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Organic acids as a botanical geographical marker of Egyptian, Chinese and German bee pollens

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Abstract:

Organic acids in bee pollen samples represented from different geographically regions of Egypt, China and Germany countries were estimated using high performance liquid chromatography (HPLC) as a chemical marker of these regions. The number of organic acids present in samples of tested bee pollens were eight in Egyptian, six in Chinese and five in German organic acids bee pollens. There was only one organic acid (Citric acid) commonly found in the all tested types of bee pollens. Oxalic acid it can be used to differentiate between German bee pollen and the other under study. Also, malonic acid was absent in Chinese pollen while it was found in other bee pollens tested. So, organic acids can be used to distinguish among the main different types of pollens (Egyptian, Chinese and German).

Introduction

Bee pollen be a healthy foodstuff with an extensive variety of therapeutic properties (Feás *et al*., 2012). Bees collect pollen from numerous plant anthers, mix it with secretions from their salivary glands or nectar, place it in specific baskets located at their hind legs, and transfer it to the hive (Komosinska-Vassev *et al*., 2015). However, many factors can contribute for the composition of bee pollen, such as the plant source, the climate and weather conditions, the characteristics of the soil, as well as the actions of the beekeeper (Pereira *et al*., 2006 and Shubharani *et al*., 2013). A mixture of flower pollen from different species is agglutinated by nectar and honeybee enzymes (e.g. amylase, catalase) secreted by salivary glands and pollen loads are formed, which are recognized as bee pollen in the form of granules , two hundred substances were found in the pollen grains from different plant species, the group of basic chemical substances is proteins, amino acids, carbohydrates, lipids and fatty acids, phenolic compounds, enzymes, and coenzymes as well as vitamins and bio elements (Campos *et al*., 2008 and 2010).

Any significant deficiency in the elemental content of rock, soil, and water affects the elemental content of plants growing in a region, which directly affects the nectar and pollen (Hernández *et al.*, 2005). The chemical composition of pollen is diverse and complex (Kroyer and Hegedus, 2001). And it varies significantly, according to the botanical and geographical origin of

the pollen grains that make up the pollen loads. The composition of the grains, in their turn, suffers the influence of the soil and climate conditions, age and nutritional state of the plant during the development of the grain. In the same plant species there can be variations in pollen composition according to region, season of the year, and even between years (Funari *et al.*, 2003; Almeida- Muradian *et al*., 2005; Melo *et al.*, 2009 and Silveira, 2012). Approximately 250 substances can be found in this product (Komosinska-Vassev *et al*., 2015). Physico-chemical composition of pollen samples collected in State of São Paulo, Brazil, average were 21.5% proteins, 2.8% ashes, 23.6% moisture, 76.3% of dry residue, 3.5% lipids, 28.4% of total sugars, 20.7 mEq/kg of titrate acidity and pH = 5.1. The bromatological characterization of bee pollen from Argentina was made by (Coronel *et al*., 2004). The organic acids composition varies depending upon the species, age of the plant and the vegetable tissue. In *E. plantagineum* bee pollen hydromethanolic extract eight organic acids were identified: oxalic, aconitic, citric, pyruvic, malonic, shikimic, acetic and fumaric acids. Although organic acids represent less than 0.5% of honey's constituent, they make important contributions to organoleptic, physical and chemical properties of honey (Inés *et al*., 2006). Malonic and acetic acids are the precursors for fatty acids biosynthesis furthermore, shikimic and malonic acids are precursors of phenolic compounds, a heterogeneous group from a metabolic point of view, presenting antioxidant, anti-inflammatory and anti-allergic activities (Avni *et al*., 2014). Certain substances, such as ascorbic acid, amines, carbohydrates, and sulfur-containing compounds, can also react with phosphomolybdic and phosphotungstic acids of the reagent used (Huang *et al*., 2005). Organic acids can also be used as predictors of fermentation, antioxidant activity and as botanical geographical markers (Mato *et al.*, 2003).

