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Influence of Microwave- and Ultrasound-Assisted Extraction on Bioactive Compounds from Pollen

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Abstract

The development of functional foods is increasingly essential in our society due to the prevention of many diseases. Among the functional foods are those that contain antioxidant activity. Bee pollen is very rich in phenolic and non-phenolic compounds with high antioxidant capacity. In this work, the antioxidant properties of bee pollen extracts obtained by Microwave-Assisted Extraction (MAE) and Ultrasound-Assisted Extraction (UAE) were studied. Marked differences were found between both methods. The content of total polyphenols was higher in the extracts obtained by MAE, and the antioxidant activity was higher in those obtained by UAE.

Keywords: functional foods, antioxidant capacity, polyphenols, bee-pollen

Introduction

Phenolic compounds are secondary metabolites of plants that possess at least one aromatic ring with one or more hydroxyl groups. They can be divided into phenolic acids and polyphenols depending on the number of phenolic units [1]. Flavonoids are classified as flavanols, flavones, catechins, flavanones, anthocyanins and isoflavones [2].

These plant metabolites have a higher antioxidant activity compared to other natural antioxidants. These give biological protection to DNA and lipids against the damages caused by ROS, retard the oxidation of low-density lipoproteins (LDL), and have been associated with the reduction of chronic diseases generated by oxidative stress [1]. In bee pollen, these natural antioxidants are the main pigments. The variation in the content of phenolic compounds is due to geographical origin, botanical, exposure to ultraviolet light and drying treatments [3].

In addition to the nutritional properties of bee pollen, antioxidant properties have been studied *in vivo* and *in vitro*. These components have antimicrobial, antimutagenic, antiestrogenic, chemoprotective, anti-inflammatory activities and for the prevention of numerous diseases, mainly those associated with oxidative stress [4-6]. All the therapeutic effects and protective effects of bee pollen have been associated with the content of antioxidant compounds, mainly phenolic compounds. In this work were studied two methods to extract bee pollen: Microwave-Assisted Extraction (MAE) and Ultrasound-Assisted Extraction (UAE).

Materials and Methods

Microwave Assisted Extraction (MAE)

A microwave of 1350 W of power and 60 Hz of modified frequency was used. The solvent was food grade ethanol, and the extractions were carried out as follows: 1 g of pollen with 10 and 50 mL of ethanol, 135, 405 and 945 W of electrical power for 6, 12 and 24 s. In each treatment, the temperature was measured. The extracts were filtered and stored in freezing (-20 °C ± 3 °C) until analysis.

Ultrasound-assisted extraction (UAE)

For this extraction, 1 g of pollen was taken with 10 mL of food grade ethanol. Ultrasound was performed in an ELMA 30H ELMA bath of 35 kHz frequency and 250 W for 15 min, centrifuged and the supernatant was filtered. The yield of both extractions was calculated.

Total Phenolic Content (TPC)

According to Slinkard and Singleton [7], 500 μL of the extract was mixed with

22 mL of distilled water and 2 mL of 10% sodium carbonate. After a 2 min rest, 500 µL of the Folin Ciocalteu reagent (Panreac®) was added and left to react for 2 h in the dark to be measured or at 765 nm. The calibration curve was made with different concentrations of gallic acid (Sigma Aldrich®). The results were expressed as mg GAE / g.

Antioxidant activity – ABTS

According to Re *et al*. [8] 10 µL of the extract and 1 mL of the solution of 2,2'-azino-bis-(3-ethyl benzothiazoline-6-sulfonate ammonium) ($ABTS^{\bullet+}$) (Sigma Aldrich®) were mixed. After 6 min of reaction in the dark, the change in absorbance was measured at 734 nm and compared to the unreacted solution. A Thermo Scientific Genesys 10S visible UV spectrophotometer (USA) was used for the determination. The calibration curve was performed with Trolox (Sigma Aldrich®). The results were expressed as Trolox mmol / g pollen.

