

Enzymatic Pretreatment of Vegetable Materials to Increase the Extraction Yield of Bioactive Compounds

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In this paper, the influence of enzymatic pretreatment on the microwave assisted extraction (MAE) of polyphenols from artichoke leaves is described. Prior to enzymatic pretreatment, the influence of different parameters (extraction time, stirring rate, and extraction temperature) on the extraction process was studied. The total phenolic content (TPC) increases with the stirring rate. To avoid degradation of polyphenols, the extraction time and temperature should not be too high. The antioxidant capacity is in concordance with the TPC results. The enzymatic pretreatment, for the best extraction conditions, enhances the concentration of polyphenols compared with the extracts obtained without pretreatment.

Keywords: enzymatic pretreatment, polyphenols, artichoke, microwave assisted extraction

Artichoke (*Cynara scolymus* L.) is originated from the southern Mediterranean parts of North Africa and is widely grown in Europe or America [1].

Globe artichoke is grown for its immature inflorescence which is consumed as vegetable, due to its healthpromoting compounds and sensory properties. The residues, namely leaf and stem, represent 80-85% of the above ground biomass [2, 3]. The bioactive compounds of artichoke are mainly polyphenols, as well as inulin [4], fibres and minerals that provide pharmaceutical and nutritional properties. Caffeic acid derivatives are the main phenolic compounds in artichoke heads and leaves. Besides them artichoke contains other phenolic acids (ferulic and coumaric acids), apigenin, luteolin, etc. [5].

Classical extraction methods of active principles are Soxhlet extraction [6], maceration [7] and heat reflux extraction [8]. These methods require expensive organic solvents with relatively high consumption, long extraction times, and high temperatures that could lead to degradation of valuable constituents. More convenient techniques such as pressurized fluid extraction (PFE), ultrasound assisted extraction (UAE), pulsed electric field (PEF), supercritical fluid extraction (SFE) and microwave assisted extraction (MAE) were recent presented [9-11].

Due to the combination of extraction technique with microwave heating, MAE is a promising technology for the extraction of bioactive compounds from vegetal material. The advantages of MAE are a shorter extraction time, high extraction efficiency and selectivity, good control of heating process, better extraction yield and low energyconsumption [11-14].

The efficiency of the extraction process is influenced by the interactions between bioactive molecules and sample matrix and by the diffusion of solvent through the plant matrix. Thus, the structure of the material is an important factor influencing the extraction efficiency and any means to modify the structure to enhance the extraction is attractive. Considering the latter, a quite new strategy to enhance the extraction of bioactive compounds from plant is the enzymatic pretreatment [15]. The purpose of the enzyme is to break or soften the cell walls. Thus, the bioactive compounds have an easier access to the solvent [16,17]. Enzymes such as glucuronidases, cellulases, hemi-

cellulases, pectinases, glucanases, amylases, and tannases have already been used to break the carbohydrate linkages and to decompose the cell wall structure [18].

The purpose of this work was to study the influence of enzymatic pretreatment and different parameters (extraction time, stirring rate, and extraction temperature) on the microwave assisted extraction of polyphenols from artichoke leaves.

Experimental part

Materials

Artichoke leaves (*Cynara cardunculus* L.) were harvested in the summer of 2014 at Hofigal S.A. in Furculesti. The fresh leaves were dried in an air flow-heating oven at 60°C to a constant weight and stored at 4-5°C until they were used for the extraction of phenolic compounds. Folin Ciocalteu reagent (Merck), ethanol and sodium carbonate were analytical purity grade. Reagents for antioxidant capacity, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), ABTS (2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), and potassium persulfate were purchased from Sigma-Aldrich. The enzymes (Celluclean 400 L, Flavourzyme 1000 L, Glucanex, and Ultrazyme 100T) were kindly donated by Novozymes A/S (Denmark).

Extraction procedure

The microwave assisted extraction (MAE) of polyphenolic compounds was carried out in a microwave applicator (Biotage Initiator). The extractions were carried out in triplicate using a 20:1 ratio of solvent to plant. The extraction solvent was a mixture of 50% ethanol in water. The experiments were performed at different temperatures (40, 60 and 80°C) and stirring rates (300, 600 and 900 rpm). Individual experiments were performed considering the following extraction times: 5, 7.5, 10, 15 min. After the extraction, the mixture was centrifuged at 3000 rpm for 10 min at room temperature and the supernatant was collected and fresh analysed every time.

