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Improving the Extraction of Juice And Bioactive Compounds from Blueberry Fruits and Their By-Products cy Application of Moderate Electric Field (MEF)

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The influence of Moderate Electric Field (MEF) pre-treatment of blueberry fruits on the yield and quality of the expressed juice as well as the subsequent extraction yield of bioactive compounds from berry by-products (press cake left after MEF-assisted pressing), was investigated. Thermal MEF pre-treatments were performed under different combinations of electric field strengths (E=18-55 V.cm^{-1}) and final heating temperature (T_f=25-80 °C) before applying a pressure of 1.32 bar for 5 min. For the sake of comparison, the effect of conventional heating (CH) (T_f=25-80 °C), was also investigated. Results showed that the Z_p values increased with increasing the field strength and heating temperature. However, at the same final heating temperature, MEF treatment achieved higher Z_p values (up to 0.66) than CH (up to 0.45). Coherently, thermal MEF pre-treatment (36.4 V.cm^{-1}, 60 °C) significantly increased the juice yield, which also showed higher TPC, TAC, and AA, as compared with either untreated or CH-treated samples. Moreover, compared to the untreated and CH-treated samples, higher amounts of total phenolics, total anthocyanins and antioxidant activity were detected in the press cake extracts. These results were explained by the combined non-thermal and thermal effect of MEF on the cell disintegration and extraction efficiency.

1. Introduction

Mechanical pressing and conventional extraction with solvents are widely used in the food industry for the recovery of juices and bioactive compounds from fruits and vegetables and their processing by-products (Bobinaite et al., 2015). However, although these methods offer a simple approach, they typically suffer from several drawbacks mainly related to the presence of the envelope (membrane and wall) surrounding the plant cell, which acts as a barrier that greatly limits mass transfer phenomena of juices, solvents and targets intracellular compounds during extraction processes (Gavahian et al., 2018). As a result, these processes are typically a slow, laborious and highly energy-consuming step in the production of fruit juices (Wang and Sastry, 2002), or require a large amount of solvent and energy and long extraction time to recover a substantial amount of valuable compounds from plant tissues (Bobinaite et al., 2015). For these reasons, different methods, based on mechanical pre-treatments of comminution of the raw material, as well as a subsequent enzymatic and/or thermal treatment, have been proposed for the improvement of extraction efficiency and yield of juice and bioactive compounds (Wang and Sastry, 2002). In this frame, electrotechnologies, such as pulsed electric fields (PEF) and moderate electric field (MEF) have been drawing attention as alternative methods for cell disintegration before pressing or extraction processes. It is known, that the presence of an external electric field causes a reversible or irreversible increase of cell membrane permeabilization of plant tissues by electroporation, leading to several benefits, such as enhanced product yield and quality as well as reduced extraction time and costs (Gavahian et al., 2018). However, unlike PEF technology, which mainly rely on the non-thermal (electroporation) effect, MEF may also combine effects of electrical and thermal (ohmic heating) phenomena to increase the membrane permeability as well as induce cell wall disruption, thus leading to further process intensification (Gavahian et al., 2018). Specifically, the MEF process involves the application (from seconds to minutes) of electric fields, typically under 100 V.cm^{-1}

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(considerably lower than PEF), applied in a form of a sinusoidal or bipolar square wave in the range of Hz up to tens of kHz, with or without ohmic heating effects, to biomaterials placed in direct contact with two electrodes of a treatment chamber (Sensoy and Sastry 2004). Interestingly, when the ohmic heating effect is involved, heat is generated rapidly and volumetrically, and the product does not experience a large temperature gradient within itself. The quick temperature increase may provide further damages to the cell structure, and release the target compounds to the surrounding area (Gavahian et al., 2018). Several research works have highlighted the benefits of the MEF technique in combination with the pressing or extraction process to enhance the quantity and quality of juice and valuable compounds from a variety of food and food processing by-products (Gavahian et al., 2018). Moreover, it has been shown that that the relative importance of each (non-thermal and thermal) mechanism, as well as the extent of cell damages induced by MEF treatment and consequent release of intracellular material, are correlated with the raw material specifications and process conditions, such as temperature, electric field strength, and frequency (Gavahian et al., 2018). In general, higher electric fields can boost the extraction yield mainly by increasing heating rate, or increasing cells' electropermeabilization in a thermal and a low (<50 °C) temperature MEF process, respectively (Lebovka et al., 2005). It was also widely reported that the additional non-thermal effects induced by the electric field in MEF are more effective at low frequencies (Sensoy & Sastry, 2004). To the best of our knowledge, only in the study of Pereira et al. (2016) the existence of non-thermal effects has been observed during thermal MEF treatment at high frequency (25 kHz) and proved effective on the extraction of phytochemicals from plant tissues. Additionally, the use of high frequencies (above 15-20 kHz) effectively eliminates or minimize the occurrence of electrochemical reactions that otherwise could lead to undesired effects such as electrode corrosion and consequent product contamination (Pataro et al., 2014).

