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Modelling the recovery of biocompounds from peach waste assisted by pulsed electric fields or thermal treatment

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ABSTRACT

The recovery of non-purified bioactive extracts (70% ethanol) from peach pomace (PP) was assisted by conventional thermal treatment (CTT, 50 °C up to 90 min) or pulsed electric fields (PEF, specific energy input, E_V, of 0.0014–2.88 kJ/kg). The maximum concentration of biocompounds and antioxidant activity, assessed with spectrophotometric and HPLC methods, was obtained upon 40 min by CTT and 0.0014 kJ/kg by PEF, which took 16 μs. A two-step mechanism was proposed when CTT was applied, considering a first step (zero-order kinetic) in which the PP biocompounds were released into the extraction media and a second degradation stage (first-order). A significant relationship was found between E_V and PP biocompound degradation during PEF extraction, and a two-term degradation model was proposed to explain obtained data.

The CTT or PEF-assisted recovery of biocompounds from PP was adequately explained by the proposed mechanistic and empirical kinetic models, which are feasible tools to understand the involved phenomena in the extraction procedures.

1. Introduction

European Union (EU) fruit juice and nectars consumption was 9.2 billion litres in 2017, with peach (*Prunus persica* L.) representing the fourth flavour profile in the market (AIJN, 2018). During peach juice production, a huge amount of waste is generated. Usually, peach pomace (PP), referring to the pressing solid residue generated from peach juicing process, accounts for approximately 10% of the initial fruit weight (Argun and Dao, 2017). Peach is rich in antioxidant biocompounds that are mainly localized in the pulp and peel tissues, which are the main constituents of PP. In particular, phenolic compounds have been found to be the major contributor to the antioxidant activity of peach, followed by ascorbic acid and carotenoids (Gil et al., 2002; Redondo et al., 2017; Redondo et al., 2018). Although this, like other vegetable by-products, PP is currently discarded in landfills, anaerobically digested or composted. These PP management options not only represent a wastage of valuable biomass, but also an environmental burden and an economic cost for companies. For these reasons, the identification of alternative management options, able to properly valorise PP, are required in order to turn this industrial discard into an added-value product. In this regard, the extraction of biocompounds could represent an effective valorisation strategy of PP (El Darra et al., 2018).

Traditional techniques employed in the extraction of antioxidant compounds from fruits involve the use of organic solvents and high temperatures (Li et al., 2006). Such extraction processes present high costs and environmental impact, and often result in the thermo-degradation of biocompounds. Moreover, the extraction may be negligible if the cells are intact. In fact, the extraction of biocompounds from vegetable tissues is a mass transfer process, whose rate principally depends on the resistance of the biocompound to migrating into the extraction solvent. The compartmentalization of biocompounds into the cells of the vegetable tissue and their interaction with the vegetable matrix increase this resistance to extraction (Donsì et al., 2010). Therefore, assisting-technologies, able to disrupt cellular tissue integrity have been increasingly proposed to enhance extraction efficiency, while preserving the bioactive compounds in the extract. Such strategies include the use of microwaves, high pressures (pressurized liquids, supercritical fluids, high pressure homogenization), ultrasounds, and pulsed electric fields (PEF) (Rombaut et al., 2014). In the case of PEF, the vegetable material is subjected to external electric fields (1–10 kV/cm) for a short time (microseconds), resulting in the so-called

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"electroporation" of the cell membranes, which leads to an increase in the cell membrane permeability, thus favouring the biocompounds extraction (Donsì et al., 2010). The application of PEF has many advantages over other assisting techniques. As compared to pressure-based technologies, PEF process has lower energy costs and does not cause the release of undesirable substances into the extraction solvent, due to the slight denaturation of the cell membranes (Redondo et al., 2018). Moreover, as compared to microwaves and ultrasounds, the loss of thermosensitive bioactive compounds is reduced because no temperature increase is generated (Cacace and Mazza, 2003). Based on these technological advantages, PEF has already been used to extract marketable compounds from different vegetable matrices (Kumari et al., 2018).