Enzymatic pretreatment procedure

Artichoke leaves were mixed with the pretreatment solution (a buffer solution containing 0.1 M citric acid and

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0.2 M $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ mixed in a 1/1.06 ratio of citric acid to sodium phosphate dehydrate) to keep the pH value at 5 during enzymatic pretreatment. Enzymes (Celluclean 400 L, Flavourzyme 1000 L, Glucanex and Ultrazyme 100T) were added on the pretreatment mixture and stirred for 30 min at 40°C. The concentration of enzymes related to substrate was 5% for all types of enzyme. After pretreatment, the mixture was submitted to MAE of polyphenols. Control samples, only with buffer solution, were also performed.

Analysis

Determination of total phenolic content

Total phenolic content of extracts was determined colorimetrically using the Folin-Ciocalteu method according to our previous work [19].

TEAC assay

The antioxidant capacity was performed using 2,2'-azino bis-3-ethylbenzthiazoline-sulphonic acid (ABTS) radical scavenging assay according to our previous work [19].

Results and discussion

Influence of different parameters on the MAE of polyphenols from artichoke leaves

In order to establish the best extraction conditions, the influence of different parameters (extraction time, stirring rate, and extraction temperature) on the MAE of polyphenols from artichoke leaves were studied prior to enzymatic pretreatment.

Temperature is an important factor which may influence the solid-liquid extraction process. Increasing temperature lead to increase in solubility of polyphenols and the mass transfer coefficient between plant matrix and solvent is enhanced. The extraction of bioactive compounds is influenced also by the extraction time. A high temperature and a long extraction time determine rapid ruptures of the cell wall and, further, facile release of phenolic compounds. However, a long extraction time and a high temperature may lead to the oxidation of polyphenols or may alter the conformation of extracted compounds.

The influence of the extraction time on MAE of polyphenols from artichoke leaves at different temperatures is shown in figures 1 and 2.

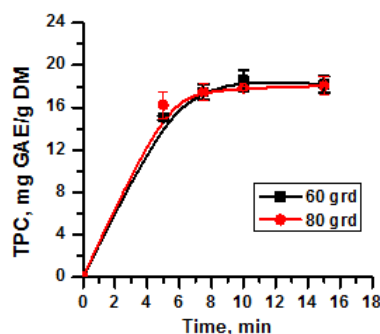


Fig.1. Influence of the extraction time on the TPC for different temperatures and a stirring rate of 900 rpm.

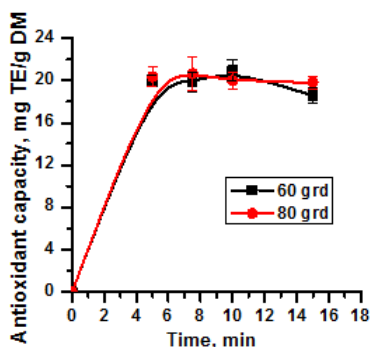


Fig.2. Influence of the extraction time on the antioxidant capacity for different temperatures and a stirring rate of 900 rpm.

As shown in figure 1, the TPC of artichoke extracts increases with increasing the extraction time. A maximum TPC is achieved after 10 min. The antioxidant capacity values have a similar behavior with the TPC; the best results being obtained after 10 min (fig. 2). Moreover, the values of the TPC and antioxidant capacity at 60 and 80°C are almost the same. This behavior confirms the degradation of polyphenols at high temperatures and long extraction times.

As shown previous, polyphenols are thermolabile compounds. Thus, for the optimum extraction time (10 min), experiments in milder condition (temperature of 40°C) were performed. The results are shown in figures 3 and 4.

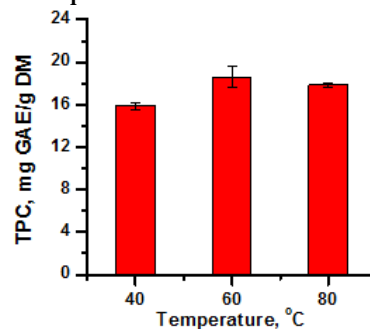


Fig.3. Influence of the extraction temperature on the TPC at 10 min extraction time.