The aim of this study was to use organic acid to distinguish among different types of bee pollen from Egypt, China and Germany.

Materials and methods

The present investigation was carried out in Bee Research Department, Plant Protection Research Institute, during year 2019 to determine organic acids in 3 types of Egyptian, China and Germany pollen's. Pollen samples were collected from different regions from Egypt, China and Germany. These pollen types were determination of organic acids by using high performance liquid chromatography (HPLC).

1. Determination of organic compounds in pollen samples by HPLC:

Approximately, 3g of freeze-dried pollen were transferred to Eppendorf tube with 5mL of distilled water. Then the extract was centrifuged (15,000 rpm, 4°C, 10min) and the supernatant was separated with membrane filter (0.45µm) and transferred to vials. Organic acids in pollen were Identification by a JASCO HPLC, using a hypersil C reversed-phase column [250x4.66 mm] with 5 µm particle size. A constant flow rate of 0.7 ml/min sulphuric acid in distilled water Ph. 2.45 was used as mobile phases; the detector set at wavelength 210 nm, the concentration of individual compound was calculated on the basis of the peak area measurements. All chemicals and solvents were used in HPLC spectral grade (Anna *et al.*, 1994).

The data were statistically analyzed using one way ANOVA. LSD was used to evaluate the significant

difference between means (comparison of means) at the level of P <0.05 level.

Results and discussion

Data in Table (1) and Figure (1) represent the organic acids that can be found in the bee pollens under investigation. Twelve organic acids were detected in analyzing of three bee pollen types under investigation by using HPLC (Figure 2). They were oxalic, formic, tartaric, shikmic, malonic, maleic, citric, succinic, proponic, butyric, lactic and adipic acids. Only one of them (Citric acid) commons and was in the all tested bee pollen; its highest concentrations was detected in Chinese bee pollen 0.5453 mg/100 followed by 0.0185, 0.0043 mg/100 in Egyptian and German bee pollen respectively. On the other hand, formic acid, it was absent in any bee pollen under investigation. However, tartaric and adipic acids was characterized of Egyptian bee pollen the concentrations were 0.3012, 0.0053 mg/100g respectively. While oxalic acid and malonic acid distinguishes alone of German bee pollen the concentrations were 0.0048, 0.0433 mg/100g. maleic acid not detected in the Chinese bee pollen but found high significant concentration in German and Egyptian bee pollen were 5.9715 and 0.0035mg/100g respectively. Four organic acids in different concentrations were detected in Egyptian and Chinese bee pollen its shikmic acid 0.0029, 0.0390 mg/100g, succinic acid 0.0940, 0.2118 mg/100g respectively without significant differences and proponic acid 2.6693, 5.2253 mg/100g, lactic acid 0.0744, 0.7181 respectively with high significant differences. Butyric acid detected only in Chinese and German bee pollen with high significant differences the concentrations were 5.8148, 0.7090 mg/100g respectively.

Finally, numbers of organic acids present in samples bee pollens was eight in Egyptian, six in Chinese and five organic acids in German bee pollens; but found highly significant differences in the sum organic acids concentrations of Chinese, German and Egyptian bee pollen were 12.5542, 6.7326 and 3.1789 mg/100g., respectivily.

Table (1): Organic acids concentration (mg/100g) in three bee pollen types (Egyptian, Chinese and German).

Organic acids	Egyptian bee pollen	Chinese bee pollen	German bee pollen	P < 0.05
Oxalic	0.0000	0.0000	0.0048±0.0010	
Formic	0.0000	0.0000	0.0000	
Tartaric	0.3109±0.0861	0.0000	0.0000	
Shikmic	0.0029±0.0005	0.0390±0.0098	0.0000	0.0031**
Malonic	0.0000	0.0000	0.0431±0.0101	
Maleic	0.0035±0.0012^{b}	0.0000	5.9715±1.0023^{a}	0.0005***
Citric	0.0185±0.0012^{b}	0.5452±0.1001^{a}	0.0043±0.0011^{b}	0.0004***
Succinic	0.0940±0.0104^{a}	0.2118±0.0993^{a}	0.0000±0.0000	0.1157^{ns}
Proponic	2.6693±0.9993^{b}	5.2253±0.9995^{a}	0.0000±0.0000	0.0315*
Butryic	0.0000±0.0000	5.8148±0.9997^{a}	0.7090±0.0997^{b}	0.0009***
Lactic	0.0744±0.0126^{b}	0.7181±0.1002^{a}	0.0000	0.0004***
Adipic	0.0053±0.0011	0.0000	0.0000	
Sum	3.1789±1.1123	12.5542±2.3085	6.7326±1.1141	0.0037***