Moreover, as per the literature survey, no studies have yet been published on the effect of MEF-assisted pressing and extraction of juice and bioactive compounds from blueberries and their by-products (skins and seeds). The blueberry is an important source of many traditional nutrients and contains a variety of phenolic compounds with high antioxidant capacity, especially anthocyanins, which are believed to be the key bioactive compounds responsible for the many reported health benefits of blueberries and also other fruits (Pataro et al., 2017a). This has led, in recent years, to a remarkable increase in market demand and consumers interest for blueberry processed products (i.e., juice) and phenolic derivatives (extracts), which show a wide range of industrial applications as natural colourants or nutraceuticals (Pataro et al., 2017a).

Therefore, the objective of this work was to assess the potential of thermal MEF pre-treatments at high-frequency to damage cell structure of blueberry tissue before pressing, with the aim of enhancing the yield and quality of the expressed juice as well as the subsequent extraction yield of bioactive compounds from blueberry by-products (press cake left after PEF-assisted pressing). For the sake of comparison, the effect of conventional thermal pretreatment was also evaluated.

2. Materials and methods

2.1 Raw materials and chemicals

The blueberries (*Vaccinium corymbosum*) were purchased from a local market in Italy and stored at 4 °C until use. Gallic acid, cyanidin 3-O-rutinoside, anhydrous sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH), concentrated hydrochloric acid, and Folin & Ciocalteu's phenol reagent were supplied from Sigma-Aldrich (Steinheim, Germany). Ethanol (70% grade), sodium dihydrogen phosphate and potassium chloride were purchased from Carlo Erba reagents (Cornaredo, Italy).

2.2 Experimental setup

MEF treatments were performed by using a laboratory-scale batch unit previously described by Pataro et al. (2014). Briefly, the system consisted of a static MEF treatment device made of an open cylindrical polycarbonate tube (3 cm in diameter, 5.5 cm in length) limited by two disk-shaped stainless steel (type AISI 316L) electrodes isolated at each edge with Teflon pressure caps. The electrodes were connected to a 15 kW power supply unit (model GR1520, Micropi Elettronica, Saviano, Italy) able to provide electric current in the form of bipolar square waves at 25 kHz with adjustable amplitude (0-1500 V). An integrated data-logger was employed to record continuously and simultaneously voltage across and current through the samples as measured by special voltage and current transducers, as well as product temperature measured by a T-type Teflon coated thermocouple (1 mm in diameter) placed at the geometrical center of the treatment device.

2.3 Experimental procedure

For each experiment, 40 g of blueberries were first partially crashed by hand, and then heated from 25 °C up to a final temperature (T_f) of 40, 50, 60, 70 or 80 °C by either MEF or conventional heating (CH) treatments. For the MEF process, the samples were loaded into the treatment device and heated up to the desired final temperature by applying a constant gradient voltages of 18.2 $V.cm^{-1}$ (MEF1), 36.4 $V.cm^{-1}$ (MEF2), or 54,5 $V.cm^{-1}$ (MEF3). For the conventional heating, the samples were packed into flexible pouches, made of a 115 µm thin polymer/aluminum/polymer film (OPP30-A19-LDPE70), and then immersed into a water heating bath set at 90 °C and heated up to the desired final temperature. The temperature rise of the samples was recorded by introducing a type-K thermocouple into the center of the packages. After either MEF or CH treatment, the samples were immediately collected and placed in an ice bath to be cooled down to ambient temperature (25 °C), before being subjected to impedance measurement and extraction process.

