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The Impact of Thermal and Electrical Pre-Treatments and Anti-Browning Solution on the Chlorogenic and Dicaffeoylquinic Acids Extraction Yield from Endive Roots

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Highlights

- PEF and MW pre-treatments lead to increased extracted yield and CQAs extraction
- Smaller cossette size and higher working pressure improve extracted yield
- ABS mitigate enzymatic oxidative effects during pressing and pretreatment stages
- Combined pre-treatments lead to 65% (diCQAs) and 80% (5-CQA) extraction yield

Abstract: Forced endive roots (FER) contain beneficial antioxidant compounds such as chlorogenic acid (5-CQA) and dicaffeoylquinic acids (diCQAs). This study compared the extraction yields of 5-CQA and diCQAs using a biomass pressing method with various pre-treatments, including pulsed electric field (PEF) and microwave (MW), against the solid-liquid extraction method. The results indicated that the MW pre-treatment achieved the highest yields, extracting 28 ± 2% of 5-CQA and 13 ± 1% of diCQAs, surpassing the solid-liquid method. Furthermore, the oxidative degradation of CQAs is studied and it appears that this reaction is enhanced by PEF pretreatment. An anti-browning solution (ABS) is successfully tested to reduce this oxidation and protects CQAs. An extraction process utilizing MW and PEF pre-treatments combined with an ABS solution achieved yields of 65 ± 1% for diCQAs and 80 ± 5% for 5-CQA, significantly outperforming the solid-liquid extraction method.

Industrial relevance: FER from agricultural processing are generated in large quantities and typically discarded due to their uselessness. Due to low CQAs content in FER, conventional extraction is energy and solvent consumption intensive. Innovative pre-treatment techniques such as MW and PEF combined with pressing can provide ways to drastically reduce extraction costs. This study provides an easy to scale up extraction method to produce highly concentrated juice of CQAs, valuable compounds for the cosmetic or the food industry.

Keywords: caffeoylquinic acids; pulsed electrical field; microwave; pressing; oxidation; forced endive roots

1. Introduction

The Belgian endive (Cichorium intybus L.) is widely cultivated across Europe, with France producing approximately 160,000 tons annually. Notably, 95% of this production occurs in the Hauts-de-France region. The cultivation of Belgian endives, also referred to as chicon, involves a two-stage process. In the first stage, the roots are grown in fields and harvested. During the second stage, these

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roots undergo a forcing process in complete darkness for 21 days at temperatures between 16 and 20 °C, resulting in the formation of chicons.

After the chicons are harvested, the forced endive roots (FER), a by-product of this process, are left behind. FER is typically used in low-value applications, such as livestock feed supplements, composting, or as field amendments. Since one root is produced for each chicon, FER constitutes the primary by-product of Belgian endive cultivation.

Despite their current uses, FER holds significant potential due to their richness in bioactive compounds, particularly phenolic compounds known for their health-promoting properties. Specifically, FER are abundant in chlorogenic acid (5-CQA) and dicaffeoylquinic acids (diCQAs) [1–4] , which are valuable for their antioxidant and anti-inflammatory benefits. The extraction of CQAs from FER represents an essential step in the valorization of this by-product, offering opportunities to enhance its economic and functional value within the framework of sustainable agriculture and circular economy practices.

Traditionally, (CQAs) have been extracted using solid–liquid extraction methods, which involve dissolving the desired compounds in a solvent and separating them from the solid plant material. This technique, though effective, has certain limitations in terms of efficiency and sustainability, particularly with respect to solvent use and energy consumption [5,6]. Over the years, various studies have focused on optimizing the extraction of CQAs using conventional methods, such as adjusting solvent type, temperature, and extraction time [7,8]. Additionally, innovative techniques like microwave-assisted extraction [9] and ultrasound-assisted extraction [10–12] have emerged as alternatives, offering the potential to improve extraction efficiency and reduce extraction times.

In recent years, the use of supercritical fluids, especially supercritical carbon dioxide (CO_2), has gained attention as a promising method for 5-CQA extraction. This approach offers advantages such as selective extraction, high extraction yields, and environmentally friendly processing [13]. Despite these advances, traditional thermal methods, including those based on diffusion, still have several drawbacks. These include high energy consumption, the potential for juice dilution, and a reduction in the quality of the final product. Thermal treatments may also lead to the degradation of sensitive compounds, such as polyphenols, affecting the overall yield and bioactivity of the extracted substances.

Another significant technique for the recovery of bioactive compounds, especially water-soluble ones like sugars and inulin, is biomass pressing. This method is widely employed in industries where the extraction of soluble compounds from plant biomass is required. Several studies have investigated the extraction of polyphenols and other bioactive molecules by pressing, demonstrating its efficiency in recovering valuable compounds from plant material [14–18]. However, to maximize extraction yields and improve the quality of the extracted compounds, pretreatment of the biomass is often necessary. Pretreatment methods can help break down cell walls, release compounds of interest, and optimize extraction efficiency.

The most commonly used pretreatment techniques include thermal treatments, such as microwave pretreatment [19–22], enzymatic-assisted extraction [23], and electrical treatments like pulsed electric field (PEF) [24,25]. These methods aim to enhance the extraction process by improving the accessibility of the target compounds within the plant tissue. However, while these techniques have shown promise, further research is required to fully understand the specific effects of thermal and electric fields on the recovery of CQAs from plant materials.