It must be noted that the high moisture content of PP is one of the main issues related to its management, since it makes it quickly prone to microbial spoilage (Ajila et al., 2012). Therefore, in a realistic scenario, it is likely that PP should be preliminary frozen or air-dried in order to be subsequently subjected to any extraction process, possibly modifying the content, composition and extractability of bioactive compounds.

Based on these considerations, the aim of this study was to evaluate the recovery of non-purified biocompounds in extracts from PP by extraction processes assisted by PEF or conventional thermal treatment (CTT) and explain the involved phenomena through kinetic models. Frozen and dried PP were subjected to PEF or CTT assisted extractions using a 70% hydroalcoholic solvent and the obtained extracts were analysed for biocompound concentration and antioxidant activity. Moreover, the extraction efficacy was evaluated to identify the more advantageous assisted extraction technology for PP valorisation.

2. Materials and methods

2.1. Reagents

The used reagents were absolute ethanol (Scharlau, Barcelona, Spain.), bidestilled water (Milli-Q system, Millipore, Bedford, USA), Fast Blue reagent (Sigma Aldrich, St. Louis, U.S.A.), Sodium carbonate (Carlo Erba, Milan, Italy) gallic acid 97% (Sigma Aldrich, St. Louis, U.S.A.), Sodium acetate anhydrous (Sigma Aldrich, St. Louis, U.S.A.), Aluminum chloride (Sigma Aldrich, St. Louis, U.S.A.), Quercetin: 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one (Sigma Aldrich, St. Louis, U.S.A.), 1,4-dithiothreitol (DTT) (Sigma Aldrich, St. Louis, U.S.A.), Potassium chloride (Sigma Aldrich, St. Louis, U.S.A.), *Meta*-phosphoric acid (Sigma Aldrich, St. Louis, U.S.A.), DPPH: 2,2-Diphenyl-1-picrylhydrazyl (Sigma Aldrich, St. Louis, U.S.A.), Trolox: (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid 97% (Sigma Aldrich, St. Louis, U.S.A.).

2.2. Peach pomace (PP)

The peach pomace (PP), residue from peach juice extraction was kindly furnished by Indulleida S.A. (Alguaire, Lleida, Spain). The PP was subjected, in the factory, to two alternative stabilization procedures, freezing and drying. PP was portioned into plastic trays and frozen at −18 °C to obtain the frozen PP. PP was air-dried at 140 °C, and ground to 30 mesh, according to the protocol patented by the company (Sorribas, 1993). Frozen and dried PPs were characterized by a moisture content of about 85.0 ± 1.1 and 4.0 ± 1.0 (g/100 g), respectively, determined using the official gravimetric method (AOAC, 1997). Frozen PP was thawed at 4 °C for 12 h prior to use. Both frozen and dried PPs were used at room temperature (20 ± 1 °C).

2.3. Preparation of PP extracts

The preparation of PP extracts was performed according to Plazzotta et al. (2020). In particular, the extracts were prepared taking into account the PP moisture content (4% and 84% for PP dried and PP frozen, respectively) and using aqueous ethanol as solvent, so that the final ethanol:water ratio became 70:30 w/w, in all cases. Namely, an amount of 20 g (dm) from each dried and frozen PP, were placed into a glass flask with the solvent to maintain a final ratio ethanol:water 70:30 w/w, in all the extractions performed. The suspension was homogenized by manual mixing for 1 min and immediately subjected to the different extraction protocols. The dispersions were then filtered using 1.2 μm filters (Sartorius 17,593–100 cellulose acetate 25 mm/1.20 μm, Filtros, Anoia, Spain) to remove the solid residue, obtaining the extracts. The latter were stored in the dark at −18 °C until analysis. Control extracts were prepared by filtering the PP dispersions immediately after the manual mixing, without any further assisted extraction.

2.3.1. Conventional thermal treatment (CTT)

PP dispersions were subjected to conventional thermal treatment (CTT) by using a thermal bath set at 50 °C. Sample temperature reached 50 °C in less than 5 min and temperature was monitored in order to maintain it at 50 °C during the extraction, that was carried out up to 90 min. Sample temperature was measured during CTT by a copper-constantan thermocouple probe connected to a portable data logger (mod. TP100, XS Instruments, Carpi, Italy). The energy consumption of the CTT procedure was of 88.9 kJ/kg, calculated according to the following Eq. (1) (El Darra, Grimi, Maroun, Louka and Vorobiev (2013).

$$E_v = C_P \Delta T \quad (1)$$

$C_P$is solvent specific heat (2.964 kJ/kg°C^{-1}) and ΔT (=30 °C) is the temperature increase to reach the experimental temperature from the room temperature.