2.4.1. Impedance measurements

Cell disintegration index (Z_P) was determined via measurements of electrical complex impedance in frequency sweep (10^2 – 10^6 Hz), and used to quantify the degree of cell membrane permeabilization of berry tissues induced by either MEF or CH treatments, according to the method described by Bobinaitė et al. (2015). For each treatment condition investigated, the Z_P value, ranging from 0 (for intact tissue) to 1 (for fully permeabilized tissue), was calculated as previously described by Bobinaitė et al. (2015).

2.4.2. Juice expression and extraction procedure of phenolic compounds from blueberry press cake

Untreated (control), CH and MEF-treated samples were pressed at a constant pressure of 1.32 bar for 5 min using the mechanical press and protocol previously described by Bobinaite et al. (2015). The liquid expressed during each experiment was collected in a plastic tube and then centrifuged at 5000 rpm for 10 min at 4 °C (PK121R model, ALC International, Cologno Monzese, Milan, Italy) in order to obtain a clear juice. The latter was weighed to evaluate the extraction yield, expressed as g of juice per 100 g of fresh weight (fw) berries, and then stored at 4 °C until analyzed. The remaining processing waste (press cake left after pressing) were subjected to solid-liquid extraction with acidified aqueous ethanol (50 % ethanol; 0.5 %HCl, v/v) at a solvent to cake ratio of 7:1 ($mL.g^{-1}$). The extraction process was carried out for 24 hours at ambient temperature with constant shaking at 150 rpm. The final extracts, obtained after filtration with a filter paper (Watman no.1), were stored at 4 °C until analyzed.

2.4 Analysis of the juice and press cake extracts

The total phenolic content (TPC) of blueberry juice and press cake extract were determined using the Folin-Ciocalteu method previously described by Bobinaitė et al. (2015). Gallic acid was used as the standard for the calibration curve, and results were expressed in mg of gallic acid equivalents (GAE) per 1 L of juice or per 1 kg of fw berry press cake. The total anthocyanin content (TAC) of the samples was determined by using the pH differential method described by Bobinaitė et al. (2015). Blueberry juice or press-cake extracts were added to buffer solutions (sodium chloride pH 1.0, and sodium acetate pH 4.5). The absorbance of the solutions was measured at 520 and 700 nm. The concentration of anthocyanins was expressed in mg of cyanidin-3-glucoside equivalents per L of juice or per kg of berry press cake. The antioxidant activity of juice and press cake extracts was determined by DPPH scavenging assay according to the method reported by Pataro et al. (2017a) and expressed as percent DPPH inhibition.

2.5 Statistical analysis

All the experiments and analyses were performed in triplicate and the results were reported as means ± standard deviations. Differences among mean values were analysed by one-way variance (ANOVA), performed with SPSS 20 (SPSS IBM., Chicago, USA) statistical package, and Tukey test was performed to determine statistically significantly differences ($p < 0.05$).