Antioxidant activity - FRAP

For this determination, 20 µL of the extract, 450 µL of the ferric complex FRAP reagent (TPTZ) (Sigma Aldrich) and 735 µL of distilled water were mixed. After 30 min of reaction in the dark, the absorbance was measured at 593 nm. The results were expressed as mmol Trolox / g pollen [9].

Statistical analysis

The completely random factorial design was used to evaluate the MAE. The data were analysed using an ANOVA and a Pearson correlation test. The Statgraphics XVII for Windows software was used.

Results and Discussion

The evaluation of extraction conditions by MAE was studied to efficiently obtain an extract with a higher concentration of antioxidant compounds. The analysis at 135 W with 10 ml of solvent reached a temperature of 30°C. This temperature is not enough to affect the extraction of polyphenols. Then this power was discarded for higher volumes of solvent.

Table 1 shows the MAE treatments applied to 1 g of pollen and the extraction temperature measured in each one. The temperature increased with the power and the time of radiation, with the increase of the power, the molecular vibration is faster and therefore, it is a factor that directly influences the temperature.

Table 1. Extraction conditions of bee pollen antioxidants by MAE

Power (W)	Time (s)	Solvent (mL)	Temperature (°C)
135	6	10	32
405	6	10	50
945	6	10	53
135	12	10	32
405	12	10	60
945	12	10	62
405	12	50	45
945	12	50	50
405	24	50	45
945	24	50	65

Yield Extraction

Table 2 shows the performance results in each treatment, the effect of the amount of solvent is observed. With 50 mL, a higher yield is obtained than with 10 mL. The factors evaluated (potency and time) show a directly proportional relationship with the weight of the extract, comparable results were reported by Pérez and Luque [10].

Table 2. Mean values and standard deviation of ethanolic extracts yields obtained at different MAE conditions and compared to EAU

Method	Power (W)	Time (s)	Solute/solvent (g/mL)	Yield (%)
MAE	135	6	1/10	26.3 ± 0.2^{a}
MAE	405	6	1/10	35 ± 3^{bc}
MAE	945	6	1/10	39 ± 2.5^{cd}
MAE	135	12	1/10	31 ± 2^{ab}
MAE	405	12	1/10	42.2 ± 1.2^{cde}
MAE	945	12	1/10	45 ± 2^{def}
MAE	405	12	1/50	49.5 ± 0.2^{efg}
MAE	945	12	1/50	50 ± 4^{gh}
MAE	405	24	1/50	56 ± 5^{h}
MAE	945	24	1/50	54 ± 4^{h}
EAU	---	---	1/10	41.16 ± 3.18^{cde}

Different superscript letters indicate significant difference ($p < 0.05$)

The lowest yield was obtained with 135 W since the power is probably not enough to generate a cell break. The yield values obtained by the UAE was like that obtained with the MAE at a solute/solvent ratio 1/10 g/mL.

Total Phenolic Content (TPC) and antioxidant activity

Table 3 shows the values of TPC and antioxidant activity of the extracts obtained by MAE and UAE. Among the MAE conditions, significant differences were found ($p < 0.05$) regarding interactions between the factors (power, time and solvent volume).

The TPC values obtained by UAE was lower than MAE at high potencies (405 and 945 W), but the antioxidant activity in the extracts obtained by the first one was higher than those obtained by EAM.

Table 3. Total Phenolic Content and antioxidant activity of pollen extracts obtained by MAE and compared to UAE

Method	**Power (W)**	**Time (s)**	**Solute/solvent (g/mL)**	**TPC* (mg GAE/g)**	**ABTS* (mmol Trolox/g)**
MAE	135	6	1/10	31.1 ± 0.5^{b}	0.0582 ± 0.0012^{a}
MAE	405	6	1/10	36.5 ± 0.5^{d}	0.0629 ± 0.0012^{a}
MAE	945	6	1/10	35 ± 1^{d}	0.063 ± 0.002^{a}
MAE	135	12	1/10	27 ± 1^{a}	0.057 ± 0.002^{a}
MAE	405	12	1/10	41 ± 1^{e}	0.070 ± 0.003^{b}
MAE	945	12	1/10	40 ± 1^{e}	0.069 ± 0.002^{b}
MAE	405	12	1/50	45 ± 1^{f}	0.089 ± 0.002^{c}
MAE	945	12	1/50	49 ± 1^{gh}	0.089 ± 0.002^{c}
MAE	405	24	1/50	48 ± 1^{g}	0.092 ± 0.003^{c}
MAE	945	24	1/50	51 ± 1^{h}	0.071 ± 0.003^{b}
UAE	---	---	1/10	33 ± 1 c	0.098 ± 0.002^{d}