A major concern in the extraction of CQAs is the potential for oxidation, which can occur during both the pressing and pretreatment stages of extraction. CQAs are particularly susceptible to enzymatic oxidation, leading to the degradation of the compounds and a reduction in their bioactivity [26,27]. Therefore, it is essential to control oxidative processes during extraction to maintain the integrity of the extracted CQAs. Studies have shown that the use of anti-oxidative solutions or inhibitors, such as an anti-browning solution (ABS), can effectively mitigate oxidation during pressing and pretreatment, preserving the quality of the extracted compounds [28].

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To the best of our knowledge, the optimization of process parameters for the extraction of CQAs from forced endive roots (FER) using pressing in combination with PEF or MW pre-treatments has not been extensively studied. These techniques, when combined, have the potential to significantly reduce energy consumption and process time compared to traditional methods, offering a more efficient and sustainable alternative for the extraction of valuable bioactive compounds. The objective of this study was to explore the potential of PEF and MW pre-treatments applied to FER in order to improve both the extraction yield and recovery of CQAs. In addition to assessing the pressing kinetics and juice characteristics, such as °Brix (soluble solids content), special attention was given to investigating the oxidative effects that may occur during the pre-treatment and pressing stages. Furthermore, the study sought to evaluate the effectiveness of an anti-browning solution (ABS) in preventing oxidation and enhancing the overall quality of the extracted CQAs.

2. Materials and Methods

2.1. Raw Materials

Forced Witloof Belgian Endive Roots (FER) (Cichorium intybus L.) of the cultivars 'Flexine – Vilmorin' and 'Laurine' are provided by the Association des Producteurs d'Endive de France's experimental station in Arras, France. The FER are washed with cold tap water and stored in the dark at 4 °C until further use. They are then cut into cossettes using food cutting equipment (Robot Coupe CL50, S.N.C, France), and three sizes of cossettes are prepared: S^1 (2 × 2 × 55 mm^3), S^2 (2 × 5 × 55 mm^3), and S^3 (3 × 5 × 55 mm^3). After cutting, the FER are mixed and randomly distributed across each experimental condition.

2.2. Analytical Reagents and Chemicals

Acetonitrile (99.9%), ethanol (99.9%), ascorbic acid (99%), and formic acid (98–100%) were purchased from Thermo Fisher Scientific (Illkirch, France). Methanol (99%) and oxalic acid (technical grade) were obtained from VWR (Fontenay-Sous-Bois, France). Ultrapure water was produced using a Milli-Q system (Millipore Corporation, USA). Chlorogenic acid standard (5-CQA) (>98%) was supplied by Sigma (Saint-Quentin-Fallavier, France), while 3,5-dicaffeoylquinic acid (3,5-diCQA), 3,4-dicaffeoylquinic acid (3,4-diCQA), and 4,5-dicaffeoylquinic acid (4,5-diCQA) were obtained from Carbosynth (Compton, United Kingdom)

2.3. Extraction Procedure

FER were cut into cossettes, and the impact of different physical (PEF, MW, cutting size) and chemical (ABS) pretreatments on the extraction yield and CQAs yield was evaluated. The experimental procedure is summarized in Figure 1. Approximately 500 g of FER were used for each experimental condition.

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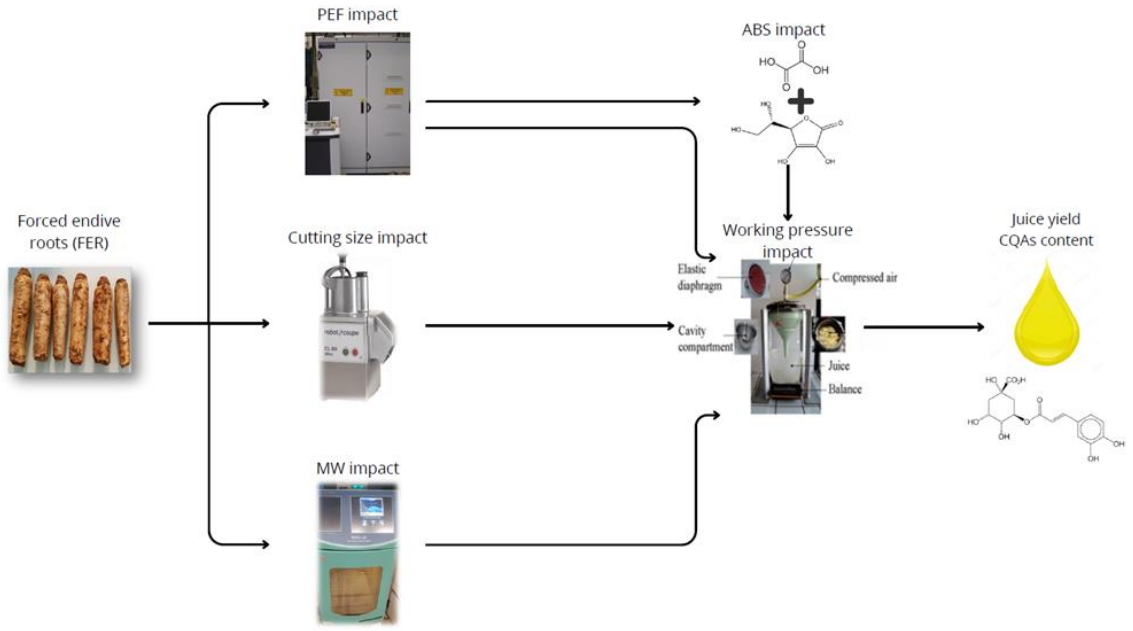

Figure 1. Experimental process scheme.

