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MICROWAVE AND ULTRASOUND-ASSISTED EXTRACTION OF MONOMERIC ANTHOCYANIN PIGMENT CONTENT FROM PLANT RESIDUALS

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Abstract

Our investigation focused on the intensification of monomeric anthocyanin pigment content (MAC) extraction from raspberry residuals by using microwave (MW) and ultrasound (US) radiation with different solvents, and to monitor the efficacy of these processes by the assessment of the dielectric behavior of the extracts. All the experiments were carried out with using 5 grams of the raspberry raw material mixed with 100 cm^3 of three different solvents – distilled water, hydrochloric acid, and the mixture of HCl and citric acid – separately. For the intensification processes, microwave and ultrasonic radiation were used in three different total irradiated energy input values: 30 kJ, 45 kJ and 60 kJ. The MAC content was analyzed according to the AOAC official method for total monomeric anthocyanin pigment content, while the dielectric behavior of the extracts was analyzed with laboratory dielectric assessment kit. Our results revealed that using MW irradiation as an intensifying process for extraction can be effectively used in the lower irradiated energy range (30 kJ), and the higher level of power (500W) is more efficient, regardless of the applied solvent. If, however, ultrasonic radiation is being used, usually the higher the energy intensity, the higher the yield of MAC can be observed. The assessment of dielectric behavior proved that a strong correlation (r=0.98-0.99) can be found between the dielectric constant (ε') and the MAC concentration of the extracts, regardless of the intensifying method and solvent used.

Introduction

The significance of anthocyanin pigments to food quality lies in their role in enhancing color and visual appeal, and growing attention is being paid to the anthocyanin levels in food products and nutraceuticals due to their potential health advantages. The concentration of these pigments serves as a valuable metric for the quality assurance and procurement guidelines of fruit juices, nutraceuticals, and natural color additives. Anthocyanins account for the red, purple, and blue shades found in most fruits, vegetables, and grains [1]. Six prevalent types of anthocyanidins exist—namely, pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin. These structures can differ based on glycosidic modifications at the 3 and 5 positions, and further diversity arises from the acylation of sugar groups with organic acids. The extraction process commonly employs solvents like water, methanol, ethanol, often acidified with hydrochloric or citric acid to stabilize the anthocyanins [2]. The extracted solution is then subjected to various purification and concentration methods, such as solid-phase extraction or liquid chromatography. The resulting anthocyanin extracts are widely used in food, pharmaceuticals, and cosmetics for their antioxidant properties and vibrant colors. During the first stage of extraction, using solvents without any intensification method usually grants low or negligible yield [3] and thus, it is usually combined with various thermal and/or mechanical treatments. Among these, the microwave or ultrasound-assisted extraction (MAE or UAE) methods have already shown promising results, compared to conventional hot-water extraction (HWE) [4]. MAE is primarily based on the dielectric heating mechanism caused by the absorption of microwaves, which induce rapid, selective heating inside the bulk material, depending on its

dielectric properties. Since plant cells contain high amount of bounded and free water, they can absorb and store electromagnetic energy easily, and due to the polar nature of water molecules, this absorbed electromagnetic energy can be easily dissipated, i.e., can be converted into heat. Therefore, when exposed to microwaves, large amount of heat is generated inside the plant cells, which comes with the increase in pressure as well. When the pressure inside the plant cell exceeds a critical value, the cell wall eventually disrupts [5], and the different substances – anthocyanins, for example - found in the intracellular space or in the cell wall are released. UAE is based on a vastly different principle, namely the ultrasound-induced cavitation. Cavitation is a physical phenomenon that occurs when a substance suddenly changes from a liquid phase to a gas phase due to pressure drop. The explanation is that if the velocity of a liquid in a particular direction increases suddenly, its pressure decreases according to Bernoulli's law. The resulting bubble, if it is placed along the flow line at a point where the pressure exceeds the saturation vapor pressure for the temperature at that point, will suddenly burst, and the liquid surfaces in sudden contact with each other will produce a shock wave. High-intensity ultrasonic irradiation induces high and low frequency cycles in the absorbing liquid. During the high-frequency cycle, small vacuum bubbles or cavities are created, which grow throughout the cycle, and then these bubbles collapse during the low-frequency cycle. When these cavitation bubbles collapse, a momentary (micro- or nanosecond-long), but extremely high increment of temperature and pressure occur. These, and the shockwaves generated by the collapsing of cavities expose the material to mechanical stress and shear, which cause the degradation and disruption of plant cell walls [6].