3. Results and discussion

3.1 Effect of CH and MEF treatment on the cell disintegration index of blueberry tissues

Figure 1a depicts the influence of the final heating temperature on the Z_P of CH (0 $V.cm^{-1}$) and MEF-treated blueberry tissues at three different field strengths. Results show that, for each value of the field strength applied, the Z_P increased significantly with increasing the temperature up to approximately a temperature value ranged between 60-70 °C. Further increments of temperature above this range caused only marginal effects, being a saturation level reached. The higher is the field strength applied, the higher the saturation level reached. Moreover, regardless of the final heating temperature, the presence of field strength yielded a greater extent of the cell damage than conventional heating, which was already observed in the low temperature range (40-50 °C), especially when a field strength higher than 18.2 $V.cm^{-1}$ was applied. Specifically, it is likely that, in the low temperature range (< 50 °C), the non-thermal damage is dominant and grows with the heating temperature, probably because cell membranes are more susceptible to an electric field at higher temperatures (Sensoy & Sastry 2004). This is clear evidence of the existence of a non-thermal effect despite the high frequency applied, which is consistent with findings of previous scientists (Pereira et al., 2016) On the other hand, for heating temperature above 50 °C, thermal damages likely starts (Sensoy & Sastry, 2004) leading to thermal denaturation and cell expansion due to the possible rupture of cell walls (in the temperature range 60-75 °C), loss of intercellular adhesion, and texture changes (Gavahian et al., 2018). Additionally, the higher heating rate detected in a thermal MEF process than CH, at least for field strength greater than 18.2 V.cm-1 (data not shown), likely induced further thermal damage to the structures of berry tissues causing sudden cell expansion and disruption (Lebovka et al., 2005). Similar effects were previously observed by Lebovka et al. (2005) when studying the electrically induced damage to apple and potato tissues during thermal MEF treatment. Therefore, it seems that there is a synergistic effect between field strength and temperature during thermal MEF treatment, which could explain the greater degree of cell damage detected during thermal MEF as compared with CH at the same heating temperature (Figure 1a). These results, however, appear in contrast with previous works where the non-thermal effect of MEF was not observed in several studies (Sensoy & Sastry, 2004), especially whenever the thermal effects overshadowed MEF effects. Interestingly, from the results of Figure 1a, it can be also inferred that the final heating temperature required to reach given permeabilization decreases with increasing the field strength applied. To better clarify the relationship between field strength and temperature, the results of Figure 1a it was also evaluated the so-called characteristic electro-thermal damage temperature value (TE) required for Zp to attain, at each field strength applied, one-half of its maximal value, i.e. 0.5. Results plotted in Figure 1b, show that the T_E value exponentially decreases with the increase of the field strength, and then tend to level off to a relatively constant value of about 60 °C when a field strength of about 36.4 V/cm was applied. Based on these results, the use of higher field strength should be preferred to obtain the desired degree of permeabilization with the minimum thermal damage. However, the estimation of the optimal value of the electric field intensity must take into account that beyond a certain value of E no appreciable reduction in the T_E value can be achieved. Similarly, it has been previously shown that there is an electric field strength threshold above which an increase in the field strength could not affect the degree of damage of chive leaves tissue (Gavahian et al., 2018).

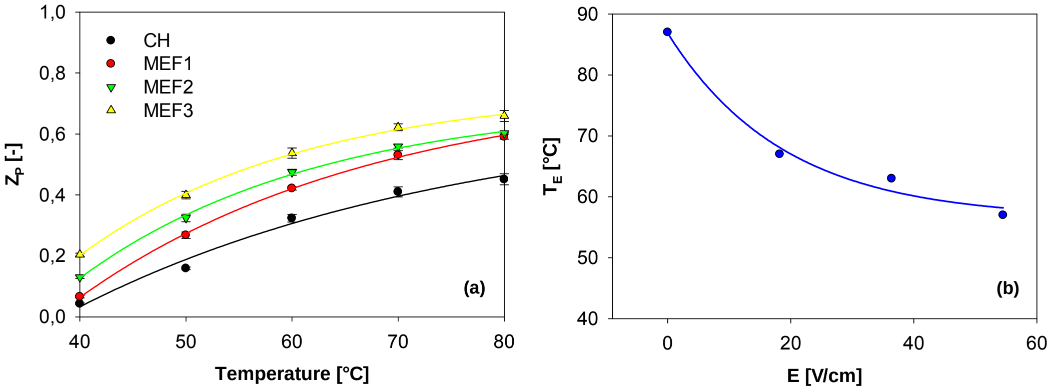

Figure 1: (a) Cell disintegration index (Z_P) of blueberries as a function of the final heating temperature and after CH (0 V/cm), and MEF treatment at different electric field strengths. (b) Characteristic thermal damage temperature T_E of berry tissues versus electric field strength applied.

According to these results, further investigations of MEF pre-treatment on the extractability of juice and phenolic compounds from berry fruits were carried out at either constant field strength (36.4 V.cm^{-1}) for different heating temperatures, or at a fixed heating temperature (60 °C) and for different field strengths.

3.2 Effect of CH and MEF pretreatment on the extraction yield and quality of berry juice and press cake extracts

Table 1 report the yield, TPC, TAC, and AA of blueberry juice obtained after pressing of berry fruits preheated up to the final heating temperature of 60 °C by applying either CH (0 V.cm-1) or MEF treatment at different field strengths (18.2, 36.4 and 54.5 V.cm^{-1}). The juice yield for the raw, untreated (0 kV/cm) berries was 8.0 %. The pre-heating of berries up to 60 °C by either CH or MEF treatments significantly ($p \leq 0.05$) increased (by 19-55 %) the juice yield as compared with the control sample. It is worth noting that, in comparison to juice obtained from CH pre-heated berries, MEF pre-treatments at 18.2, 36.4, and 54.5 V.cm^{-1} increased the juice yield by 6.3, 27.4, and 30.5 %, respectively. However, among the pre-treated samples a significant ($p<0.05$) difference was observed only when the field strength was increased from 18.2 to 36.4 V.cm^{-1}.