2.3.1. Pressing Experiments

A laboratory press chamber (hemispherical in shape, with a radius of R = 114 mm and a surface area of A = 817 cm²), equipped with an elastic diaphragm, was used for the pressing experiments. The FER cossettes were pressed at 3 bar, except in Section 3.1.2. The extracted juice was regularly weighed using a balance (Sartorius Entris II, Göttingen, Germany) to generate a pressing kinetic curve.

As previously described [29], the extracted yield Y_i was calculated as follows:

$$Y_i(\%) = \frac{m_{juice}}{m_{FER}} \times 100 \qquad \text{(Eq 1)}$$

where m_{juice} is the mass of extracted juice during pressing (g); and m_{FER} is the initial mass of FER before pressing (g).

2.3.2. Diffusion Experiments

As previously described [29], the conventional solid-liquid extractions were performed for 30 minutes in a 500 mL Erlenmeyer flask covered with aluminum foil to prevent water loss. Water was used as the solvent. The extractions were conducted at 90 °C with a solid-liquid ratio of 1:10 to extract all CQAs. In all experiments, the agitation speed was maintained at 400 rpm

2.4. Physical and Chemical Pretreatments

2.4.1. Pulsed Electrical Field Pretreatment

For the PEF treatment of cossettes, a pilot PEF generator (5 kV - 1 kA; Hazemeyer, Saint-Quentin, France) was used. The treatment chamber consisted of two flat stainless-steel electrodes (230 mm × 265 mm), which generated near-rectangular monopolar pulses.

The distance between the electrodes was 8 cm. Cossettes were placed between the electrodes either without any solvent (for Sections 3.1 and 3.2) or with solvent (for Sections 3.3, 3.4, and 3.5) at a solid/liquid ratio of 1:1.

A fixed electric field strength of 600 V.cm^{-1} is used for all the experiments. The samples are PEF-treated with N = 20 train of impulsions. Each train consisted of n =100 pulses, with a pulse duration of t_i = 100 µs and a rest time of 10 s after each train of impulsions. The total time of PEF treatment was calculated as follow:

$$t_{PEF}(s) = n \times N \times t \qquad \text{(Eq 2)}$$

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With t_{PEF} fixed at 0.2 sec. The temperature of the FER does not rise by more than 5°C during treatment. (maximum of 25 °C after PEF treatment).

2.4.2. Microwave Pretreatment

The MW treatment was performed using a Milestone EOS-G microwave laboratory oven (Sorisole Bergamo, Italy). This device is a multimode microwave reactor operating at 2.45 GHz with 10 W increments and a maximum power output of 900 W. FER cossettes were heated at a constant power density of 1 W/g. An optical fiber was used to monitor the temperature inside the plant material.

Cossettes were either cooled to room temperature before pressing or directly used for pressing experiments, as described in Section 3.5. The initial temperature of the FER was 22 °C, rising to 100 °C after 10 minutes of MW treatment (Figure S2, supplementary data)

2.4.3. Anti-Browning Treatment

A solution containing 1% (w/w) ascorbic acid and 0.2% (w/w) oxalic acid was prepared, as described by Son et al. (2001). This solution is referred to as ABS. FER cossettes were immersed in the solution for 10 minutes at a solid–liquid ratio of 1:1. Subsequently, the cossette-solution mixture was used for the pretreatment and pressing stages of the extraction process in Sections 3.3 and 3.5.

2.5. Analytical Measurements

2.5.1. Moisture Determination

As previously described [31], the moisture content of the FER is determined in duplicate using a MB-35 moisture balance (Mettler Toledo, Viroflay, France). Approximately 1 g of FER is heated to 105 °C until its weight remained stable. The moisture content (MC) is calculated using the following equation:

$$MC(\%) = \frac{m_i - m_d}{m_i} \times 100 \qquad \text{(Eq 3)}$$

where m_i is the mass of the initial sample and m_d is the mass of the dried sample.

2.5.2. Soluble Matter

The total soluble matter (°Brix) in the juice was measured using an AR digital refractometer (Leica Microsystems Inc., Buffalo, New York, USA).

2.5.3. HPLC Analysis

Quantification of CQAs was performed as previously described [31]. The identification and quantification of 5-CQA, 3,5-diCQA, 3,4-diCQA, and 4,5-diCQA content were achieved by comparing their relative retention times to those of the corresponding standard compounds. The sum of 3,5-diCQA, 3,4-diCQA, and 4,5-diCQA is considered as dicaffeoylquinic acids (diCQAs).

2.6. CQAs Extraction Yield

In this study, the 5-CQA and diCQAs extraction yield compared to solid liquid extraction were calculated as followed:

$$Y_{5-CQA}(\%) = \frac{m_{5-CQApressing}}{m_{5-CQAS:L}} * 100 \qquad \text{(Eq 4)}$$

$$Y_{diCQAs}(\%) = \frac{m_{diCQAspressing}}{m_{diCQAsS:L}} * 100 \qquad \text{(Eq 5)}$$

Where, $m_{5-CQApressing}$ and $m_{diCQAspressing}$ are respectively the masses (g) of 5-CQA and diCQAs extracted by pressing with or without pre-treatment. $m_{5-CQAS:L}$ (g) and $m_{diCQAsS:L}$ are respectively the mass of 5-CQA and diCQA extracted by the solid-liquid extraction presented before. $Y_{molecule}$ was used to refer to either $Y_{5\text{-CQA}}$ or Y_{diCQAs}.