Applying measurements to identify the dielectric behavior of certain compounds or media have been widely investigated as a promising tool for process monitoring. Dielectric assessment is rapid, sensitive, non-destructive and doesn't necessitate the use of strong chemicals, therefore it can be a great alternative to conventional analytic methods. The fundamental mechanisms behind the dielectric behavior are to be found in electrodynamic properties, namely that an electromagnetic field with any strength E causes a so-called dielectric shift D in real materials, which can be understood as a kind of phase shift δ. In case E is not excessively high, the proportionality between E and D remains, and as the frequency increases the emerging δ phase difference becomes more and more prominent. The frequency at which the phase shift becomes noticeable depends – among others - on the temperature and the chemical, physical properties of the medium, and can be written as:

$$\varepsilon^* = \frac{D}{E} = |\varepsilon| e^{-j\delta} \tag{1}$$

Using the Euler-identity and separating the real and imaginary parts we get:

$$\varepsilon^*(f) = \left|\frac{D}{E}\right| (cos\delta - jsin\delta) = \varepsilon'(f) - j\varepsilon''(f) \tag{2}$$

Based upon the previous equation, the material's response can be characterized by the so-called dielectric properties, e.g. dielectric constant (ε‘), dielectric loss factor (ε‘‘) or loss tangent (tanδ). ε‘, the real part of the complex permittivity shows the electric (or electromagnetic) energy absorbing and storing capability of materials, ε‘‘ – the complex part of the permittivity – shows how much of the absorbed and/or stored energy is converted into heat or other types of energy, while tanδ incorporates the latter two ($tan\delta = \frac{f\varepsilon'' + \sigma}{\varepsilon'}$) and denotes quantitatively the dissipation of the electrical energy due to different physical processes such as electrical conduction, dielectric relaxation, dielectric resonance and loss from non-linear processes.

Experimental

The raw material for extraction was raspberry expeller / press cake, a residue originated from the juice-pressing of raspberry fruits. The average water content *W%* was 68.42%, while the dry matter content (*DMC*) 1.579 g. For the extraction process, three different solvents were applied (separately): pure distilled water (pH=6.8), hydrochloric acid (pH=2) and the mixture of HCl and citric acid (pH=2). Using HCl can help disrupt the plant cell wall by depolymerizing it, however the oxidative environment above a certain extent can be harmful for the target components as well. Citric acid can help stabilize the target molecules and making them more resilient to oxidation and/or thermal impacts. For the intensifying operation, microwave (P= 250W and 500W) and ultrasound (200W power) irradiation were being used with different operational times, achieving 30 kJ, 45 kJ and 60 kJ total irradiated energy, respectively. After the extraction step, the total anthocyanin content was analyzed by the standard AOAC differential pH spectrophotometric method. Simultaneously, the dielectric properties of the extracts were determined as well, using a DAK 3.5 (SPEAG GmBh, *Germany*) open-ended dielectric sensor connected to a vector network analyzer (ZVL-3, Rhode&Schwarz GmBh, *Switzerland*) with a 50-ohm coaxial power supply line.

Results and discussion

In the first part of our investigations, we wanted to see how the different solvents and utilized intensifying operations affect the yield of MAC in the extracts. Figure 1 shows the results obtained for the pure water extraction during MAE and UAE with three different total energy outputs, incorporating the dielectric constant of the extracts at f=700 MHz.