Different superscript letters indicate significant difference ($p < 0.05$)

The TPC values were between 27.3 ± 0.7 and 51.04 ± 0.55 mg GAE / g. In the MAE process, the power was the most important variables since it directly influenced the temperature and the breakdown of the cell walls in the matrix. The higher the power, the greater the TPC. This phenomenon was more evident when the volume of the solvent increased due to a greater drag of compounds, as was reported by Shofinita and Langrish [11] in a study on orange peels. In the same way, time increased the TPC because of the higher radiation. However, this behaviour was not observed at 135 W; probably this power did not achieve a significant breakdown of the cell wall.

Table 3 also shows the values of antioxidant activity of the extracts measured by ABTS. With a solute: solvent ratio of 1: 10 g/mL, the antioxidant activity increases proportionally with the exposure time and power, except for the treatments at 135 W. Whereas with 1: 50 g/mL, exposure time and power do not increase this parameter.

When low potencies were applied to extracts 1:10 g / mL (solute: solvent), the antioxidant activity was very low. The same happened at a power of 945 W, may be due to the steric hindrance of the ABTS radical generated by an excess of -OH groups [12].

Table 4 shows the results of antioxidant activity measured by FRAP (ferric reducing antioxidant power); this had a different behaviour than ABTS. With a relation of 1: 10 g / mL (solute/solvent) the increase in potency promotes a decrease in the antioxidant activity. Also, at 1: 50 g/mL (solute/solvent) the time and the amount of solvent do not affect.

Table 4. Mean values and standard deviation of antioxidant activity (FRAP) of pollen extracts obtained by MAE and compared to UAE

Method	**Power (W)**	**Time (s)**	**Solute/solvent (g/mL)**	**FRAP**
MAE	135	6	1/10	0.053 ± 0.002^{b}
MAE	405	6	1/10	0.0497 ± 0.0009^{b}
MAE	945	6	1/10	0.0489 ± 0.0009^{c}
MAE	135	12	1/10	0.036 ± 0.002^{d}
MAE	405	12	1/10	0.0450 ± 0.0010^{c}
MAE	945	12	1/10	0.0448 ± 0.0007^{c}
MAE	405	12	1/50	0.047 ± 0.002^{c}
MAE	945	12	1/50	0.052 ± 0.003^{bc}
MAE	405	24	1/50	0.048 ± 0.003^{bc}
MAE	945	24	1/50	0.055 ± 0.004^{b}
UAE	---	---	1/10	0.094 ± 0.005^{a}

Different superscript letters indicate significant difference ($p < 0.05$)

High antioxidant activity was expected in pollen extracts due to its high TFC. However, the results were reversed when comparing the two technologies. This is possible because pollen contains phenolic compounds, phenolic acids, phenylpropanoids and flavanols whose antioxidant power can vary. The identification of phenolic profiles in pollen has been made, finding that it is not the amount of phenols that defines its antioxidant capacity but its nature [13].

Conclusion

Antioxidant compounds from bee pollen were extracted by MAE and UAE. In the first case, the increase in the temperature of the system was observed with the increase of the power and the time of radiation, which favoured the extraction of phenolic compounds evidenced by the high TPC values. It might be because the increase in temperature promotes the breakdown of cell walls allowing the solvent to carry more compounds. In general, the TPC values were higher in the extracts

obtained by MAE and the antioxidant activity was higher in those obtained by UAE. It is possible that MAE degrades some non-phenolic compounds that have a high antioxidant capacity.

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