2.7. Energy Consumption Measurement

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Used software records the energy consumption of the PEF generator. For the MW treatment, the power was fixed for the experiment. Thus, as previously described [29] the energy consumption was calculated as follows:

$$E(kJ/kg) = \frac{P*t}{m} \quad \text{(Eq 6)}$$

Where E is the energy consumption (kJ/kg fresh weight), P is the power of the MW or PEF, and t is the time (s) and m is the mass of fresh FER (kg).

2.8. Statistical Analyses

All extractions are performed at least in duplicate. The average values and standard errors were calculated with Excel, 2016 version. Significant differences ($p<0.05$) between samples were determined using an ANOVA. A Tukey test was applied to identify significant differences between groups, represented by different letters in the figures.

3. Results and Discussion

3.1. Extracted yield

3.1.1. Effect of Cossette Size on the Extracted yield

The size of the cossettes has a significant impact on juice release; thinner cossettes offer less resistance, thereby facilitating juice extraction [32]. The effect of cossette size on the extracted yield is illustrated in Figure 2.

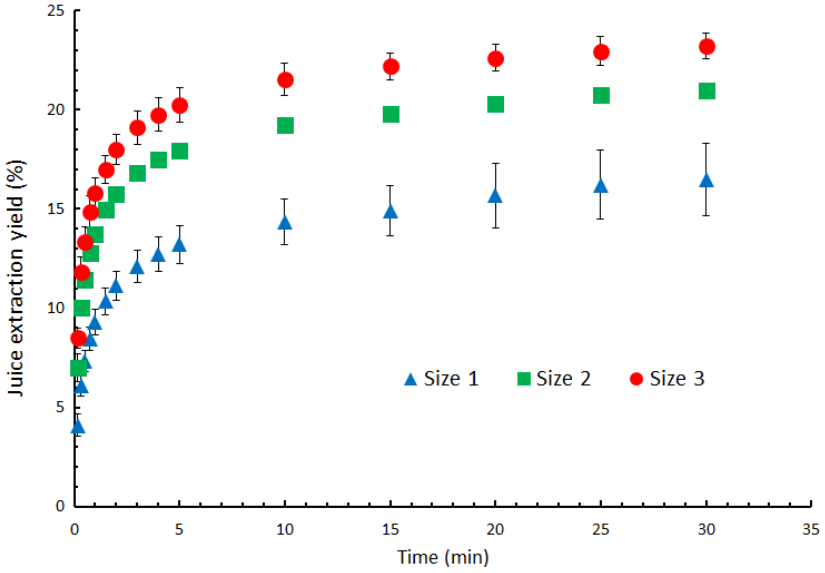

Figure 2. Extraction yield kinetics for different cossette sizes under 3 bar pressing.

A smaller cossette size is associated with a higher extraction yield. For instance, after 30 minutes of pressing, the yield for the smallest size (size 1) is 6% higher than that of size 3. A similar trend has been observed in apples and carrots [25]. However, due to the minimal difference in yield between size 1 and size 2, size 2 was selected for subsequent experiments.

3.1.2. Effect of Working Pressure on Extracted yield

The impact of working pressure on the extracted yield was also investigated (Figure 3). Three different pressures were applied: 1 bar, 2 bar, and 3 bar.

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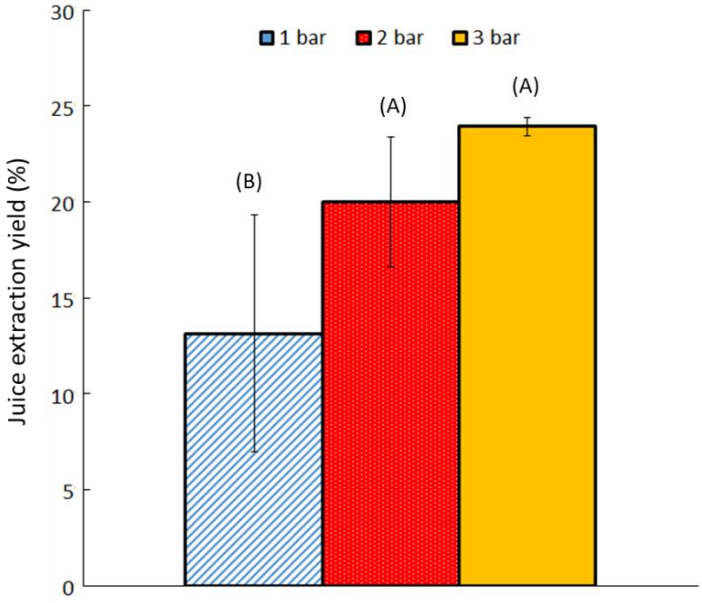

Figure 3. Yield obtained at varying working pressures after 30 minutes of pressurization. Bars with different letters indicate significant differences in the yield of extracted juice ($p > 0.05$) according to the Tukey test at 95%.

Extracted yield increased with increasing working pressure. Increasing the pressure from 1 to 3 bar enhance the extracted yield from 13 ±1 % to 24 ±1 %. The same effect of pressure has been observed for chicory roots [32]. A working pressure of 3 bar is used for subsequent experiments. The yield remains low; incorporating pretreatment steps before pressing might enhance it. Cell denaturation, in particular, seems to be a promising approach for increasing the extraction yield.

3.1.3. Effect of Pretreatment on Extracted yield

The effect of PEF and MW pretreatments prior to pressing is shown in Figure 4. A comparison with pressing without pretreatment was also conducted.

