

# Flavonoids in Georgian Bee Bread and Bee Pollen

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**Abstract:** The proposed work aims to study phenol components (flavonoids) of bee bread and pollen obtained in Georgia using the HPLC (high-performance liquid chromatography) method, and to determine the content of biologically active compounds in them. The samples were taken in Imereti region (west Georgia). After extraction of samples, there were carried out spectral and HPLC analysis of compounds for the sake of qualitative and quantitative research of them. There are studied the flavonoid compounds of bee bread and pollen by using the HPLC methods, and naringin, rutin and quercetin are identified. Their amount counts about 20% of full content of flavonoids. The content of flavonols in bee bread and pollen has been established. It also has been determined that the amount of flavonoids during the storage period of products reduces to 6.17-5.03 g·kg<sup>-1</sup>.

**Key words:** Bee bread, bee pollen, phenols, flavonoids, HPLC.

## 1. Introduction

Among the main factors determining preservation of health and prolongation of human life, a crucial role is played by regular provision of the human organism with indispensable nutrient-rich, environmentally safe and multi-functional mainly local production foods, from which the most importance should be given to bee products.

The therapeutic, health-improvement and consumer properties of bee products had been well-known to Georgian people from early times.

Modern basic researches confirm that the bee products are distinguished by unique content of biologically active substances, and they act as the biogenic stimulators and have ability to positively influence on the human organism that conditions expedience of their use in foods industry, apiotherapy and pharmacological industry [1-5].

Over the last few years, among the bee products, popular have become pollen and bee bread especially. An interest in these products is very high in many countries in the world [3-6]. There is studied the

content of hydrocarbons [1], amino acids [2] and phenol compounds [7] in them. Special attentions deserve the biologically active compounds in these products [8-16].

Despite a lot of available information, unfortunately, there were no carried out yet the analogous research works studying the bee bread and pollen obtained in Georgia. The proposed work aims to study phenol components (flavonoids) of bee bread and pollen obtained in Georgia using the HPLC (high-performance liquid chromatography) method, and to determine the content of biologically active compounds in them. The samples were taken in Imereti region. After extraction of samples, there were carried out spectral and HPLC analysis of compounds for the sake of qualitative and quantitative research of them.

## 2. Materials and Methods

The samples were taken in Georgia, in various districts of Imereti region (west Georgia).

### 2.1 Extraction

Bee pollen was blended to a puree using commercial blender. Subsamples (5 g) extract (solution Hexane) in Soxhlet apparatus. 8-9 h later, the mass is dried in

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fume hood, and dried mass was extracted by 70%-ethanol (the ratio of sample and extracting agent is 1:15), first at room temperature, and then in the boiling chamber with the same solvent. Extracts were filtered and the filtrates were centrifuged for 5 min at 5,000 rpm. All samples were passed through 0.45  $\mu\text{m}$  filters (Acrodist LC (liquid chromatography) PVDF (polyvinyl difluoride) Syringe Filters Waters) prior to HPLC analysis.

In cases of flavonols extraction of samples was carried out with 70% ethanol. For qualitative analysis following reagents (Folin Ciocalteu) have been used [17], and their composition has been defined by spectric method by setting standard calibre curve. Flavonols quantified rutin (400 nm) equivalents. The results are shown in Table 1.

### 2.2 HPLC Analysis of Flavonoids

Samples (10  $\mu\text{L}$ ) were analyzed using a Waters HPLC system equipped with a model 525 pump, UV/VISIBLE is detector. Separation was carried out using a  $4.6 \times 150$  Symmetry C 18 column (Waters Corp, Milford, MA, USA) with a  $3.9 \text{ mm} \times 20 \text{ mm}$  C 18 guard column. The mobile phase was a linear gradient of 5% formic acid (A) and methanol (B). From 20% to 30% B for 0-7 min, 30%-50% B for 7-14 min, 50%-70% B for 14-21 min, 70%-90% B for 21-28 min, 90%-20% B for 28-32 min, at  $0.7 \text{ mL}\cdot\text{min}^{-1}$ . The system was equilibrated for 10 min at the initial gradient prior to each injection. Detection wavelengths used were 270 nm and 350 nm for flavonols. Total flavonols derivatives were calculated as rutin equivalents [18].

