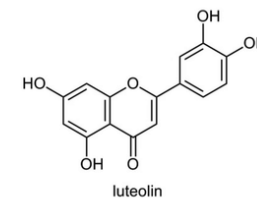
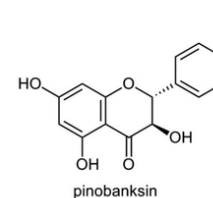
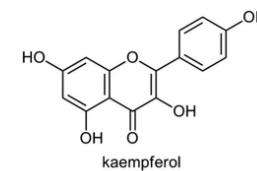
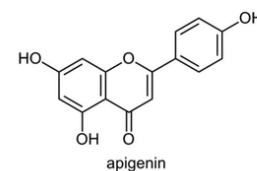
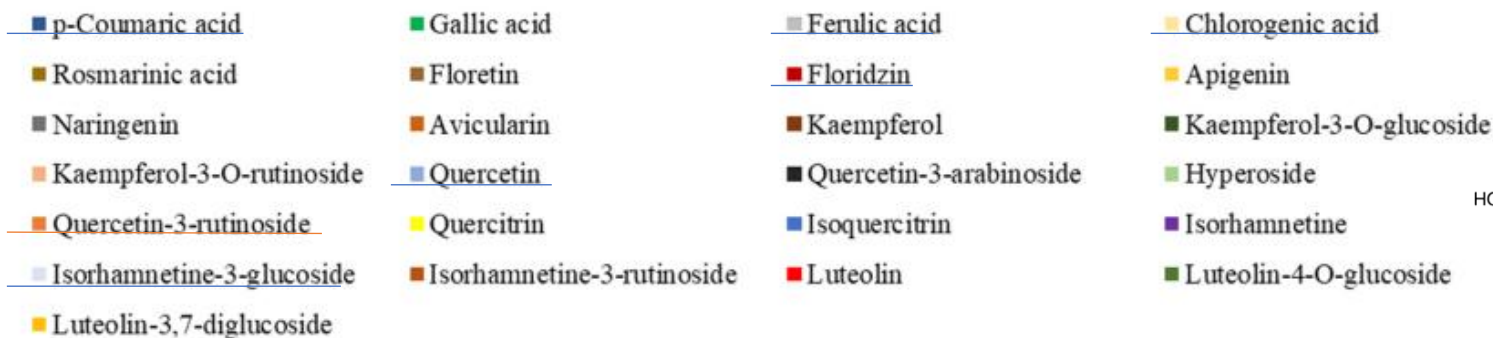
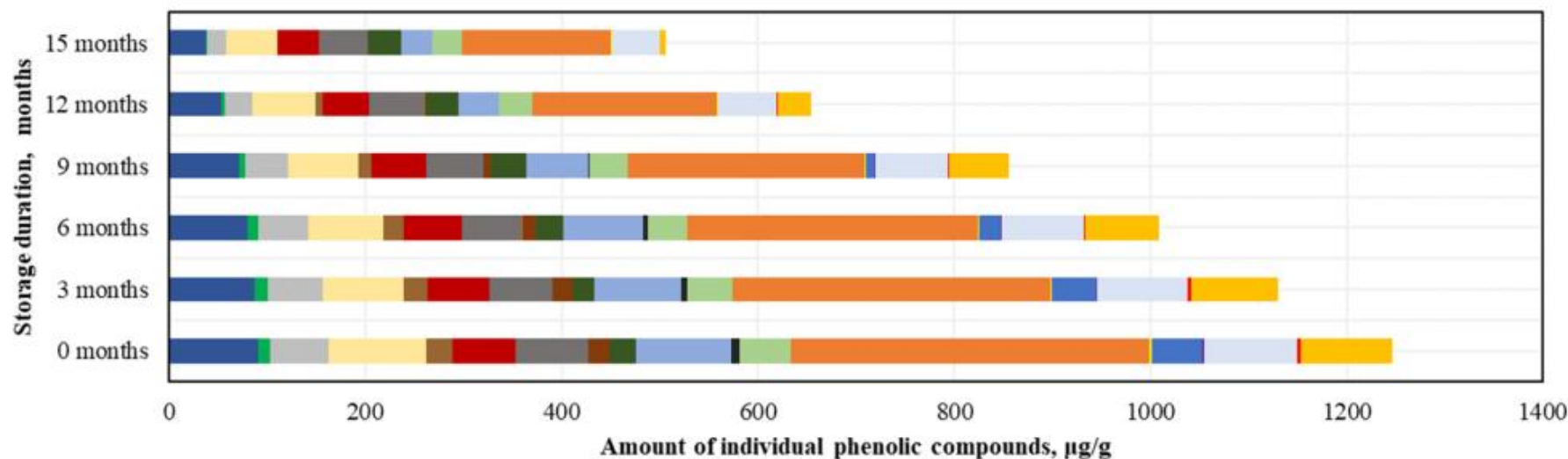
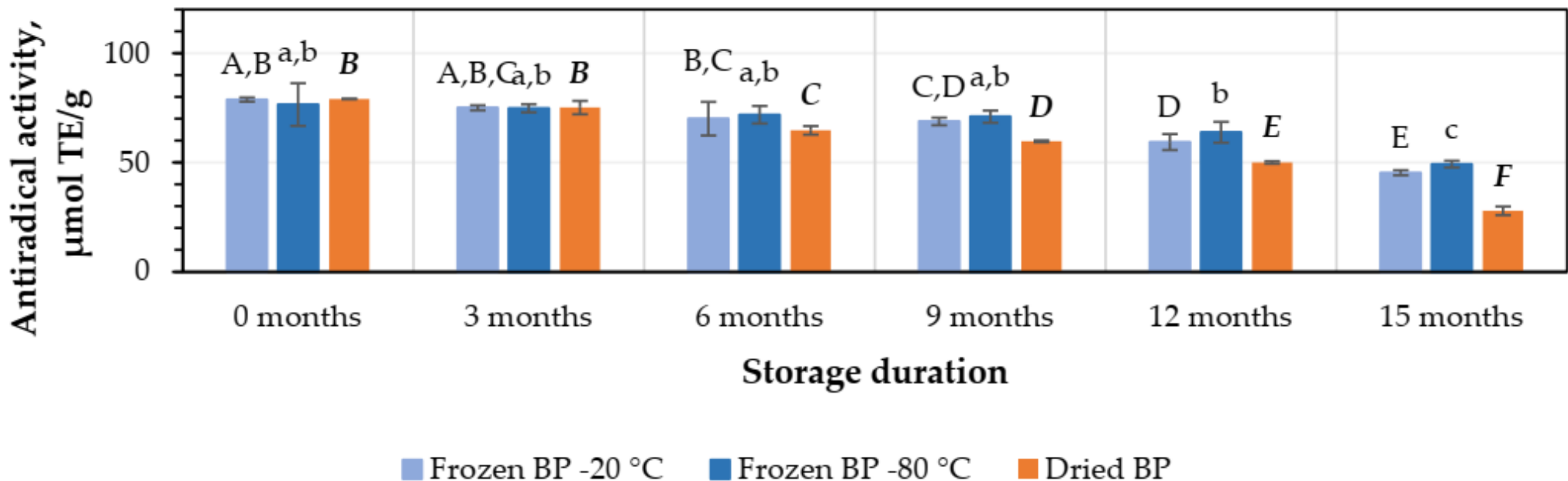