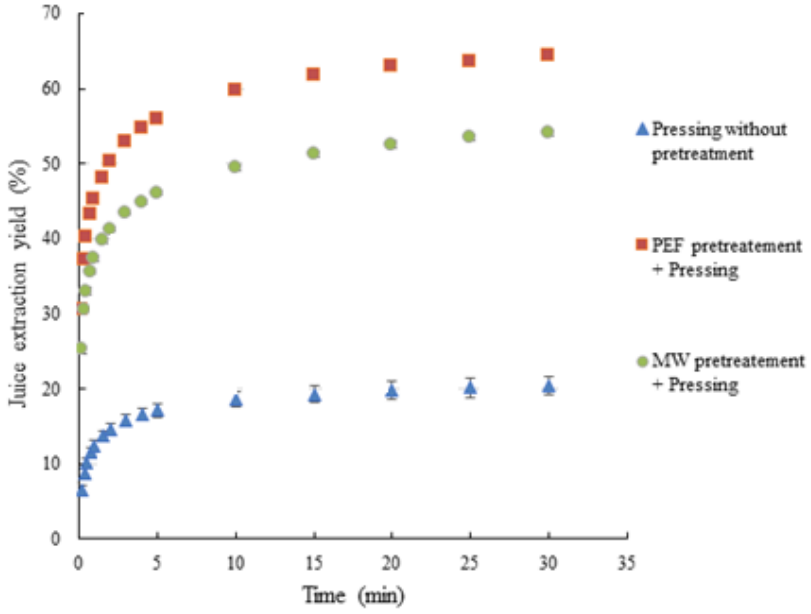

Figure 4. Effect of MW and PEF pretreatments on extracted yield kinetics.

Figure 4 shows that both pretreatments have a significant effect, resulting in a much higher extracted yield. This improved yield can be attributed to cell membrane degradation, which occurs

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with MW when temperatures exceed 60°C due to thermal treatment caused by liquid expansion [33,34], or with PEF through the electro-permeabilization of cells [35]. However, PEF pretreatment leads to better cell denaturation than microwave pretreatment, with lower energy consumption (40 kJ/kg and 600 kJ/kg, respectively). For example, after 30 minutes of pressing, only a 20 ± 1% extracted yield was obtained without pretreatment, while PEF and MW pretreatments resulted in yields of 64 ± 1% and 54 ± 1%, respectively. Thus, the extracted yield is more than three times higher with PEF pretreatment and two and a half times higher with MW pretreatment.

3.2. Juice Characterization

3.2.1. Total Soluble Matter (°Brix)

The soluble matter content in the juice primarily comprises sugars and organic acids, which are predominantly found in the vacuole sap. MW or PEF pre-treatments promote the effective breakdown of intact cells and vacuoles, facilitating the release of juice and subsequent extraction of the vacuole sap [33,36,37]. Soluble matter values in ° Brix are listed in Table 1.

Table 1. Characteristics of FER juice obtained from different pretreatment methods. * for statistical different ($p<0.05$) with pressing alone.

Condition	Soluble matter (°Brix)
Pressing without pretreatment	6.0 ± 0.0
PEF pretreatment + Pressing	7.5 ± 0.6*
MW pretreatement + Pressing	9.3 ± 0.6*

Compared to the untreated samples, both the PEF and MW pre-treatments exhibited a statistical increase ($p<0.05$) in soluble matter content. A comparable trend is observed when PEF-assisted pressing is applied to the extraction of carrot [32]. Increasing temperature using conventional solid-liquid extraction of sugar beets also leads to an increase in the soluble matter content [37].

3.2.2. CQAs Content

The extraction yields of 5-CQA and diCQAs compared to conventional solid-liquid extraction (calculated with Eqs 4 and 5) are displayed in Figure 5.

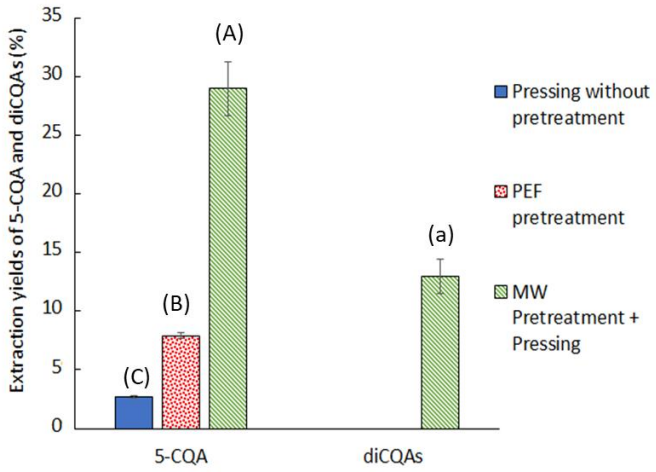

Figure 5. CQAs extraction yield with different pretreatment condition. The extraction yield is given relative to a conventional solid-liquid extraction. Bars with different small letters indicate significant differences in the yield of diCQA ($p > 0.05$) according to the Tukey test at 95%. Bars with different capital letters indicate significant differences in the yield of 5-CQA.

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MW pretreatment results in the extraction of approximately 29 ± 2% of 5-CQA and 13 ± 2% of diCQAs, whereas untreated and PEF-pretreated pressing extracts yield less than 8% of 5-CQA and no diCQAs. The primary hypothesis is that interactions between CQAs and the biomass hinder extraction. These interactions can only be disrupted by thermal treatment [19,38]. Numerous studies have reported interactions between cell wall polysaccharides and chlorogenic acid in apples and cellulose-pectin composites [39,40]. Hydrogen bonds, hydrophobic interactions, and electrostatic interactions between chlorogenic acid and cellular structures have also been documented. Investigating the effect of thermal pretreatment duration on CQAs extraction is essential to optimize energy consumption during MW pretreatment.