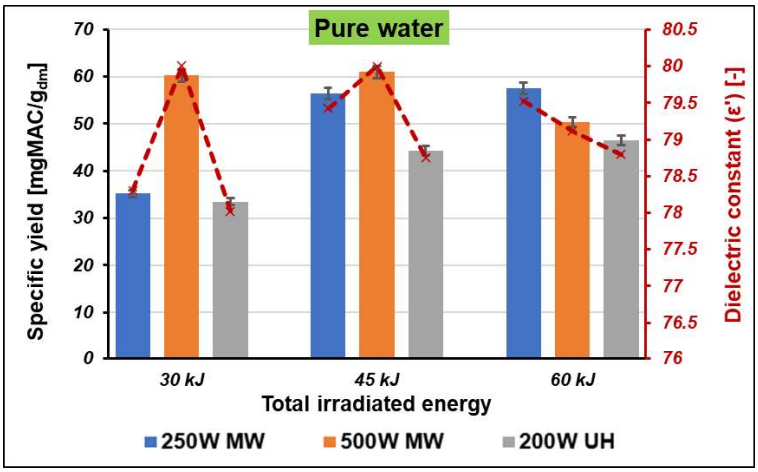

Figure 1. *Specific yield of MAC during the pure water extraction with the different intensifying operations, along with the values of ε'(700 MHz).*

Results indicate that a prominent difference can be found between the different utilized power levels of MAE, especially at low (30 kJ) irradiated energy outputs. Using 500W MW radiation for extraction at 30 kJ energy level resulted in approx. 60 mgMAC/g_{dm} (g_{dm} = grams of dry matter), which is almost two times higher than that of 250W MW or US. The reason behind might be that using higher power means increased electromagnetic (MW) field strength, which ultimately results in higher and faster bulk heating, compared to the 250W power level of MW. Since the ultrasound-assisted extraction is not based on heating mechanisms, the results suggest that a 30 kJ total irradiated energy was not enough in case of ultrasonication to cause enough mechanical stress to disrupt the plant cells. Increasing the total energy from 30 kJ to 60 kJ causes no significant change in terms of 500W MW treatment, whereas the yield obtained with 250W MW is higher. When the total irradiated energy exceeded 60 kJ, the specific yield

suddenly decreased in the case of 500W MW, which can be explained by the fact that MACs are usually sensitive to heat, and the extent of heat energy might have induced thermal degradation. This phenomenon can not be observed when the level of MW power was set to 250W, indicating that the profile of temperature increase was steadier, but slower. Using ultrasonication does not increase the temperature of the material considerably, and therefore thermal decay can not be observed there, however the higher the energy output, the higher the disintegration of the cell walls, providing increased yield of MAC. Considering all the different approaches, it can be stated that from an energetic point of view, using 30 kJ total energy with 500W MW irradiation has the greatest efficiency in terms of MAC yield per unit dry matter. Observing the results of the dielectric constant, it can be seen that a similar tendency can be found, i.e., if the yield of MAC was higher, the value of ε' was also higher, indicating a relationship between these two parameters.

Among the acidic solvents, the MAC yield was slightly higher when HCl + citric acid was used regardless of the applied intensifying operation or total irradiated energy. Based on the results, it can be also observed that the addition of citric acid could indeed preserve the target substance to some extent, especially at higher energy outputs (Figure 2). The highest efficiency, like in the case of pure water extraction, was achieved when using 500W MW radiation at 30 kJ total energy.