2.3.2. Pulsed electric field treatment (PEF)

PEF treatments were carried out using a unit with 0.1 μF capacitor with an exponential decaying waveform (Physics International, San Leandro, CA, USA). The chamber, where the electric field (E, kV/cm) is created, consists in two parallel stainless-steel plates with a gap of 5.0 cm. The PP dispersions were thus treated at 0.8–10 kV/cm, using 4–30 monopolar pulses of 4 μs, at a frequency of 0.1 Hz, which corresponded to a specific energy input (E_v) up to 72 kJ/kg, calculated according to Eq. (2) (Bot et al., 2018):

$$E_v = \frac{V^2 t}{R\ m} \quad (2)$$

where V is the voltage (V), t is the time (s) calculated as pulse duration (4 μs) multiplied by pulse number, R is the electrical resistance (Ω) and m (kg) is the PP mass.

Sample temperature was measured just before and immediately after PEF treatment (to avoid E distortion during the treatment) by a copper-constantan thermocouple probe connected to a portable data logger (mod. TP100, XS Instruments, Carpi, Italy). It was always kept in the range 20–22 °C.

2.4. Determination of bioactive compound concentration

2.4.1. Total polyphenolic (TPC)

Total phenolic content (TPC) was determined using the Fast Blue method (Medina, 2011), adapted to a 96-well microplate. A portion of 200 μL of ethanolic extract, properly diluted, was mixed with 20 μL of Fast Blue reagent (1 mg/mL) and 20 μL of Na_2CO_3 solution (0.05 g/mL). Samples were mixed and stored at room temperature in darkness for 90 min. Absorbance was measured at 420 nm using a UV/VIS Thermo Multiskan Spectrum spectrophotometer (Thermo Scientific, Waltham, USA). Ethanol blanks (70%, w/w) were run in each assay. Calibration curve was built with gallic acid (0–500 mg/L). Results were expressed as mg of gallic acid equivalents (GAE) per 100 g of dry matter (dm).

2.4.2. Total flavonoid (TF)

Total flavonoids (TF) were evaluated using the method of Humadi and Istudor (2008), adapted to a 96-well microplate. An amount of 25 μL of the ethanolic extract was added with 140 μL of deionized water, 10 μL of $C_2H_3NaO_2$ (1 g/L) and 10 μL of $AlCl_3$ (0.1 g/mL). The mixture was homogenized and stored in the dark for 40 min. The absorbance was determined at 405 nm using a UV/VIS Thermo Multiskan Spectrum spectrophotometer (Thermo Scientific, Waltham, USA). Blanks containing water instead of $C_2H_3NaO_2$ and $AlCl_3$ were run in each assay. Calibration curve was built with quercetin (0–1000 mg/L). Results were expressed as mg of quercetin equivalents (QE) per 100 g of dry matter (dm).

2.4.3. Total anthocyanin (TA)

Total anthocyanin (TA) content was evaluated by differential pH method (Chaovanalikit and Wrolstad, 2004). A portion of 2.5 mL of extract was added to 2.5 mL of 45 g/L metaphosphoric acid solution containing 1,4-Dithiothreitol (7.2 g/L). An aliquot of the sample (1 mL) was added with 1 mL of pH 1.0 buffer (0.025 M potassium chloride). Similarly, 1 mL of the sample was added with 1 mL of pH 4.5 buffer (0.4 g/L sodium acetate). Absorbance (A) was measured using a CECIL 2021 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK) at 520 and 700 nm. Absorbance was calculated as reported in Eq. (3):

$$A = [(A_{520} - A_{700})pH1.0] - [(A_{520} - A_{700})pH4.5] \quad (3)$$

The total anthocyanin content (TA) (mg/L) was calculated using Eq. (4):

$$TA = \frac{A \times W \times DF \times 1000}{\varepsilon \times L} \quad (4)$$

where ε is the cyanindin-3-glucoside molar absorption coefficient (26,900), L is the cell path length (1 cm), W is the molecular weight of cyanindin-3-glucoside (449.2 Da), DF is the dilution factor. Data were expressed as mg of cyanindin-3-glucoside equivalents (CGE) per 100 g of dry matter (dm).