3.3. Effect of Thermal Pretreatment

The MW pretreatment time before pressing is studied. Results are shown in Figure 6.

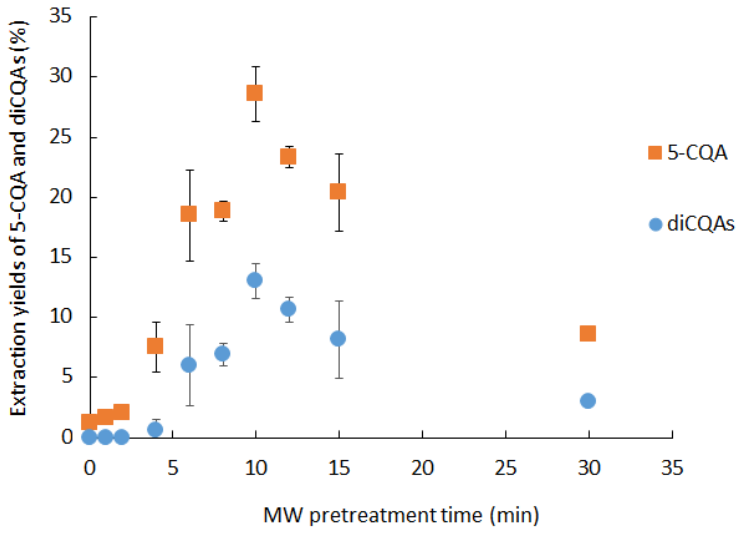

Figure 6. 5-CQA and diCQAs extraction yield with different MW pretreatment time. The extraction yield is given relative to a conventional solid-liquid extraction.

The optimal MW pretreatment time is 10 minutes for both 5-CQA and diCQAs, yielding 29 ± 2% for 5-CQA and 13 ± 2% for diCQAs. After 10 minutes of MW treatment, a temperature of 100°C is reached (Figure S1), consistent with the literature [38]. After 10 minutes, the extracted yield decreased due to water evaporation during MW treatment, which in turn reduced the CQAs yield. The diCQAs yield is half that of 5-CQA, which can be attributed to the double caffeic acid moiety, a highly hydrophobic component. This part of the CQAs interacts with the cell wall through non-covalent bonding, which likely limits its extraction [31,39]. To estimate the re-adsorption of CQAs on the FER cell wall during cooling, pressing is conducted immediately after 10 minutes of MW pretreatment and after cooling to room temperature. The results are shown in Figure 7.

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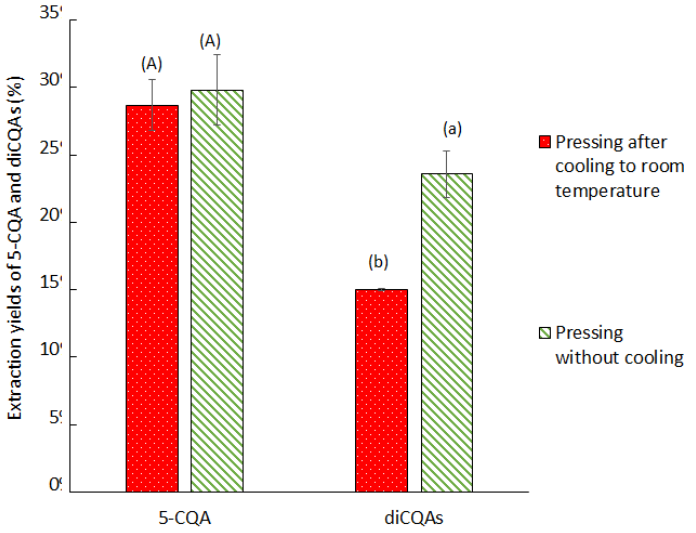

Figure 7. Effect of FER cooling on CQAs extraction yield. The extraction yield is given relative to a conventional solid-liquid extraction. Bars with different small letters indicate significant differences in the yield of diCQA ($p > 0.05$) according to the Tukey test at 95%. Bars with different capital letters indicate significant differences in the yield of 5-CQA.

During cooling to room temperature, some of the diCQAs appear to re-adsorb onto the cell wall, making them less accessible for extraction by pressing. As a result, 5-CQA seems to be less affected, with no statistically significant difference in extraction yield ($p > 0.05$). This is likely due to its better water solubility compared to diCQAs [41]. While pressing with MW pretreatment enhance CQAs extraction, a threshold appears to be attained with pressing. Consequently, it seems imperative to introduce a solvent to enhance the recovery of a larger quantity of CQAs. Water was choosing as a green and inexpensive solvent.

3.4. Effect of Water Addition

The main objective is to increase yield while maintaining eco-efficient extraction. The effect of water addition on pressing, assisted by pretreatment, was measured (Figure 8). Water was added to the FER at a ratio of fresh weight to liquid (1/1) immediately after cutting.

