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SHORT COMUNICATION

INFLUENCE OF LYOPHILIZATION AND CONVECTIVE TYPE DRYING ON ANTIOXIDANT PROPERTIES, TOTAL PHENOLS AND FLAVONOIDS IN POLLENS

Ingmars Cinkmanis, Fredijs Dimins, Velga Mikelsone

Department of Chemistry, Faculty of Food Technology, Latvia University of Agriculture, Liela iela 2, Jelgava, Latvia, e-mail: ingmars.cinkamnis@llu.lv

Abstract

Pollen is one of the most popular beekeeping products surpassed by honey and wax. In nature there is no food analogue, which can compare to pollen in terms of those biologically active substances, which are necessary for normal development and functioning of human body. The aim of the research was to determine and compare the total antioxidants, total flavonoids, total phenolic compounds and antiradical activity content in fresh, lyophilized and dried pollen samples. Content of total antioxidants (DPPH quercetin equivalent), total flavonoids, total phenols and antiradical activity (with DPPH absorption) was determined by spectrophotometric method. Three different pollen samples were analysed – fresh pollen, dried pollen at +42 °C and lyophilized pollen. Results of the analysis showed that the highest total antioxidant content is in fresh pollen – 29.75 mg QC 100 g^{-1}, but the lowest in both dried samples. Total phenol content in dried pollen was 56.89 mg GAE 100 g^{-1}, in lyophilized pollen – 54.11 mg GAE 100 g^{-1}. Total phenol content in fresh pollen was 61.64 mg GAE 100 g^{-1}. Both thermal treatment and lyophilisation decreased flavonoid content in pollen. Drying pollen at +42 °C affected by lower losses of total antioxidants, total flavonoids, total phenols and antiradical activity than in lyophilisation process.

Keywords: drying, lyophilisation, pollens, total phenols.

Introduction

The chemical composition of pollen is diverse and complex. Nowadays it is used not only as a dietary ingredient, but also as an alternative medical remedy (Kroyer, 2001). Pollen contains carbohydrates, fats, proteins (composed of all the essential amino acids), minerals (containing 28 chemical elements, especially significant amounts of K, Cu, Fe and Co) about 50 enzymes and hormones (Siņakovs, 2009). Natural antioxidants are also found in pollen (de Arruda et al. 2013; LeBlank et al., 2009). Vitamins A, E, C, B-vitamins, niacin, rutin, polyphenols, and selenium compounds are widely represented in pollen (Bonvehi, 2001). Pollen contains 20 times more vitamin A than, for example, carrots (Šteiselis, 2013).

There are various parameters characterizing antioxidants. Flavonoids are a significant group of plant secondary metabolites having a different biochemical and antioxidant effects. Flavonoids possess antiradical activity, thus radicals, as atomic oxygen, hydrogen peroxide, superoxide anion radical, resulting in the plant from UV radiation and natural plant metabolism, are neutralized and eliminated from the plant (Galeotti et al., 2008). Because of this essential factor people are interested in flavonoid rich foods, because human metabolic intermediates are also radicals that cause damage to cells, disrupting the phospholipid membranes. Flavonids have anti-virus, anti-allergic, anti-platelet, anti-inflammatory and antioxidant effects on the human body (Purviņš, Purviņa, 2011).

Antioxidants stops the oxidation in its early stage, preventing ongoing chain reactions, but antiradical activity begins to work later. Also, it neutralizes free radicals. Anti-radical activity is caused by a variety of compounds, including polyphenols that are capable of neutralizing the radicals (Feas et al., 2012).

Polyphenols are sum of phenol compounds in pollen. They are biologically active substances that are found in nature. Polyphenols main feature is the presence of many phenolic structures. This structure is the basis of large number and diversity of this group's unique physical, chemical and biological properties (Quidau et al., 2011).

Pollen composition depends on the plant type and pollen harvesting conditions (Almaraz-Abaraca, et al., 2004).