2.4.4. Vitamin C (VIT C)

The extraction of vitamin C (VIT C) was based on the procedure proposed by Odriozola-Serrano et al. (2007). A portion of 2.5 mL of extract was added to 2.5 mL of 45 g/L metaphosphoric solution containing 1,4-Dithiothreitol (7.2 g/L). The mixture was homogenized, passed through a Millipore 0.45 μm membrane and injected in the HPLC system. The HPLC system was equipped with a 600 Controller and a 486 Absorbance Detector (Waters, Milford, MA) working at 245 nm. Samples were introduced onto the column through a manual injector equipped with a sample loop (20 ll). The flow rate was fixed at 1.0 mL/min at room temperature. A reverse-phase C18 Spherisorb® ODS2 (5 μm) stainless steel column (4.6 mm 250 mm) was used as stationary phase. The mobile phase was a 0.1 g/L solution of sulphuric acid adjusted to pH 2.6 (Sánchez-Mata et al., 2000). Calibration curve was built with L-ascorbic acid (0–50 mg/L). Results were expressed as mg of VIT C per 100 g of dry matter (dm).

2.5. Determination of antioxidant activity (AA)

DPPH assay was used to assess the free radical scavenging activity. The assay was performed according to Redondo et al. (2018) adapted to a microplate reader. The reaction was initiated by addition of 280 μL/well DPPH solution in ethanol (25 μg/mL) to 20 μL/well extracts, properly diluted. The reaction mixture was kept in the dark for 10 min and its absorbance was then measured at 517 nm using a UV/VIS Thermo Multiskan Spectrum spectrophotometer (Thermo Scientific, Waltham, USA). Ethanol blanks (70 g/100 g) were run in each assay. The antioxidant activity (AA) was evaluated by measuring the variation in absorbance at 515 nm after 15 min of reaction. Calibration curve was built with trolox (0.005–0.250 mg/L). Results were expressed as mmol of trolox equivalents (TE) per 100 g of dry matter (dm).

2.6. Extraction efficacy

The extraction efficacy was calculated according to Eq. (5):

$$Efficacy\ (\%) = \frac{C_{max} - C_{control}}{C_{control}} \times 100 \quad (5)$$

where C_{max} represents the maximal concentration or maximal antioxidant activity reached by PEF or CTT assisted extractions for PP extracts, and $C_{control}$ is the extract control concentration or antioxidant activity obtained from untreated PP extracts. It should be taken into account that the C_{max} of each group of compound in the extracts from any assisted process (CTT or PEF) is always considered in relationship with the related control extract. ANOVA test (section 2.8) was used to identify the C_{max} value in the case of CTT. In the case of PEF, the C_{max} values corresponded to the C_{B0} values obtained by the model described in section 2.7.2.

2.7. Kinetic models

2.7.1. Extraction kinetic considerations

The content variation of the different biocompounds extracted by applying CTT processes was evaluated through a kinetic mechanism consisting of two consecutive and simultaneous stages. The proposed mechanism considers a first step in which the bioactive compounds (B) are released from the substrate (S) and pass from the food matrix to the solution according to a zero kinetic order, and a second step, in which the bioactive compounds may disappear from the solution, leading to degraded derivatives of bioactive compounds (B_D), following a first kinetic order (eq. (6)):

$$S - B \xrightarrow{k_0} S + B \xrightarrow{k_1} B_D \quad (6)$$

For CTT, the first and second stage of the extraction mechanism have been reported to have a kinetic constant k_0 and an order n = 0 and a kinetic constant k_1 and an order n = 1, respectively (Ibarz et al., 1999). Thus, the variation of the concentration of each bioactive or the extract antioxidant activity (C) as a function of extraction time (t) may be derived from Eq. (7):