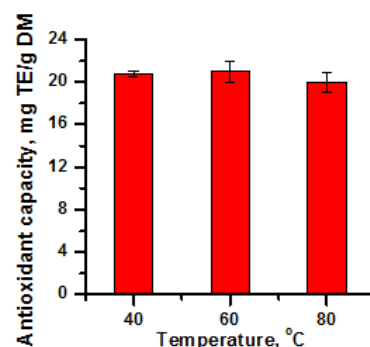


Fig.4. Influence of the extraction temperature on the antioxidant capacity at 10 min extraction time.

It can be notice that at 40°C, although the antioxidant capacity of the extracts is almost the same for all temperatures (fig. 4), the TPC values are lower than those obtained at 60 or 80°C. This can be explained by the fact that a temperature of 40°C is not high enough to break the cell wall and to release the compounds of interest. Moreover, due to the existence of some complexes between glycosides and polyphenols, a higher temperature is required in order to break those bonds. Thus, the extraction of polyphenols from artichoke leaves is more efficient at a medium value of temperature (60°C).

A good contact between the solvent and vegetal material may enhance the efficiency of extraction. Thus, the stirring of the extraction medium has to be very good to allow the diffusion of the solvent between plant tissues. The influence of the stirring rate on the extraction process is presented in figures 5 and 6.

As shown in figure 5 and 6, the extraction of polyphenols is more efficient when the stirring rate is higher. The best

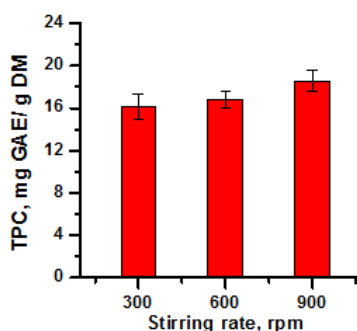


Fig.5. Influence of the stirring rate on the TPC at 10 min and 60°C.

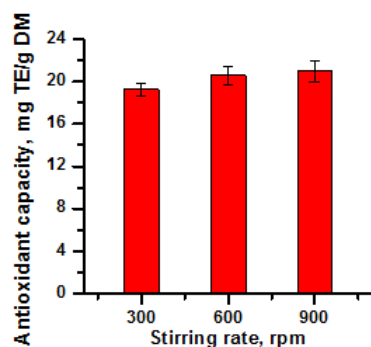


Fig.6. Influence of the stirring rate on the antioxidant capacity at 10 min and 60°C.

results are obtained at 900 rpm for both TPC and antioxidant capacity analyses.

Influence of enzymatic pretreatment on the MAE of polyphenols from artichoke leaves.

As shown previous, the best extraction condition of polyphenols from artichoke leaves are a temperature of 60°C, a stirring rate of 900 rpm and an extraction time of 10 min. Thus, the influence of the enzymatic pretreatment on the extraction process was studied for these conditions.

The influence of enzymatic pretreatment is shown in figure 7 and 8.

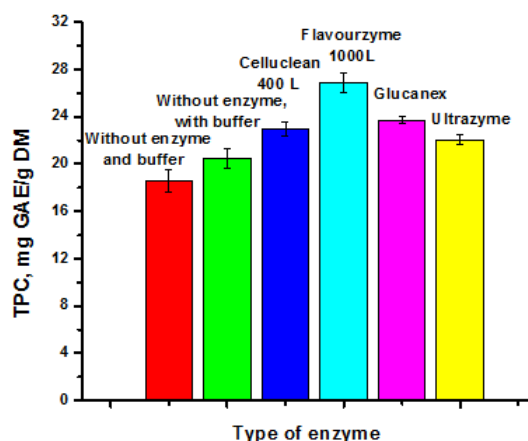


Fig.7. Influence of enzymatic pretreatment on the TPC (stirring rate of 900 rpm, temperature of 60°C, and extraction time of 10 min)