Values within the same row with different superscript letters are significantly different, P < 0.05

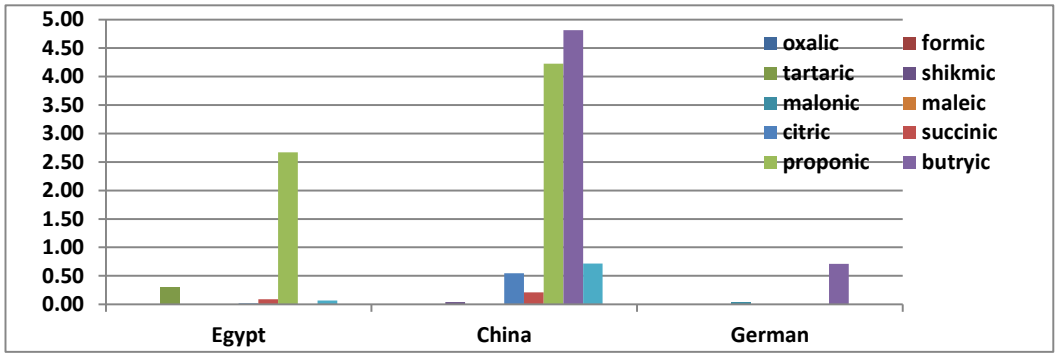
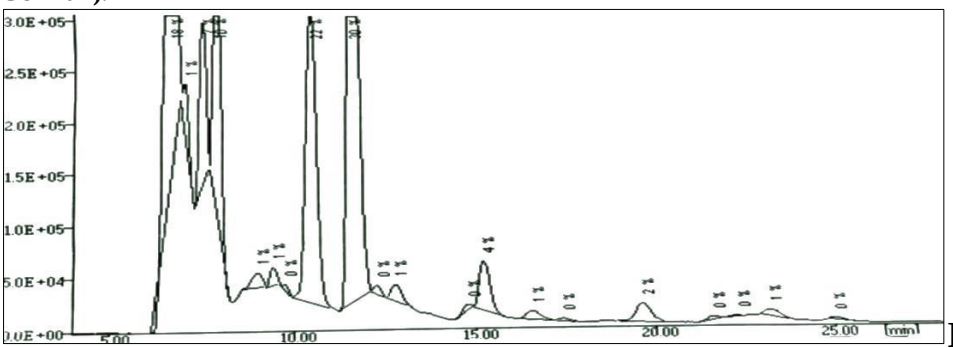
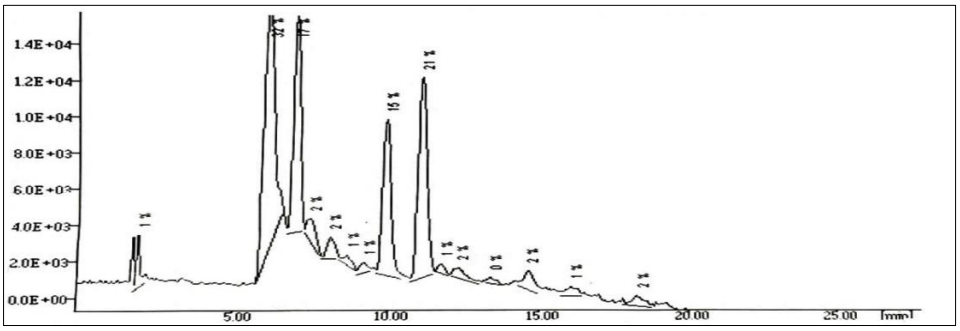


Figure (1): Organic acids concentration in three bee pollen types (Egyptian, Chinese and German).





