Journal of Pharmaceutical and Biomedical Analysis 147 (2018) 110–124

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Review

Extraction and determination of bioactive compounds from bee pollen

Ana M. Ares, Silvia Valverde, José L. Bernal, María J. Nozal, José Bernal*,[1]

I.U. CINQUIMA, Analytical Chemistry Group, University of Valladolid, E. 47011 Valladolid, Spain

ARTICLE INFO

Article history:
Received 6 June 2017
Received in revised form 3 August 2017
Accepted 5 August 2017
Available online 8 August 2017

Keywords:
Bee pollen
Bioactive compounds
Determination methods
Food supplements
Extraction techniques

ABSTRACT

Since ancient times bee pollen has been considered a good source of bioactive substances and energy. Taking into account the current demand for healthy and natural foods, it is not surprising that bee pollen has been attracting commercial interest in recent years, making it one of the most widely consumed food supplements. It has been extensively reported that bee pollen contains several health-promoting compounds, such as proteins, amino acids, lipids, phenolic compounds, vitamins or minerals. Thus, this study aims to give an overview of the extraction and determination techniques of several of the above-mentioned compounds which have been published in the last few years (2011-2017). The design of the study is in accordance with the different families of bioactive compounds, and the extraction procedures together with the analytical techniques employed and their determination are discussed. A list of some of the most relevant applications is provided for each category, including a brief summary of the experimental conditions. The references included will provide the reader with a comprehensive overview of and insight into the analysis of bioactive compounds from bee pollen.

Contents

Abbreviations and chemical formulas: AAA, amino acid analyzer; AA, samino acids; AAS, atomic absorption spectroscopy; AD, amperometric detector; AES, atomic emission spectrometry; $AlCl_3$, aluminium trichloride; $Al(NO_3)_3$, aluminium nitrate; BAB, radford assay; BCA, bicinchoninic acid; BCEC-Cl, 2-(11H-benzo[α]-carbazol-11-yl) ethyl chloroformate; BD, by difference; BPE, bound phenolic extracts; BHT, butylated hydroxytoluene; CE, capillary electrophoresis; CO_2, carbon dioxide; DAD, diode array detector; DNS, 3,5-dinitrosalicylic acid; DPPH, 1,1-diphenyl-2-picrylhydrazyl; DTT, 1,4-dithiothreitol; EA, elemental analyzer; ECD, electrochemical detector; EDTA, ethylenediaminetetraacetic acid; FAAs, free amino acids; FAMEs, fatty acids methyl esters; FFAs, free fatty acids; FCM, Folin-Ciocalteu method; FID, flame Ionization detector; FLD, fluorescence detector; FLM, fluorimetric method; FPE, free phenolic extracts; FTRPC, free tryptophan content; GC, gas chromatography; GLSs, glucosinolates; GVM, gravimetric method; HCl, hydrochloric acid; $HClO_4$, perchloric acid; HNO_3, nitric acid; H_2O_2, hydrogen peroxide; IC, individual carotenoids; ICP, inductively coupled plasma; IEF, isoelectric focusing; IF, individual flavonoids; IP, individual phenolics; IPRO, individual protein; K, Kjeldahl; KOH, potassium hydroxide; LC, liquid Chromatographic; LEM, Lane-Eynon method; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; MEC, 2-mercaptoethanol; MPA, metaphosphoric acid; MS, mass spectrometry; MS/MS, tandem mass spectrometry; NaOH, sodium hydroxide; $NaNO_2$, sodium nitrite; NH_2, amino; NIRS, near infra-red spectroscopy; NPLC, normal-phase liquid chromatograph; NA, not applied; NS, not specified; OCC, open column chromatography; OES, optical emission spectrometry; OPA, *o*-phthaldialdehyde; PAGE, polyacrylamide gel electrophoresis; PDA, photodiode array detector; PITC, phenylisothiocyanate; RID, refractive index detector; RPLC, reverse-phase liquid chromatography; RS, reducing sugar; SDS, sodium dodecyl sulfate; SFN, sulforaphane; SFE, supercritical fluid extraction; SPE, solid phase extraction; SSA, 5-sulfosalicylic acid; ST, sterols; TAAs, total amino acids; TANC, total anthocyanin content; TC, total carotenoids; TCHC, total carbohydrate content; TFC, total flavonoid content; TFLC, total flavonol content; TL, total lipids; TN, total nitrogen; TPC, total phenolic content; TPROC, total protein content; TRP, tryptophan; Tris, tris(hydroxymethyl)aminomethane; TSPP, 1-[2-(p-toluenesulfonate) ethyl]-2-phenylimidazole [4,5-f]9,10-phenanthrene; TTM, titrimetric method; TTRPC, total tryptophan content; UHPLC, ultra-high performance liquid chromatography.