### 3. Statistical Analysis

Analysis of variance was used to determine significant differences ( $P < 0.05$ ) in total anthocyanin and flavonoid contents and antioxidant capacity among genotypes of each fruit analysis [19].

### 4. Results and Discussion

Bee bread and bee pollen phenol compounds depend on their growing area (plant cover) and vary during the storage periods (Table 1). The content of flavonoids in bee bread and bee pollen is maximum in a new raw material ( $10.1 \pm 0.15$ ,  $8.2 \pm 0.14$ ). Non-cooling storage of raw material reduces several times its content ( $6.37 \pm 0.11$ ,  $7.34 \pm 0.12$ ). HPLC confirms that quantitative changes after storage period does not reflect in quantitative ones. There is a certain difference between bee bread and bee pollen phenol compounds that is naturally caused by technology of producing these products by bee. Bee pollen is a concentrated product, while the bee bread is a product designed for recycling and storage.

Flavonoids are presented in their diversity in all products. HPLC reveals the presence of at least 12-15 compounds. The dominant compounds are peak 8 (Figs. 1-4).

The interesting fact is observed during extraction of products. Cold extraction of samples (the ratio between sample and solvent is 1:15) ensures almost 80%-extraction of flavonoids. The remaining 20% enter into the hot extract. The retention times of dominant compounds Peak 8 and Peak 11 are within 17.668 min and 20.449 min (Table 2). Unfortunately,

**Table 1** Content of flavonoids in bee bread and bee pollen.

Name	Flavonols 70% $\text{C}_2\text{H}_5\text{OH}$ ( $\text{mg}\cdot\text{kg}^{-1}$ ), raw weight ( $\text{g}\cdot\text{kg}^{-1}$ )	Flavonols 70% $\text{C}_2\text{H}_5\text{OH}$ ( $\text{m g}\cdot\text{kg}^{-1}$ ), dry weight ( $\text{g}\cdot\text{kg}^{-1}$ )
1 Bee bread 2012	$5.03 \pm 0.09$	$6.37 \pm 0.11$
2 Bee pollen 2012	$6.17 \pm 0.1$	$7.34 \pm 0.12$
3 Bee bread 2013	$7.4 \pm 0.14$	$10.1 \pm 0.15$
4 Bee pollen 2013	$7.1 \pm 0.13$	$8.2 \pm 0.14$