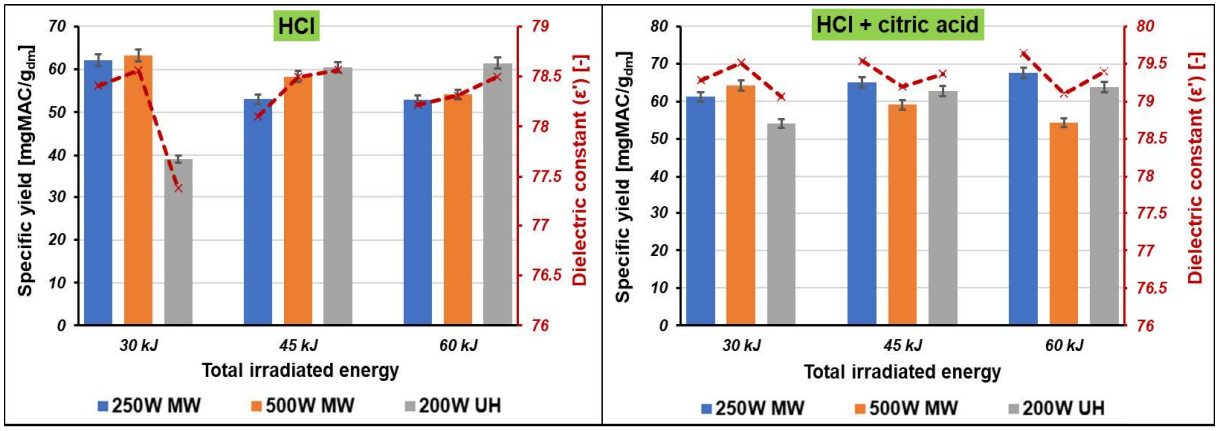

Figure 2. *Specific yield of MAC during the acidic (HCl & HCl + citric acid) with the different intensifying operations, along with the values of ε'(700 MHz).*

Like in the case of water solvent extraction, the trend of the dielectric constant rigorously followed the MAC yield, regardless of the applied treatment, and/or energy output value. The differences in the 'absolute' value of ε' is mostly due to the application of different solvents: the dielectric properties of HCl and citric acid are significantly different than that of pure water. In order to see the strength of this connection, we constructed linear functions between the concentrations of anthocyanin, and the corresponding dielectric constant (Figure 3). These results suggest a strong linear correlation between the specific MAC yield and the dielectric constant of the extracts (with coefficient of correlation *r* being 0.98-0.99), regardless of the applied solvent, or intensifying method. This ultimately also means that measuring the dielectric behavior of these samples are "non-selective", i.e., it only depends on the exact yield itself, and not on the method or process it was achieved with, making it a potential universal tool for monitoring extraction processes.

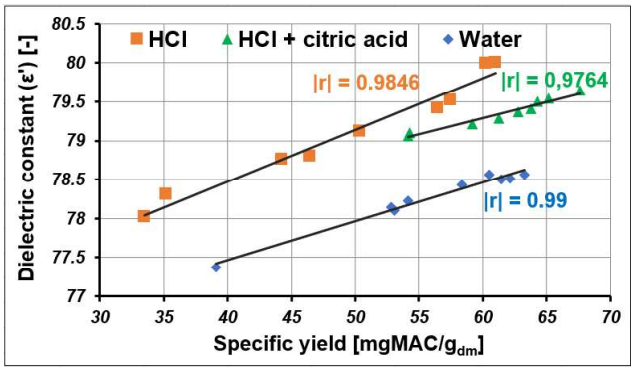

Figure 3. *Correlation between the 700MHz dielectric constant and MAC yield*

Conclusions

Our study focused on the microwave and ultrasound-assisted extraction of anthocyanin from raspberry by using different solvents, and comparing the results for yield with the dielectric behavior of the extracts. The experiments showed that regardless of the applied solvent, using relatively low (30 kJ) total energy output and 500W microwave irradiation resulted in the highest MAC yield, and when the energy level increased, the total yield decreased in case of the MAE – potentially due to thermal degradation. In case of the ultrasound process, the higher the total energy, the higher the MAC yield turned out to be, implying that this method mostly governs mechanical effects in the disruption of plant cell walls. Independent from the solvent or intensifying operation, the 700MHz dielectric constant showed a strong linear correlation with the MAC yield of the extracts, making it a promising, precise monitoring tool.

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