* Corresponding author.
E-mail address: jose.bernal@qa.uva.es (J. Bernal).
[1] web: tesea.uva.es

http://dx.doi.org/10.1016/j.jpba.2017.08.009
0731-7085/

1. Introduction

Apicultural products have long been used in phytotherapy as well as in diet for their positive health consequences. Nowadays, bee products (honey, royal jelly, propolis, bees wax or bee pollen) are gaining prominence due to the presence of bioactive compounds that are associated with beneficial properties to health [1,2]. Bee pollen in particular is gaining attention as functional food for human consumption due to its high content of compounds with health-promoting effects, such as essential amino acids, antioxidants, vitamins and lipids [3]. It is collected by foraging honey bees (*Apis* sp. including *Apis mellifera*) and stingless bees, and is a combination of mainly floral pollen with some nectar or honey, enzymes, wax and bee secretion. The pollen mixture is transported as a small pellet in the pollen basket of the beeís legs to the bee hive, where it is stored and used as food for all the developmental stages in the hive [4,5]. From the moment in which the bees add their secretions to this pollen, it acquires certain peculiar characteristics which make it different from pollen collected by hand or that which is dispersed by wind [6]. Bee pollen contains several nutrients and bioactive compounds: proteins, which are among the main components of bee pollen, include enzymes and both essential and nonessential amino acids. In fact, bee pollen is referred to as the "*only perfectly complete food*", as it contains all the essential amino acids needed for the human organism [5]. A large number of carbohydrates are also found in this substance, for instance, reducing sugars, polysaccharides, starch, and soluble and non-soluble fibers. In addition, lipids, such as fatty acids, sterols, and triglycerides, minority compounds, for example, minerals and vitamins (water and fat soluble), and several other bioactive compounds, like the phenolic type, are present [7,8]. However, the composition of bee pollen is particularly dependent on plant origin, together with other factors such as climatic conditions, soil type, beekeeper activities, and the different processes or storage treatments in commercial production [5,9]. It is worth mentioning that the bioactive quality of bee pollen declines over time, and that conditioning carried out on fresh pollen before storage is influential in its nutritional and functional value [3]. Since bee pollen displays a high level of moisture in its composition, a dehydration process (artificial drying) is necessary to avoid rapid fermentation and spoilage, which is crucial in order to extend the shelf life time [10].

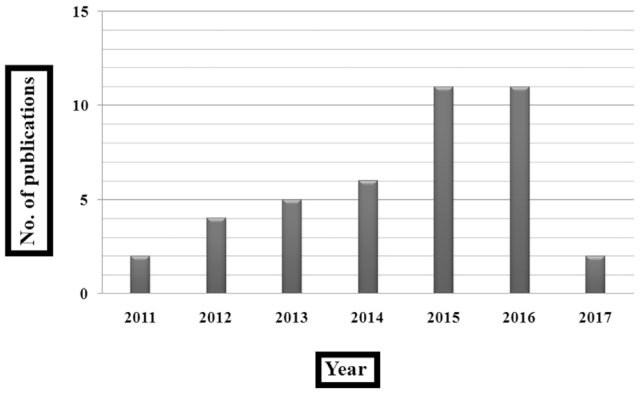

Fig. 1. Evolution of the published works in the last years (2011–2017) related to extraction and determination of bioactive compounds from bee pollen (data up to May 2017). The sources of information were the databases: ISI-Web of Knowledge, Scirus, Scopus and Science Direct. The search has been done using as keywords [(bee pollen) or (honey bee collected pollen)] and [(bioactive) or (lipids) or (vitamins) or (proteins) or (phenolic) or (essential elements) or (sugars) or (amino acids) or (carbohydrates) or (peptides) (extraction) or (isolation) or (quantification) or (separation) or (determination) or (analysis) or (chromatography)] among several others.

Over the past years, the rising interest in the extraction and determination of these beneficial bee pollen compounds has been demonstrated by the number of published research papers dealing with this issue (See Fig. 1), and the large list of countries in which such studies were carried out (See Fig. 2). As can be observed in Fig. 3, several of these compounds have been examined in this matrix during this period of time, most of the studies being devoted to the analysis of proteins, peptides and amino acids (31% of the

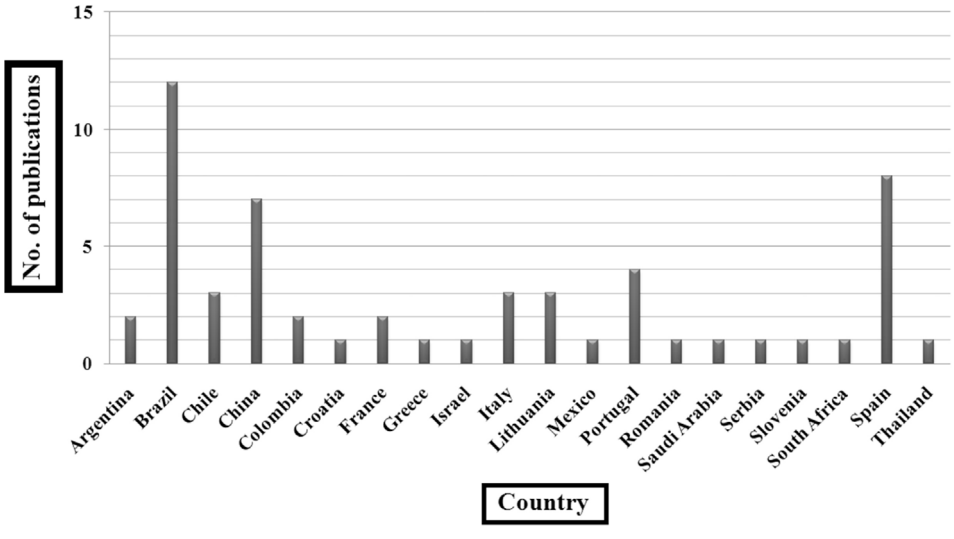

Fig. 2. Summary of the number of publications per country related to the analysis of bioactive compounds from bee pollen.