Table 1: Yield, TPC, TAC, AA of blueberry juice obtained after pressing of untreated (Control), and pre-heated (T_f=60 °C) berries by either CH or MEF under different field strength. Different letters in the same line indicate significant differences between the samples ($p \leq 0.05$)

Property	Control (25°C-0V.cm^{-1})	CH (0 V.cm^{-1})	MEF1 (18.2 V.cm^{-1})	MEF2 (36.4 V.cm^{-1})	MEF3 (54.5 V.cm^{-1})
Yield (g/100g fw)	8.0±0.4a	9.5±0.4b	10.1±0.5b	12.1±0.4c	12.4±0.2c
TPC (mgGAE/L)	2028±15a	2149±37b	2178±33b	2352±58c	2240±32bc
TAC (mg/L)	824±52a	1455±172b	1683±151c	2277±158d	2099±169d
AA (% inactivation)	19.9±1.3a	32.7±3.2b	38.3±2.3c	45.1±2.1d	42.8±2.3d

These results confirm the existence of a non-thermal effect induced above a certain electric field applied, which cause higher damage to the berry tissues leading to a higher juice yield. The content of total phenolics and anthocyanins in the control juice was 2028 mg.L^{-1} and 824 mg.L^{-1}, respectively. The application of CH or thermal MEF pre-treatment significantly ($p \leq 0.05$) increased both TPC and TAC values, which rose approximately 5.6 %, 7.4 %, 16.0 %, and 10.4 % for TPC, and 76.6 %, 104.2 %, 176.3 %, and 154.7 % for TAC, when the berry fruits were pre-treated at 0, 18.2, 36.4, and 54.5 V.cm^{-1}, respectively. However, it is worth noting that significant ($p<0.05$) differences among pre-treated samples were observed only when the field strength was increased from 18.2 to 36.4 V.cm^{-1} for TPC, and in the range between 0 and 36.4 V.cm^{-1} for TAC. Further increase of field strength above 36.4 V.cm^{-1} led to a slight decrease in both TPC and TAC. This is somehow consistent with the findings of Pereira et al. (2016), who observed that the increase in extraction yield of anthocyanins from MEF-treated coloured potato at 90 °C was reversed at electric fields strength above 20 V.cm^{-1}. Interestingly, the results of Table 1 also revealed a selective release of anthocyanins than other phenolic compounds due to MEF application to berry fruits, especially when a field strength of 36.4 V.cm^{-1} was applied. The antioxidant activity of the berry juice is also reported in Table 1. Compared to the juice obtained from the untreated berries, both CH (0 V/cm) and MEF pre-treatments at 18.2, 36.4, and 55.4 V/cm increased the AA of the juices by 64.3, 92.5, 126.6, and 115.1 %, respectively. Moreover, it is worth noting that these results were well correlated with those of TPC (R^2 = 0.894) and, especially, of TAC (R^2 = 0.978), confirming that anthocyanins contained in blueberry fruits predominantly contribute to the antioxidant activity of juices (Pataro et al., 2017a). The effect of the final heating temperature of berry fruits by either CH or MEF (34.6 Vcm^{-1}) pretreatment on the yield, TPC, TAC, and AA of expressed juice is reported in Table 2. Results show that pre-heating of berry fruits, especially in presence of an electric field, may significantly increase the extraction yields of juice, as well as enhance the release of total phenolic and total anthocyanin compounds with high antioxidant activity, as compared with the control sample.