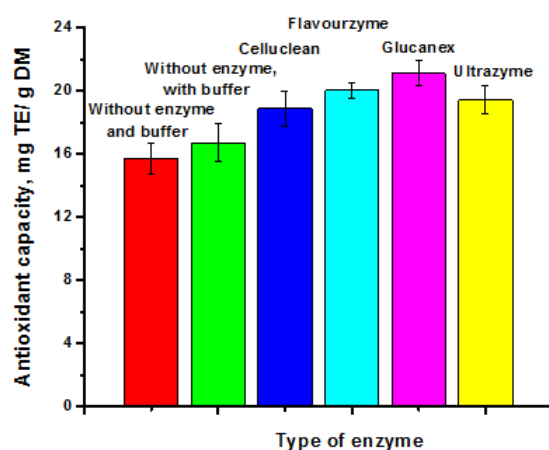


Fig.8. Influence of enzymatic pretreatment on the antioxidant capacity (stirring rate of 900 rpm, temperature of 60°C, and extraction time of 10 min)

It can be noticed that the TPC values for all enzymes are higher (approximately 20-45%) than those obtained without pretreatment or than those performed only with the buffer solution. The best results are obtained for Flavourzyme. Also, the TPC is higher for the extracts with buffer solution compared with classical ones. This is due

to the presence of citric acid in the buffer solution which can enhance the extraction by denaturing the cell membrane and implicit easily releasing of polyphenols. The antioxidant capacity of the extracts has almost the same behavior with the TPC values. Compared with polyphenols concentration, the best results on the antioxidant capacity are obtained for Glucanex (fig. 8). This difference can be explain by the fact that Flavourzyme is a specific protease enzyme type. Thus, the TPC analysis by Folin-Ciocalteu method can be influence by the proteins found in artichoke leaves.

The enzymes, which lead to the best results (Flavourzyme and Glucanex), were also tested in a mixture of 50% each one. Figures 9 and 10 show that, a mixture of these two enzymes, has a synergetic effect on the TPC. As shown is figure 10, the antioxidant capacity is in concordance with the TPC values. The synergetic effect is also noticed in the case of antioxidant capacity of the extracts.

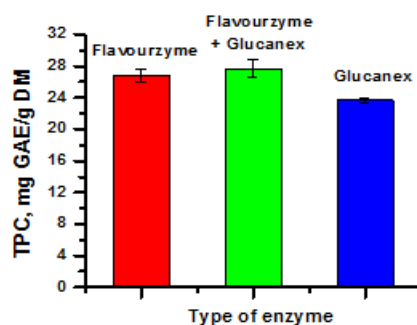


Fig.9. Influence of enzymatic pretreatment for a mixture of enzymes on the TPC (stirring rate of 900 rpm, temperature of 60°C, and extraction time of 10 min)

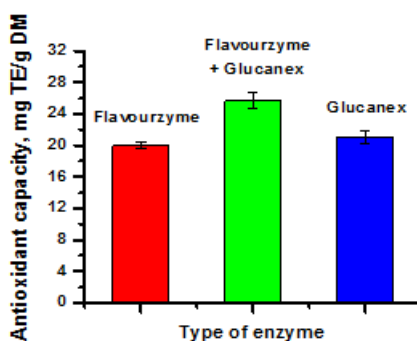


Fig.10. Influence of enzymatic pretreatment for a mixture of enzymes on the antioxidant capacity (stirring rate of 900 rpm, temperature of 60°C, and extraction time of 10 min)

Conclusions

In this work the influence of enzymatic pretreatment and different parameters (extraction temperature, stirring rate, and extraction time) on the polyphenols extraction from artichoke leaves was studied. The best results for MAE of polyphenols from artichoke leaves were obtained for a temperature of 60°C, a stirring rate of 900 rpm, and an extraction time of 10 min. The enzymatic pretreatment (for the extraction conditions mentioned above) lead to higher TPC and antioxidant capacity values (approximately 20-45%) compared with those obtained without pretreatment or only with the buffer solution. Using a mixture of enzymes (50% of Flavourzyme and Glucanex) had a synergetic effect on the TPC and antioxidant capacity of the extracts. Thus, the enzymatic pretreatment was a good strategy to enhance the yield of polyphenols from artichoke leaves.

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