$$\frac{d\ C_B}{d\ t} = k_0 - k_1\ C_B \quad (7)$$

The constant relation K is defined as the ratio between k_0 and k_1 ($K = \frac{k_0}{k_1}$). This differential equation can be integrated with the initial condition that, for t = 0, the concentration of the bioactive compound is C_{B0}. This integration allows obtaining Eq. (8):

$$C_B = K - (K - C_{B0}) \cdot e^{-k_1 t} \quad (8)$$

where C_B is the concentration of bioactive compounds or the antioxidant activity of the extract at time t during thermal extraction and C_{B0} is the initial concentration ($t = 0$).

2.7.2. Influence of applied energy density

When food matrices are treated for extraction purposes by assisted technologies, commonly, some of the extractable biocompounds are degraded depending on the effect of the treatment variable (time, temperature, energy density, etc.). This treatment variable is the one that during the process exerts the role of independent variable, while the concentration of the component that undergoes degradation is the dependent variable. In many processes, the dependent variable (C) varies decreasing according to a degradation function starting from the initial value of the dependent variable (C_0), until reaching an equilibrium value (C_{eq}).

In order to obtain a model for those processes, we consider an exponential decrease variation, according to Eq. (9):

$$C = C_{eq} + (C_0 - C_{eq}) \cdot e^{-k_i' x} \quad (9)$$

In the case of PP extracts obtained by PEF the dependent variable C_B represents the concentration of different biocompounds, C_{eq} is the equilibrium value, C_{B0} is the initial value, k_i' is the kinetic constant or constants of the degradation process and the energy density (E_v) is the independent variable, defined as reported in Eq. (2). In the PEF extraction processes, an overshoot was observed at low E_v values, allowing biocompound release into the extraction media. By contrast, with the increase of E_v, a progressive decrease of biocompound concentration was observed. It is assumed that the biocompounds extracted from PP can be divided in at least two categories, showing different sensitivity to the PEF treatment conditions, so that a two-term exponential decay model was proposed, which explains the biocompound degradation in our study (Eq. (10)):

$$C_B = C_{eq} + (C_{B0} - C_{eq}) \cdot \left(e^{-k_1' E_V} + e^{-k_2' E_V}\right) \quad (10)$$

where k_1' and k_2' are the degradation kinetic constants of the biocompounds with different sensitives to the applied E_v.

2.8. Statistical analysis

All determinations were expressed as the mean ± standard error of at least three repeated measurements from two experiment replicates. Statistical analysis was performed by using R v. 3.0.2 (The R Foundation for Statistical Computing). Bartlett's test was used to check the homogeneity of variance, one-way ANOVA was carried out and the Tukey test was used to determine statistically significant differences among means ($p < 0.05$). Experimental data were fitted to the kinetic expressions by non-linear regression procedures using TableCurve2D software (Jandel Scientific, ver. 5.01). The fittings were calculated at a 95% significance level and the goodness of fit was evaluated based on statistical parameters of fitting (R^2).

3. Results and discussion

3.1. Characterization of frozen and dried PP

In this study, extracts rich in antioxidant biocompounds were obtained from frozen and dried PP. Freezing or drying would be required in a realistic PP valorisation scenario to stabilize fresh PP and allow its further processing.