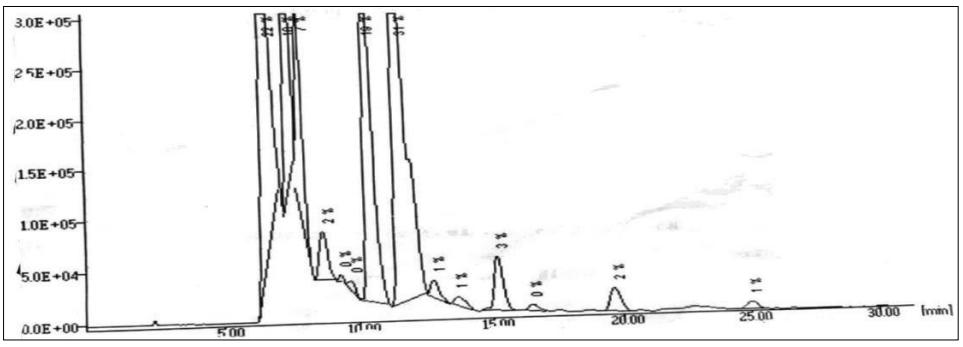


Figure (2): Peak intensity HPLC chromatogram of organic acids separation from Egyptian (E), German (G) and Chinese (C) bee pollens.

Many factors can contribute for the composition of bee pollen, such as the plant source, the climate and weather conditions, the characteristics of the soil, as well as the actions of the beekeeper (Pereira *et al*., 2006 and Shubharani *et al*., 2013). The organic acids composition varies depending upon the species, age of the plant and the vegetable tissue. In *E. plantagineum* bee pollen hydromethanolic extract eight organic acids were

identified:oxalic, aconitic, citric, pyruvic, malonic, shikimic, acetic and fumaric acids were 51.05, 6.89, 10.24, 890.27, 8152.87, 2.20, 1132.02, 0.48 mg/kg respectively (Moita *et al*., 2014). Malonic and acetic acids are the precursors for fatty acids biosynthesis. Furthermore, shikimic and malonic acids are precursors of phenolic compounds, a heterogeneous group from a metabolic point of view, presenting antioxidant, anti-inflammatory and anti-allergic activities (Avni *et al*., 2014). And according Tomas-Lorente *et al*. (1992) bee pollen mde up of natural flower pollen mixed with nectar and bee secretions, and was rich in sugars, proteins, lipids, vitamins and flavonoids (3–5% dry weight). Pollen may get to the nectar or honey by many ways (Jones and Bryant, 2014). Diversity of the physical and chemical properties of honey depends on the nectar and pollen of the original plant, colour, flavour, moisture and contents of proteins and sugars (Barth, 1989 and Pascoal *et al*., 2014). Honey contains naturally organic acids (Bogdanov *et al.* , 1997). According to honey origin it is a wide range, found the organic acid of honey (Crane, 1990). The acids from clover honey were butyric, acetic, formic, lactic succinic, pyroglutomic, maloic, citric and gluconic, while oxalic acid was tentatively identified when isolated by ion exchange adsorption (Stinson *et al*., 1960). Zhu *et al*., (2010) determined 5 organic acids (l- malic acid, medicine acid, succinic acid, citric acid and d-malic acid) in honey. El Mohandes (2011) she found that Formic acid and Malonic acid were present in all Egyptian floral honeys. By Nelson and Mottern (1931) found that citric acid was present in all floral honeys. Also, Suarez-Luque *et al*., (2002) identified malic, maleic, citiric, succinic and fumaric acids in honeys. (Nafea *et al*., 2013) oxalic, shikimic, butiric and benzoic acids were not detected in any of the eight tested honeys of Saudi Arabia formic and malonic acid were present in all tested honeys and suggested that percentages of organic acid in Saudian honeys their differing due to the differences in location and botanical origin of honey.