Figure S3. First-order degradation model of ascorbic acid in bee pollen samples

Changes in the profile of phenolic compounds (**flavonoids, phenolic acids**) in bee pollen samples during storage

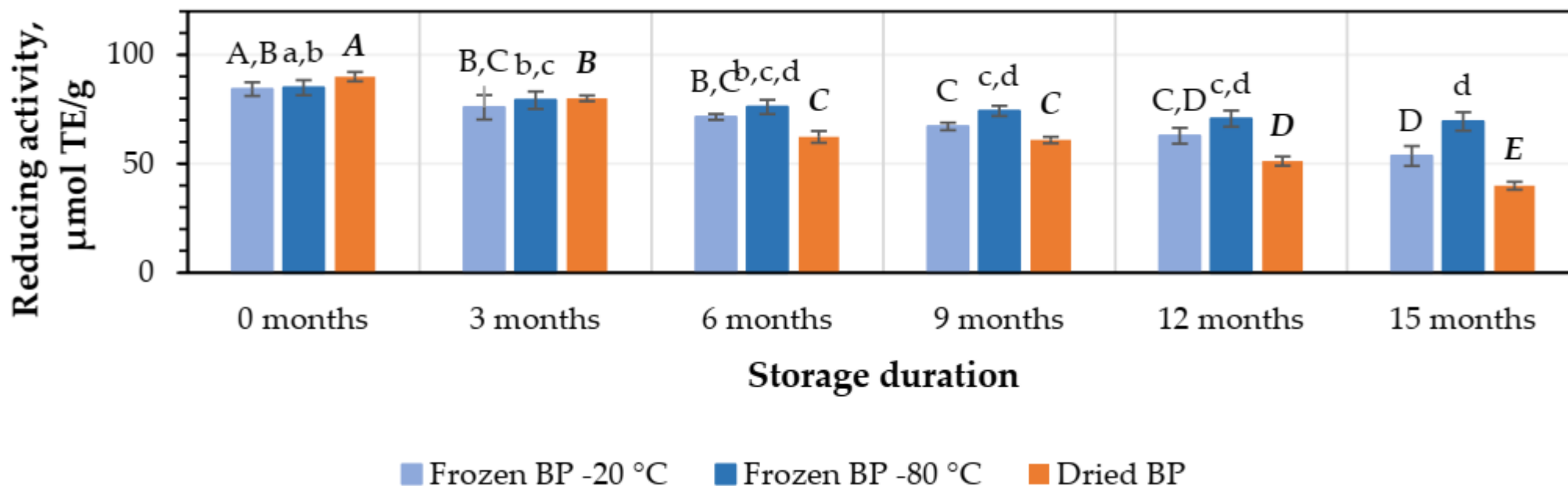


Changes in antioxidant (antiradical) activity during storage



	3 months	6 months	9 months	12 months	15 months
Dried	-5 %	-18* %	-25* %	-37* %	-65* %
Frozen -20 °C	-5 %	-11 %	-13* %	-25* %	-42* %
Frozen - 80 °C	-2 %	-6 %	-7 %	-17* %	-36* %

Changes in antioxidant (reducing) activity during storage



	3 months	6 months	9 months	12 months	15 months
Dried	-8* %	-24* %	-29* %	-50* %	-63* %
Frozen -20 °C	-14 %	-20 %	-24* %	-41* %	-59* %
Frozen - 80 °C	-4 %	-8 %	-9* %	-20* %	-29* %