Control extracts were obtained from frozen and dried PP and their biocompound composition and antioxidant activity is reported in Table 1. Interestingly, the total phenolic content (TPC) of the extracts from dried PP (416 ± 7 mg GAE/100 g dm) resulted significantly higher ($p < 0.05$) than that of the frozen one (204 ± 4 mg GAE/100 g dm) (Table 1). These results are most likely attributable to the matrix drying treatment. The latter, in fact, not only increases the porosity, and thus the extractive surface, of the vegetable matrix (Londoño-Londoño et al., 2010), but is also responsible for the formation of thermal-induced novel compounds. In particular, dried PP extracts may include Maillard reaction derivatives, able to react with the Folin reagent used for TPC determination (Echavarria et al., 2013; Mrkìc et al., 2006). By contrast, total flavonoids (TF) resulted higher ($p < 0.05$) in the frozen PP extract (32 ± 3 mg QE/100 g dm) than in the dried PP (12.1 ± 0.8 mg QE/100 g dm) (Table 1). This can be due to flavonoid degradation during peach pomace air-drying, which has been observed in different vegetables (Sharma et al., 2015; Zainol et al., 2009). Moreover, anthocyanins (TA), and vitamin C (VIT C) content of the frozen PP extract were 0.30 ± 0.04 mg CGE/100 g dm and 61 ± 5 mg VIT C/100 g dm, respectively, while they were not detectable in the dried PP extracts. This is surely attributable to the fact that the high temperature applied (140 °C) to produce PP flour degraded these compounds. In this regard, Kara and Erçelebi (2013) found a 90% anthocyanin reduction in mulberry juice upon thermal treatment at 80 °C. Similarly, Vikram et al. (2005) reported a 50% VIT C degradation in orange juice upon only 3 min heating at 90 °C. These compounds are known to exert a prominent antioxidant activity (AA) and their almost complete absence in the dried PP extract strongly affected its AA (97 ± 9 mg TE/100 g dm), which resulted significantly lower ($p < 0.05$) than that of the frozen PP extract (131.0 ± 0.3 mg TE/100 g dm, Table 1).

Table 1
Biocompound concentration and antioxidant activity of control extracts obtained from untreated frozen and dried peach pomace. Data shown are a mean ± standard deviation. TPC: Total phenolic content (mg GAE/100 g dm); TF: Total flavonoid content (mg QE/100 g dm); TA: Total anthocyanin content (mg CGE/100 g dm); VIT C: Vitamin C (mg VIT C/100 g dm); AA: Antioxidant scavenging activity (mg TE/100 g dm) measured by DPPH assay; ND: not detectable.

Peach pomace	TPC	TF	TA	VIT C	AA
Frozen	204 ± 4	32 ± 3	0.30 ± 0.04	61 ± 5	131.0 ± 0.3
Dried	416 ± 7	12.1 ± 0.8	ND	ND	97 ± 9

3.2. Extraction assisted by conventional thermal treatment (CTT)

The treatment temperature for CTT assisted extraction was set at 50 °C, which is commonly indicated as the minimum temperature for thermal processes (Patras, Brunton, O'Donnell and Tiwari, 2010), in order to preserve as much as possible bioactive compounds from thermal damage.

Fig. 1 reports the evolution of biocompound concentration and antioxidant activity during CTT. For both frozen and dried PP, the biocompound concentration and the AA of the extracts increased with the extraction time up to a certain time, after which, a subsequent decrease in the extraction rate, until reaching a plateau value, was observed. The only exception was represented by VIT C, whose extraction was not promoted by CTT extraction, probably due to the thermal sensitivity of this biocompound (Rawson et al., 2011). This extraction pattern largely agrees with literature studies relevant to the extraction in hydro-alcoholic solutions (50–80% ethanol) of bioactive compounds from vegetable by-products including grape seeds and soybeans (Bucić-Kojić et al., 2007; Jokic et al., 2010). Similar results were also obtained by El Darra et al. (2018), who subjected PP (pressed skins and pulp residues obtained by peach processing into jams and purees) to ethanolic solid-liquid extraction at 50 °C in order to extract polyphenols. These authors, in fact, observed an initial fast extraction stage, followed by an almost stabilization of the extraction yield. The maximum biocompound concentration and the maximum antioxidant activity (C_{max}) obtained upon the CTT extraction procedure was identified based on ANOVA. To this aim, the biocompound concentration and AA values obtained upon increasing CTT time were compared in order to identify the treatment time leading to the significantly highest value. Since in all cases a plateau was reached, the C_{max} was identified as the first plateau value, beyond which a further increase in CTT time did not promote a statistically significant increase. The extract from frozen PP presented C_{max} values for TPC, TF, TA and AA of 400 ± 21 mg GAE/100 g dm, 147 ± 3 mg QE/100 g dm, 2.74 ± 0.09 mg CGE/100 g dm and 185 ± 2 mg TE/100 g dm, respectively, which resulted about 2.0, 4.7, 9.1 and 1.4 times higher than those of the corresponding control extract (Table 1). By contrast, the C_{max} value relevant to VIT C (61 ± 5 mg/100 g dm) resulted comparable to that of the control extract ($p \geq 0.05$, Table 1). In the case of CTT of dried PP extracts, the C_{max} values for TPC, TF and AA were 524 ± 27 mg GAE/100 g dm, 108 ± 1 mg QE/100 g dm and 143 ± 7 mg TE/100 g dm, respectively. Such values resulted 1.2, 8.9 and 1.5 times higher than those of control extracts (Table 1). In addition, in the case of dried PP, independently on CTT time, TA and VIT C were always not detectable.