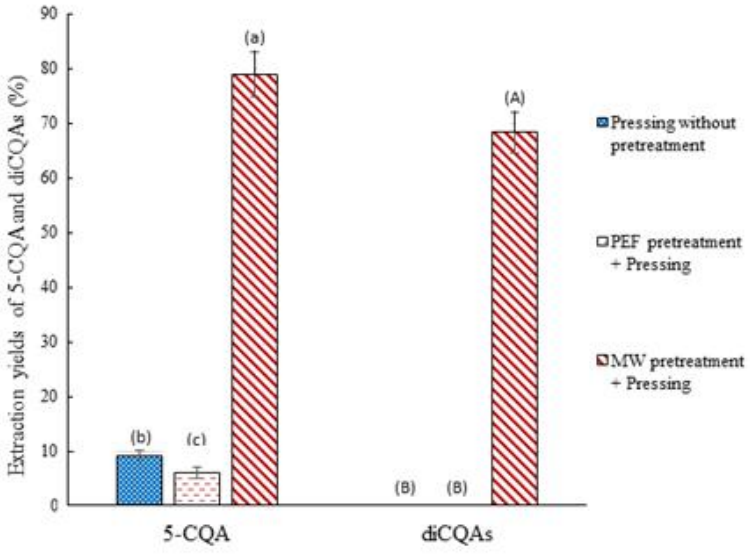

Figure 8. Effect of water addition on CQAs extraction yield with different pretreatment condition. The extraction yield is given relative to a conventional solid-liquid extraction. Bars with different small letters indicate

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significant differences in the yield of 5-CQA ($p > 0.05$) according to the Tukey test at 95%. Bars with different capital letters indicate significant differences in the yield of diCQA.

The addition of water with a MW pretreatment significantly increased the extraction of 5-CQA (from 30 ± 2% to 79 ± 4%) and diCQAs (from 24 ± 2% to 68 ± 2%) compared with extraction without solvent. In addition, pressing alone with the addition of solvent improved the extraction of 5-CQA from 2 ± 1% to 9 ± 1%. The addition of water seems to help the diffusion of the CQAs in the extraction medium and to limit the re-adsorption of the CQAs by the plant material, explaining the improved yields. However, for diCQAs, extraction by pressing without pre-treatment showed no improvement in yield. Similarly, the use of pretreatment by PEF did not bring any significant benefit on the extraction efficiency of CQAs, compared with solvent-free extraction (Figure 5). Heat treatment seems necessary to desorb the CQAs. Moreover, low yields with PEF can be caused by oxidation of CQAs by polyphenol oxidase (PPO), which leads to their degradation into brown components (allomelanins). In our previous work [42], this oxidation is assumed to occur during drying, but also seems to occur during pre-treatments and pressing [43,44]. An anti-browning solution (ABS) can be used to prevent this oxidation (Altunkaya & Gökmen, 2009).

3.5. Effect of PEF + ABS Pretreatment on FER CQAs Content

The effect of the antioxidant solution on extracted CQAs yields was studied (Figure 9). Water or the antioxidant solution was added to the FER at the same ratio as before after cutting. The antioxidant solution is made by ascorbic acid (1%) and oxalic acid (0.02%).

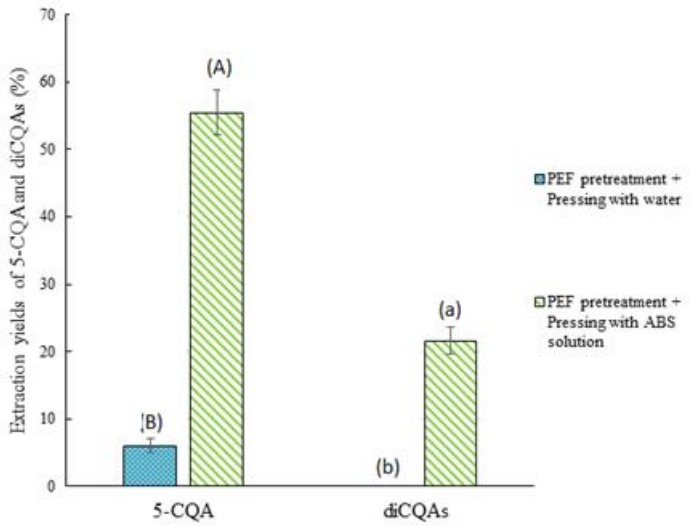

Figure 9. Effect of an anti-browning solution or water addition on CQAs extraction yield. The extraction yield is given relative to a conventional solid-liquid extraction. Bars with different small letters indicate significant differences in the yield of diCQA ($p > 0.05$) according to the Tukey test at 95%. Bars with different capital letters indicate significant differences in the yield of 5-CQA.

The ABS solution boosts the CQAs extraction, increasing diCQAs extraction from 0 to 22% and 5-CQA from 6% to 55%. ABS treatment appears to prevent PPO activity and CQAs degradation after PEF pre-treatment. Thus, the partial release of CQAs can be achieved by electric treatment to disrupt the weak interactions between CQAs and cell wall polysaccharides. With ABS treatment, FER are brighter even after PEF pretreatment with a less visible brown part (Figure S2). Browning prevention is an indication of PPO activity prevention and preservation of CQAs [45].

3.6. Coupling Pre-Treatments and Anti-Browning Treatment

The CQAs yield is limited by the extracted yield, adsorption of CQAs onto the FER cell wall, and PPO degradation. To overcome these limitations, MW and PEF pretreatments can be coupled with

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pressing to increase CQAs yield. The results are presented in Figure 10A and B. FER are mixed with the ABS before any pretreatment. A comparison without prior PEF pretreatment is also conducted to assess the effect of adding PEF on extraction.