Table 2: Yield, TPC, TAC, and AA of blueberry juice obtained after pressing of untreated (Control, 25 °C), CH, and MEF (E=36.4 V.cm^{-1})-heated berries at different final heating temperature. Different letters in the same line indicates significant differences between the samples ($p \leq 0.05$)

Property	Pre-treatment	Final heating temperature (°C)					
		25	40	50	60	70	80
Yield	CH	8.0±0.4a	8.4±0.5ab	9.3±0.2b	9.5±0.4b	9.8±0.3b	9.5±0.6b
(g/100g fw)	MEF2	8.0±0.4a	10.1±0.5b	11.3±0.4c	12.1±0.4d	11.4±0.5c	11.3±0.4c
TP	CH	2028±15a	2057±22a	2096±11a	2149±37b	2131±20b	2105±25a
(mgGAE/L)	MEF2	2028±15a	2189±71ab	2244±44b	2352±58c	2321±58c	2309±42c
TAC	CH	824±52a	1015±151b	1198±31b	1455±172c	1419±90c	1372±74c
(mg/L)	MEF2	824±52a	1683±151b	2037±114c	2277±168d	2181±157d	2099±169c
AA	CH	19.9±1.3a	26.3±2.2b	29.7±1.6bc	32.7±3.2c	31.3±1.5bc	30.9±1.6bc
(% inactivation)	MEF2	19.9±1.3a	35.1±3.1b	38.4±0.6c	45.1±2.1d	40.7±2.3c	39.6±1.2c

A similar effect was previously noted by other scientists, who observed an increase in the yield of juice (Wang and Sastry, 2002), TPC and TAC (Pereira et al., 2016) with increasing the heating temperature. However, regardless of the pre-heating method, the highest yields were detected at a final heating temperature of 60 °C, which is consistent with the results of Figure 1. Above this temperature, it is likely that the excessive tissue softening due to electroporation and/or thermal effect, caused compaction and closing of the capillaries in press cake leading to unfavourable de-juicing conditions (Pataro et al., 2017b). Additionally, it has been also reported that anthocyanin degradation is time and temperature dependent and that these compounds are especially sensitive to temperatures above 70 °C (Sarkis et al., 2013). From these results, it can be concluded that the permeabilization effect induced by MEF treatment at 36.4 V/cm at 60 °C, resulted in the most favourable de-juicing properties of berry fruits. According to these results, the extractability of residual phenolic compounds from press cake left after pressing of untreated, CH and MEF (36.4 V/cm)-pre-treated berries at 60°C was also evaluated, and results are shown in Table 3 in terms of TPC, TAC and AA detected in the extracts. TPC and TAC of untreated extracts were 1564 and 511 mg/kg, respectively, which confirmed that a substantial amount of valuable compounds was still retained inside the berry skins and seeds. However, as compared with the untreated sample, the total amount of these compounds increased by 9.1 and 32.0 % for TPC, and by 62.8 and 132.5 % for TAC, when they were extracted from press cakes obtained after CH or MEF-assisted pressing of fresh fruits, respectively. In comparison to the control extract, CH and MEF treatments significantly ($p \leq 0.05$) increased the antioxidant activity of the press-cake extracts by 33.2, and 107.2 %, respectively. This is consistent with the higher concentration of phenolic compounds, and especially anthocyanins, detected in the press cake extracts of MEF pre-treated samples, as compared with those achieved from untreated and CH pre-treated fruits.

Table 3: Concentrations (in mg/kg press cake) of TPC and TAC, and AA of press cake extracts obtained after pressing of untreated (Control), and pre-treated (T_f=60 °C) berries by either CH (0 V/cm) or MEF (36.4 V/cm). Different letters in the same column indicates significant differences between the samples ($p \leq 0.05$)

Sample	TPC (mgGAE/kg)	TAC (mg/kg)	AA (% inactivation)
Control	1564±80a	511±46a	20.8±2.7a
CH	1706±98b	832±37b	27.7±3.5b
MEF2	2065±51c	1188±106c	43.1±4.1c

4. Conclusions

The results of this study have demonstrated that the combined non-thermal and thermal effects induced by high frequency MEF pre-treatment at relatively low field strength (E=36.4 $V.cm^{-1}$) and moderate temperature (T_f=60 °C) appeared to be sufficient for the improvement of a juice yield as well as for the intensification of the phenolic and anthocyanin compounds extraction from both berry fruits and their by-products (press cakes).

Finally, these promising results confirm the potential of MEF coupled with mild heating for enhancing the efficiency of classical unit operations of the food industry, such as mechanical pressing and extraction, as well as to add value to food products and by-products.

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