Variable according to relative humidity of air is the water content - in freshly harvested it is 20–30%. Fresh pollen must be used within a short period of time because of the increased moisture content, they begins to grow mould and microorganisms, which produce toxins are developed. For long-term storage of pollen they need to be treated – water content must not exceed 12.5%, the optimal water content is 8–10% (Ritmanis, 2004). Beneficial nutritional properties of biologically active substances in various honey products, including pollen, are widely studied but rarely changes in composition and properties based on type of storage and treatment are discussed.

Most conventional way to treat pollen is to dry it in convective type dryers. Pollen is laid in a thin layer and dried at 42 °C, the existing water is continuously discharged by forced ventilation (Bogdanov, 2011). Thermal drying is not the only way to achieve the desired result.

In lyophilisation process pollen is not dried by heating but by freezing it under reduced pressure. This causes sublimation - water in pollen immediately goes into the gaseous phase, skipping the liquid phase (Giordano et al., 2011). This technique is indispensable if it is required to dry heat-sensitive substances, which during drying in the oven (42 °C) is significantly affected by temperature and lose its high-value properties (de Melo, de Almeida-Muradian, 2010).

Pollen high-value properties, characterized by biological activity indicators such as total phenol, total flavonoids, total antioxidant content and antiradical activity, is reduced if product is dried or lyophilized (Giordano et al., 2011).

The aim of the research was to determine and compare the total antioxidants, total flavonoids, total phenolic compounds and antiradical activity content in fresh, lyophilized and dried pollen samples.

Materials and Methods

Research was carried out at the Department of Chemistry, Faculty of Food Technology at the Latvia University of Agriculture. The object of the research was bee pollen. Pollen were harvested in April of 2016 from Saldus district Ltd. „ULMUS-MEDUS".

Characterization of drying process

Bee pollen were convective dried with air circulation at 42 ºC using Memmert UFE-400 and lyophilized using Christ Freeze Dryer Alpha 1-2 LD plus at - 60 °C for 24 hours at 0.046 mbar.

Extraction of pollen samples

1.500 g of fresh, dried and lyophilized pollen was extracted with 50 mL methanol. Extraction was carried out for 1 hour. Extract were centrifuged at 13 000 rpm in a centrifuge for 5 min, the supernant was used for further analysis.

Determination of total phenolic content (TPC)

Total phenol compound content was determined by spectrophotometry (Kaškoniene, 2009). Method is based on phenol compound reaction with the Folin-Ciocalteu reagent. Coloured solution is formed, which is measured in the light absorption using a 760 nm wavelength. Total phenolic content levels are expressed as gallic acid equivivalents mgQE 100 g^{-1} dry weight (Singleton, 1999).

Determination of total flavonoids content

The total flavonoid content of pollen was determined by spectrophotometry. Method was based on the pollen flavonoids reaction with $AlCl_3$. Coloured solution was formed, its light absorption was measured using 415 nm wavelength light. Total flavonoid content are expressed as quercetin equivalent mgQE 100 g^{-1} dry weight (Singh, 2012).

Determination of total antioxidants content

Pollen antioxidant properties was characterized by spectrophotometry (Bertoncelj, 2007). The method is based on reagent DPPH (2,2-diphenyl-1-picrylhydrazyl) reaction with antioxidants by light absorption maximum of 517 nm wavelength. Total antioxidants content is expressed as quercetin equivalent mg QE 100 g^{-1} dry weight.

Determination of antiradical scavenging activity

Antiradical scavenging activity was determined by spectrophotometric method with DPPH (2.2-diphenyl-1-picrylhydrazyl) absorption of 517 nm wavelength.

Statistical analysis

The results were processed by mathematical and statistical methods (mean, standard deviation) using Microsoft Office Excel 2016.

Results and Discussion

The highest total flavonoid content was found in fresh pollen: 196 mg QE 100 g^{-1}, while the lowest was in lyophilized pollen: 154 mg QE 100 g^{-1} (Fig.1).