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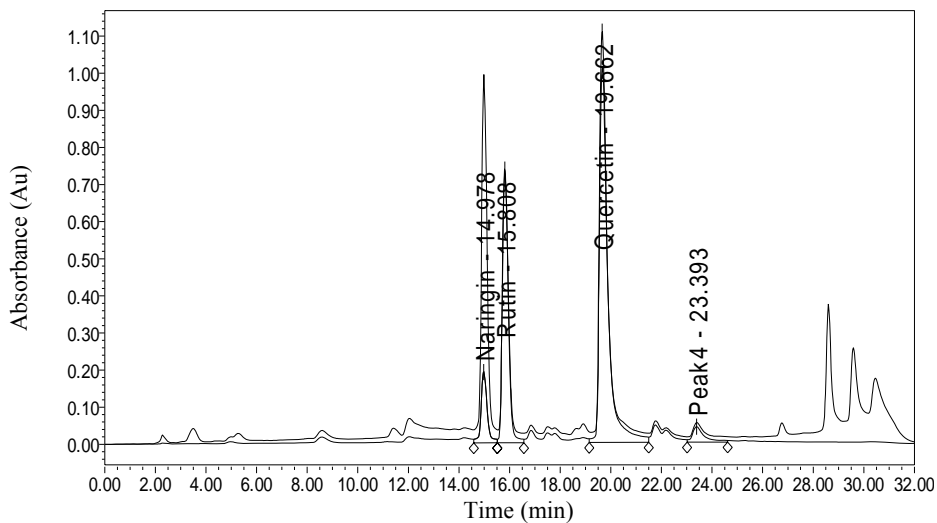
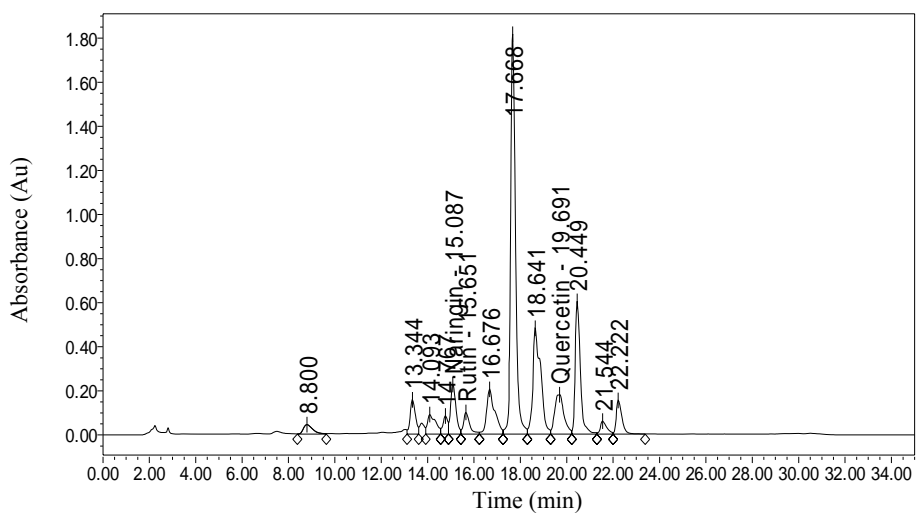
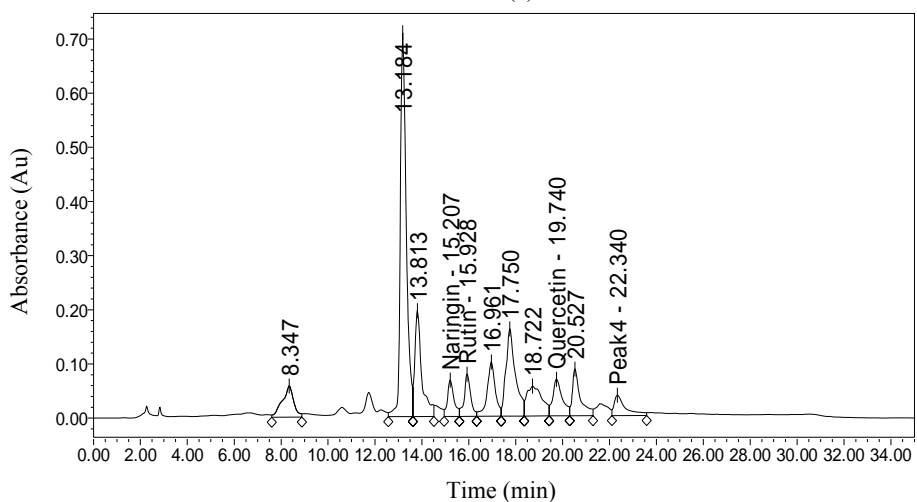


Fig. 1 HPLC chromatogram (270 nm, 350 nm) of flavonols standards.