Types and quantity of organic acids in Egyptian, Chinese and German bee pollen were due to the differences in location and botanical origin of bee pollen. Finally, organic acids may be used to distinguish among the main types for Egyptian, Chinese and German bee pollen.

References

Almeida-Muradian, L.B.; Pamplona, L.C.; Coimbra, S. and Barth, O.M. (2005): Chemical composition and botanical evaluation of dried bee pollen pellets. J. Food Compos Anal., 18:105-111.

Anna, C. ; Lorenz , S.; Carlo, T. and Paolo, C. (1994): Solid-phase extraction and highperformance liquid chromatographic determination of organic acids in honey. Journal of chromatography a, 669:59-64.

Avni, D.; Hendriksma, H.P.; Dag, A.; Uni, Z. and Shafir, S. (2014): Nutritional aspects of honey bee-collected pollen and constraints on colony development in the eastern Mediterranean. Journal of Insect Physiology n.d.doi:10.1016/j.jinsphys. 07.001.

Barth, O.M. (1989) : O Pólen no mel brasileiro. Rio de Janeiro: Editora Luxor, p .151 (A, HO).

Bogdanov, S.; Martin, P. and Iullmann, C. (1997): Harmonised methods of the European honey commission. Apidologie. APIDGB5 (Extra issue), 1-59.

Campos, M.C.; Firgerio, J.; Lopes, S. and Bogdanov, S. (2010): What is the future of Bee-Pollen ?. Journal of Analytical Atomic Spectrometry, 2:131-144.

Campos, M.G.R.; Bogdanov, S.; de Almeida-Muradian, L.B.; Szczesna, T.; Mancebo, Y.; Frigerio, C., *et al*. (2008) : Pollen composition and standardization of analytical methods. Journal of Apicultural Research, 47:154-61.

Campos, M.G.R.; Bogdanov, S. ; de Almeida-Muradian, L.B. *et al*. (2008):Pollen composition and standardisation of analytical methods. Journal of Apicultural Research, 47, 2:154-61.

Coronel, B.B.; Grasso, D.; Pereira, S.C. and Fernández, G. (2004): Caracterización bromatológica del polen apícola argentino. Ciencia, DocenciayTecnología. http://www.redalyc.org/resumen.oa?id=14502906 .

Crane, E. (1990): Bees and Beekeeping. Science, Practice and World Resources. Heinemann; Oxford, UK. pp. 614.

El Mohandes, S.S. (2011) : Organic acids in different types of Egyptian honey. J. of Plant Protection and Pathology Mansoura University, Egypt. 2 (10) :865-872.

Feás, X.; Vázquez-Tato, M.P.; Estevinho, L.; Seijas, J.A. and Iglesias, A. (2012): Organic Bee Pollen: Botanical Origin, Nutritional Value, Bioactive Compounds, Antioxidant Activity and Microbiological Quality. Molecules, 17:8359–8377.

Funari, S.R.C.; Rocha, H.C.; Sforcin, J.M.; Filho, H.G.; Curi, P.R. and Gomes Dierckx, S.M.A. (2003): Composiçes bromatologica e mineral do polen coletado por abelhas africanizadas (*Apis mellifera* L.) em Botucatu, Estado de Sȁo Paulo. Arch Latinoam Prod Anim., 11:88–93.

Hernández, O.; Fraga, J.M.G.; Jiménez, A. I. and Jiménez, F. (2005): Characterization of honey from the Canary Islands: determination of the mineral content by atomic absorption spectrophotometry. Food Chem., 93(3):449-458.

Huang, D.; OU,B. and Prior, R.L. (2005): The chemistry behind antioxidant capacity assays. J. Agric Food. Chem., 53:1841-1856.

Inés, M.; José, H.F.; Jesús, S. and Teresa, S.M. (2006): Analytical Methods for the determination of organic acids in honey. Critical Reviews in Analytical Chemistry Publish date: 1st January.