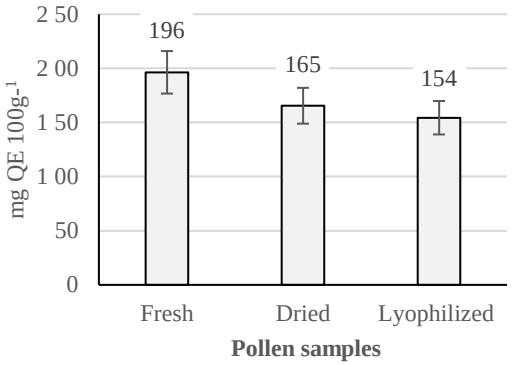

Figure 1. Content of total flavonoids

Flavonoid content in pollen is decreased by both thermal treatment and lyophilisation of pollen. Heat treatment destroyed 15.7%, while the lyophilisation 21.3% of flavonoids, compared to flavonoid content in fresh pollen. When comparing the flavonoid content of the thermally treated and lyophilized pollen, differences are not significant.

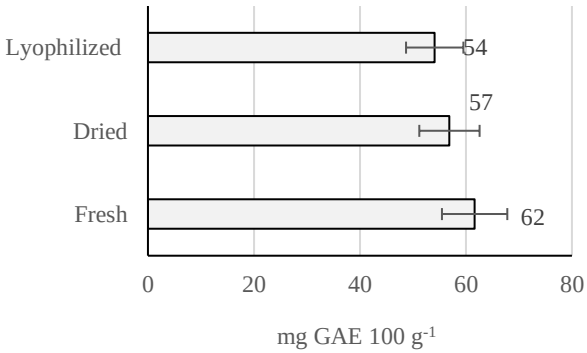

Figure 2. Content of total phenols

In dried pollen total phenol content in the dry matter is 57 mg GAE 100 g^{-1}, in lyophilized 54 mg GAE 100 g^{-1}, in fresh 62 mg GAE 100 g^{-1} (Fig. 2). The differences between the total phenolic content of dried and lyophilized pollen is similar – differing only by 2.1%, which is within the method errors limits.

The highest antiradical activity was detected in dried pollen – 85%, slightly lower in the freeze-dried pollen – 82%, while the lowest was in fresh pollen – 72% (Fig. 3).

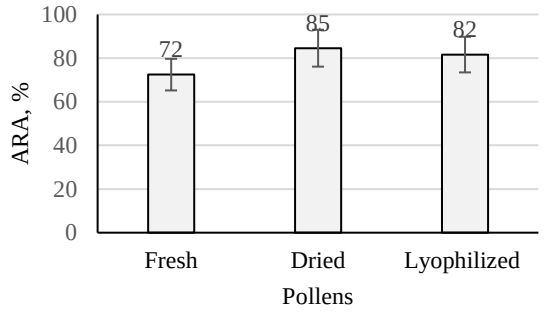

Figure 3. Antiradical scavenging activity

However, the differences between data was within error limit of the method i.e. ±10%. If highest and lowest error detection margin is taken in account regarding the data, then the differences are negligible.

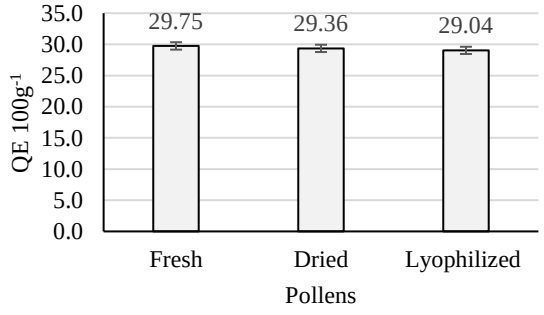

Figure 4. Content of total antioxidants

The total content of antioxidants (Fig.4) in dried and lyophilized pollen dry matter is similar - differing only by 2%, which is within the margin of method error.

Conclusions

The highest total flavonoid - 196 mg QE 100 g^{-1} and phenol content – 62 mg GAE 100 g^{-1} was found in fresh pollen, antiradical activity in analysed samples was insignificantly higher in dried and lyophilized samples, but the total antioxidants content of all samples was similar and in all the pollen ranged from 29.04 to 29.75 QE 100 g^{-1}.

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