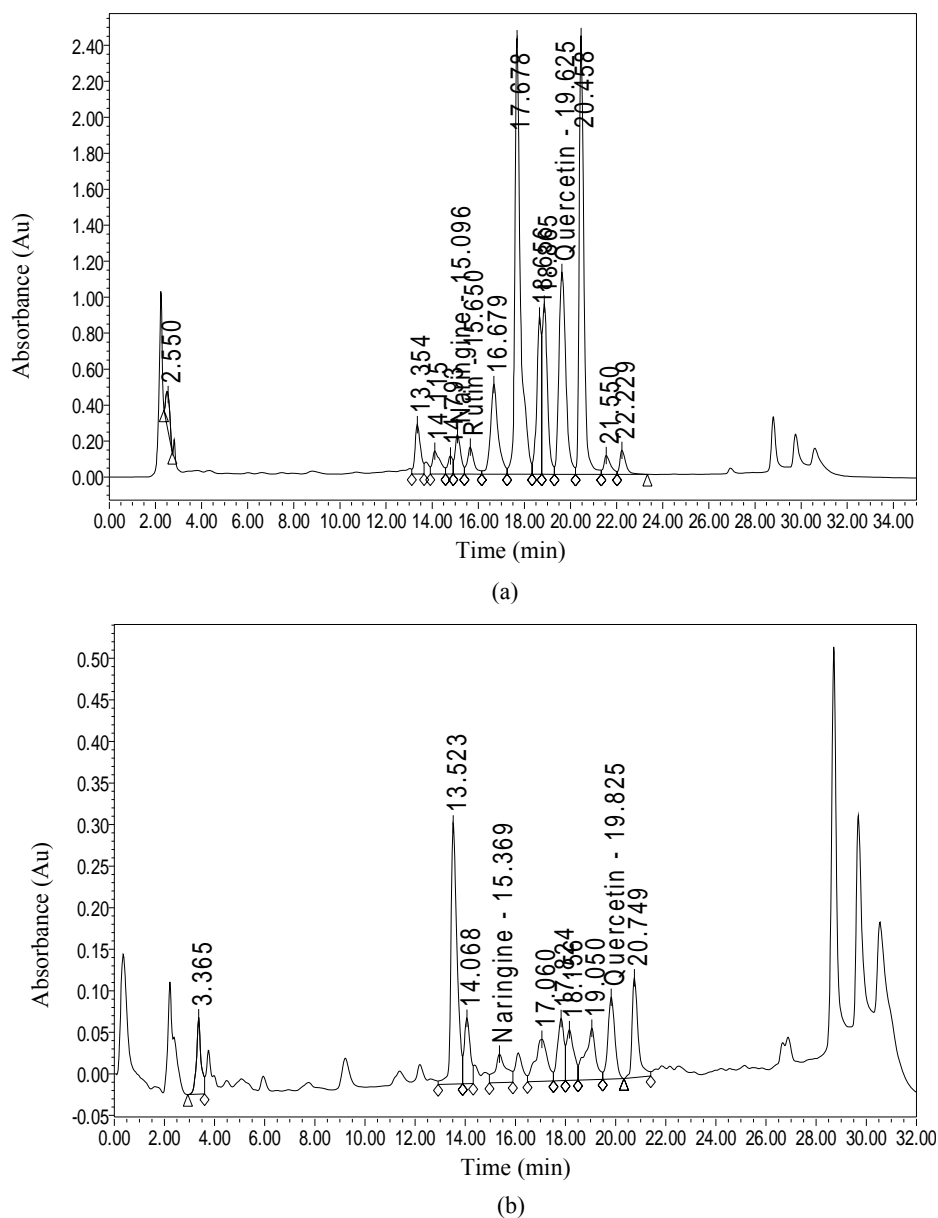


(a)



(b)