Jones, G.D. and Bryant, V.M. (2014): Pollen Studies of East Texas Honey. Palynology, 38(2) :242-258.

Komosinska-Vassev, K.; Olczyk, P.; Kafmierczak, J.; Mencner, L. and Olczyk, K. (2015): Bee pollen: chemical composition and therapeutic application. Evid. Based Complement Alternat. Med., 1–6.

Kroyer, G. and Hegedus, N. (2001): Evaluation of bioactive properties of pollen extracts as functional dietary food supplement, innovative food. Science and Emerging Technologies, 2:171–174.

Mato, I.; Huidobro, J.F.; Simal-Lozano, J. and Sancho, M.T. (2003): Significance of nonaromatic organic acids in

honey. Journal of Food Protection, 66 (12):2371-2376.

Melo, I.L.P.; Freitas, A.S.; Barth, O.M. and Almeida-Muradian, L.B. (2009): Relação entre a composição nutricional e an origem floral de pólen apicola desidratado. Rev. Inst. Adolfo Lutz., 68:346-353.

Moita, E.; Sousa, C.; Paula, B.; Andrade Fernandes, F. ; Brígida, R.; Pinho, L. ; Silva, R. and Valentão, P. (2014) : Effects of *Echium plantagineum* L. bee pollen on basophil degranulation: Relationship with metabolic profile. Molecules, 19:10635-10649.

Nafea, E.A.; Zidan, E.W.; Asmaa, M. and Sehata, F. I.A.A. (2013): Determination of organic acids in Saudian bee honey types. Egypt. Acad. J. Biolog. Sci., 5 (2):117-120.

Nelson, E.K. and Mottern, H.H. (1931) : Some organic acids in honey. Industrial and engineering chemistry, 23 (3):335-336.

Pascoal, A.; Rodrigues, S.;Teixeira, A.; Feás, X. and Estevinho, L.M. (2014): Biological activities of commercial bee pollens: Antimicrobial, antimutagenic, antioxidant and antiinflammatory. Food and Chemical Toxicology, 63:233-239.

Pereira, F.M.; Freitas, B.M.; Vieira Neto, J.M.; Lopes, M.T.R. and Barbosa, A.L. and de Camargo, R.C.R. (2006): Desenvolvimento de colˆonias de abelhas com diferentes alimentos prot´eicos. Pesquisa Agropecu´aria Brasileira, 41(1):1–7.

Shubharani, R; Roopa, P. and Sivaram, V. (2013): Pollen morphology of selected bee forage plants. Global Journal of Bioscience and Biotechnology, 2 (1):82–90.

Silveira, T.A. (2012): Caracterização sazonal do pólen apícola quanto à origem botânica, aspectos físico-químicos e elementos traços como bioindicadora de poluição ambiental http://www.teses.usp.br/teses/disponiveis/11/11146/tde-20032012-090016/pt-br.php. Accessed 26 Feb 2017.

Stinson, E.E.; Subers, M.H.; Petty, J. and White, J.W. (1960): The Composition of Honey. V. Separation and Identification of the organic acids. Archives of Biochemistry and Biophysics, 1:6-12.

Suarez-Luque, S.; Mato, I.; Huidobro, J.F.; Simal-Lozano, J. and Sancho, M.T. (2002) : Rapid determination of minority organic acids in honey by highperformance liquid chromatograndaphy. J. chromatography, 955 (2):207-214.

Tomas-Lorente, F.; Garcia-Grau, M.M.; Nieto, J.L. and Tomas-Barberan, F.A. (1992): Flavonoids from Cistus-Ladanifer bee Pollen. Phytochemistry, 31:2027-29.

Zhu, X.L.; Ye, F.; Yang, J.; Xiau, X.; Wen, H. and Liu, R. (2010): Determination of Organic acids in honey by solid Phase extraction-high performance liquid chromatography. Sepu., 28 (10) : 945-949.