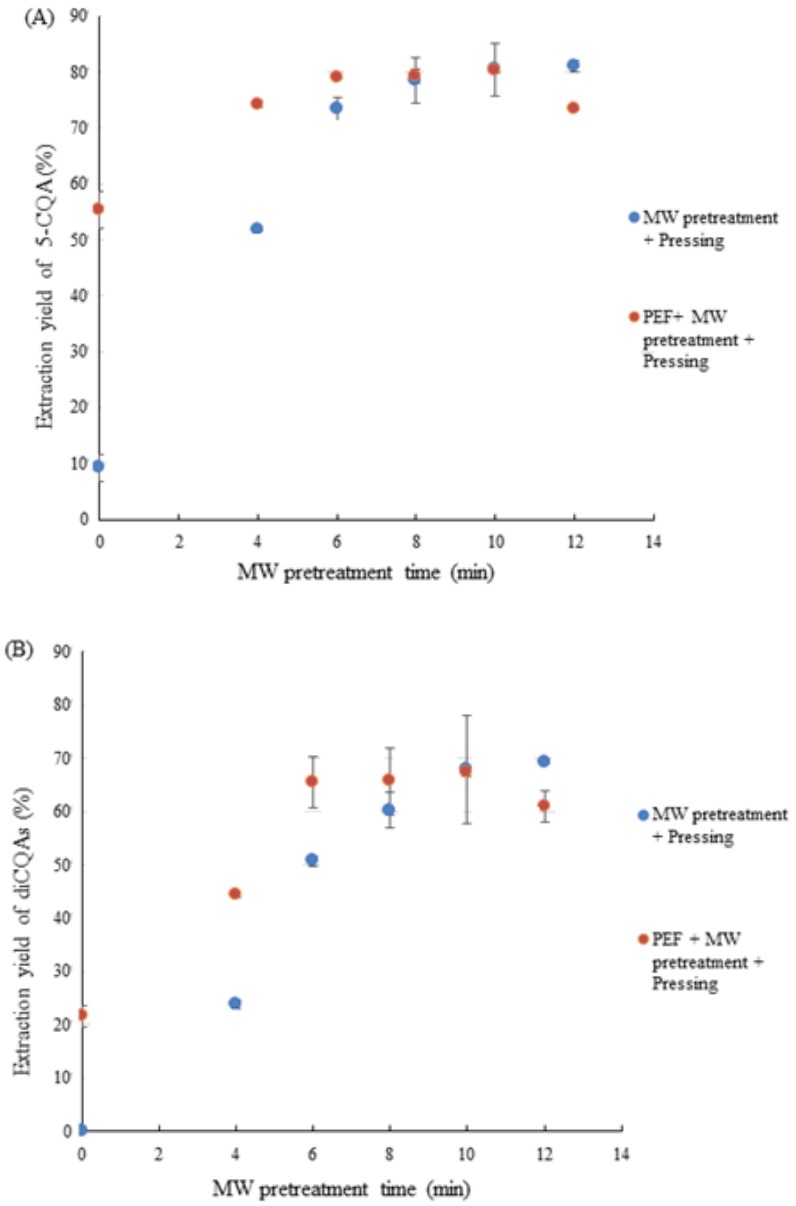

Figure 10. Effect of PEF and MW Pretreatments on 5-CQA (A) and diCQAs (B) Extraction Yield, with FER treated with an ABS Solution prior to pretreatment.

As observed previously, the yield of diCQAs is lower than that of 5-CQA. However, the yields of 5-CQA and diCQAs with coupled pretreatments are significantly higher than those obtained with only one pretreatment, up to 6 minutes of MW pretreatment. This increase can be attributed to the cell permeabilization effect caused by PEF, which allows CQAs to be released more easily into the medium. The combination of PEF and MW pretreatments does not improve the maximum CQAs yield, but it helps achieve the maximum yield with a reduced MW pretreatment duration, from 10 to 6 minutes. PEF pretreatment also reduces energy consumption (from 600 kJ/kg to 400 kJ/kg) while achieving optimal extraction yield.

4. Conclusions

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This study highlights the significant impact of thermal and electrical pre-treatments on juice extraction and the recovery of chlorogenic acids (CQAs) from FER. The application of pulsed electric field (PEF) pre-treatment resulted in a higher extraction yield compared to microwave (MW) pre-treatment, demonstrating the superior cell membrane permeabilization effect of the electrical field over thermal treatment. However, the recovery of CQAs was higher with MW pre-treatment, suggesting that thermal treatment plays a critical role in desorbing CQAs from the plant cell wall matrix, thereby facilitating their release into the juice.

An interesting observation was the oxidative effect associated with pressing and extraction, which was amplified by PEF treatment, leading to CQAs degradation. In contrast, treatment with aqueous biphasic systems (ABS) effectively mitigated oxidative degradation, significantly enhancing CQAs recovery from the biomass.

The combined use of MW, PEF, and ABS pre-treatments before pressing produced outstanding results, achieving recovery rates of 65% diCQAs and 80% 5-CQA compared to conventional solid-liquid extraction. This innovative approach not only yielded highly concentrated juice but also reduced energy consumption. The extracted juice could serve as a precursor for the production of high-value bioactive compounds, underscoring the potential of this method for industrial applications.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Authors contribution: E.D: Conceptualization, Methodology, Formal analysis, Investigation, Data acquisition, Writing – original draft; M.C: Writing – review, Funding acquisition; N.G : Writing – review, Funding acquisition, Project administration.

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Abbreviations

5-CQA, chlorogenic acid; ABS, anti-browning solution; diCQAs, dicaffeoylquinic acids; FER, forced endive roots; MC, moisture content; MW, microwave; PEF, pulsed electrical field; PPO, polyphenol oxidase

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