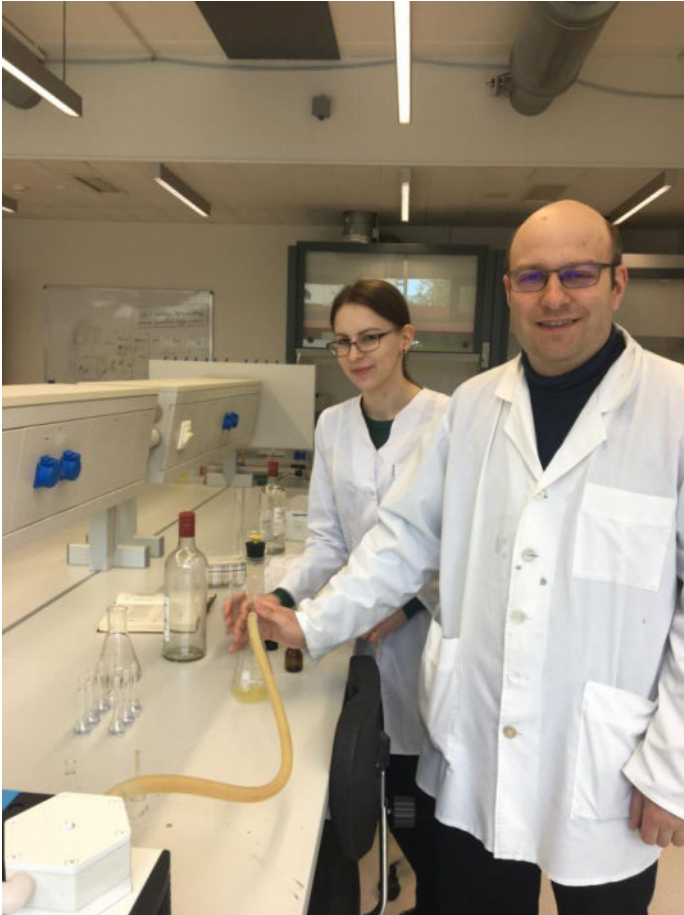
Summary of results

- **Storage conditions strongly affect bee pollen quality.**
- Dried pollen showed the most pronounced degradation of phenolic compounds, flavonoids, and ascorbic acid.
- **Freezing is superior to drying.**
- Storage at $-20\text{ }^{\circ}\text{C}$ slowed down degradation, **while $-80\text{ }^{\circ}\text{C}$ provided the best preservation of bioactive compounds (phenolics, vitamin C and antioxidant activity).**

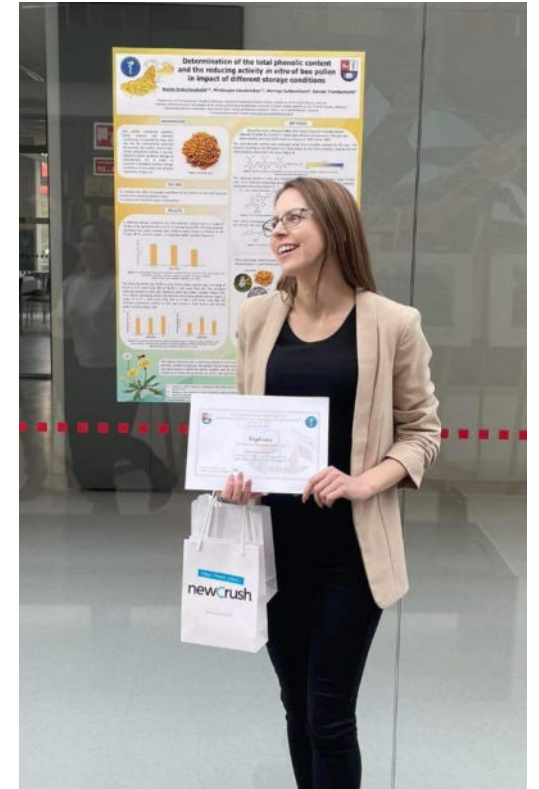
Conclusions

- Bee pollen is **highly sensitive to storage and processing**
- **Vitamin C:** decline during **drying and storage, especially in dried pollen; best** preserved in **frozen bee pollen** samples.
- **Phenolic compounds:** **more stable, best preserved in frozen samples;** but reduced amounts with prolonged storage;
- **Antioxidant activity: best preserved in frozen samples,** decline with prolonged storage;
- To ensure high-quality bee pollen, **appropriate preparation, storage conditions, and storage duration are of critical importance.**

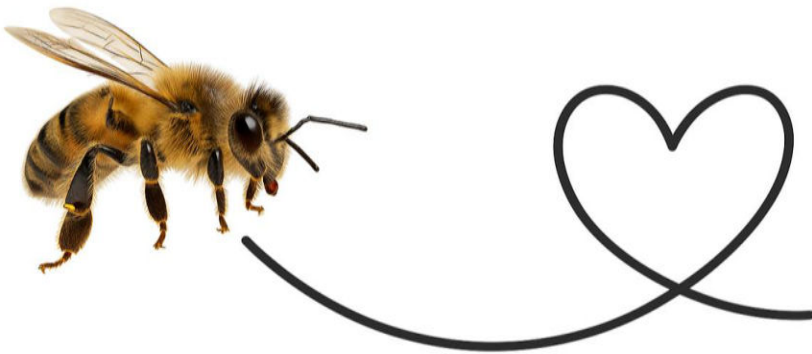
Thanks to the whole research team!



- Rosita Stebuliauskaitė
- Dr. Mindaugas Liaudanskas
- Dr. Neringa Sutkevičienė
- Dr. Violeta Čeksterytė
- Dr. Vaidotas Žvikas
- Dr. Mindaugas Marksa
- Beekeeper Žygimantas Jankevičius



Thank You!
Mange Tak!
Ačiū!



Sveikiniai iš Lietuvos ! Welcome to Lithuania!



Trakai Castle



Šiauliai, Hill of Crosses



Baltic sea coast