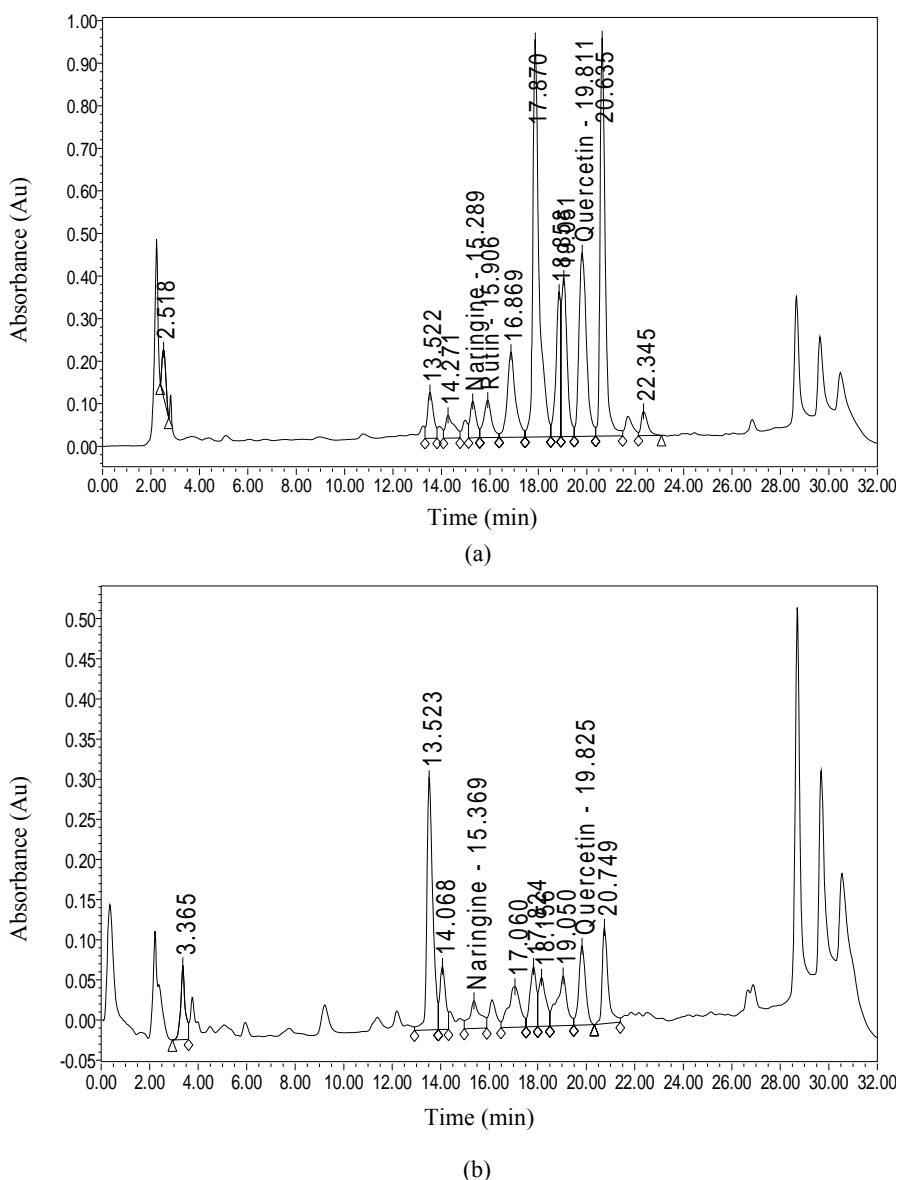
Fig. 2 HPLC chromatogram (350 nm) of flavonols bee pollen cold extracts bee bread and bee pollen.



**Fig. 3** HPLC chromatogram (270 nm) of flavonols bee pollen cold extracts bee bread and bee pollen.

**Table 2** HPLC characteristic of bee bread and bee pollen flavonoids.

No.	Name	Retention time (min)	Cold bee bread				Hot bee bread			
			Area (%)	Amount	Area (%)	Amount	Area (%)	Amount	Area (%)	Amount
5	Naringin (mg·kg <sup>-1</sup> )	15.087	4.96	76.87	3.6	18.69	4.4	18.44	2.3	0.87
6	Rutin (mg·kg <sup>-1</sup> )	15.651	2.48	3.33	4.13	4.18	4.7	4.66	3.09	2.59
7		16.676	6.78		6.09		6.98		9.03	
8		17.668	36.18		12.63		35.51		26.14	
9		18.641	15.39		7.32		14.85		6.66	
10	Quercetin (mg·kg <sup>-1</sup> )	19.691	6.78	15.95	5.29	19.32	6.71	3.86	14.52	5.10
11		20.449	12.73		10.43		21.46		10.04	



**Fig. 4 HPLC chromatogram (270 nm) of flavonols bee pollen hot extracts bee bread bee pollen.**

at this stage of research, this compound is not identified yet. It has become possible to identify naringin, rutin and quercetin in composition of products. Their amount counts about 20% of full content of flavonoids.

## 5. Conclusions

For the first in Georgia, there are studied the flavonoid compounds of bee bread and pollen by using the HPLC methods, naringin, rutin and quercetin are identified.

It has been established the content of flavonols in bee bread and pollen.

It also has been determined that the amount of flavonoids during the storage period of products reduce to  $6.17-5.03 \text{ g}\cdot\text{kg}^{-1}$ .

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