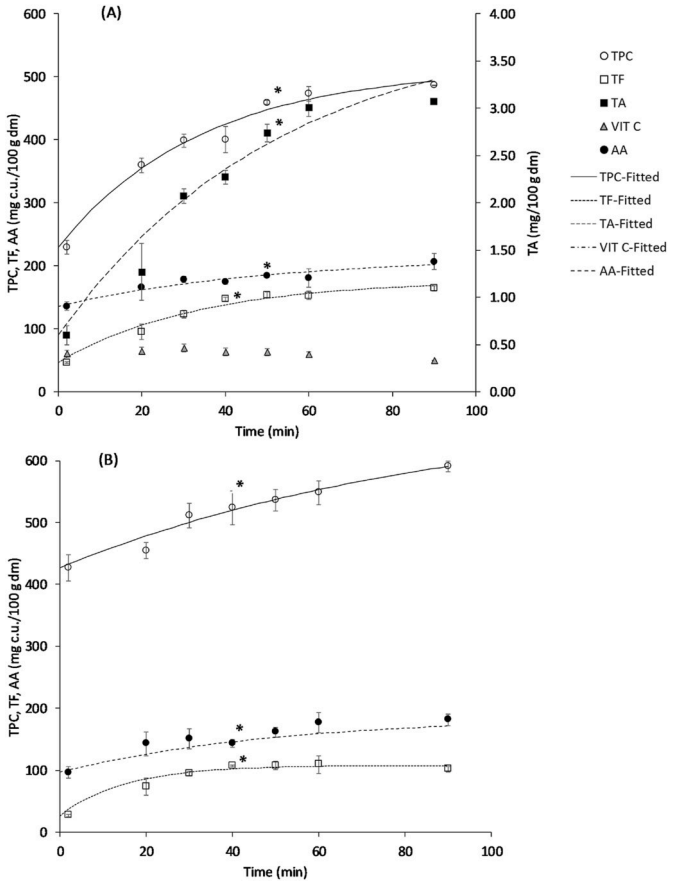

Fig. 1. Evolution of antioxidant biocompounds concentration assisted by CTT extraction process for frozen (A) and dried (B) peach waste. Data shown are a mean ± standard deviation. TPC: Total phenolic content (mg GAE/100 g dm); TF: Total flavonoid content (mg QE/100 g dm); TA: Total anthocyanin content (mg CGE/100 g dm); AA: Antioxidant scavenging activity (mg TE/100 g dm) measured by DPPH assay; c. u.: concentration units; * maximal concentration (C_{max}) identified by ANOVA.

The two-stage mechanism proposed in Eq. (6) well-fitted the obtained data in the CTT assisted extraction of biocompounds and their antioxidant activity, as it is shown in Fig. 1. In the first extraction stage, the solvent diffuses inside the cellular structure and biocompounds are extracted from the matrix, increasing their concentration in the extraction solvent; in the second one, an almost stabilization of the extraction yield is reached, which means that biocompound disappearance from the extract occurs concomitantly to extraction, possibly due to biocompound thermal degradation (Ibarz et al., 1999).

CTT extraction data were modelled using the kinetic model presented in Eq. (8), which well-fitted experimental data, with a R^2_{adj} higher than 0.90 (Table 2). The estimated parameters showed a value of K always higher than 1, indicating a predominance of extraction step (associated to the kinetic constant k_0) over thermal degradation (k_1). The latter seemed to occur similarly for all the biocompounds, as indicated by comparable k_1 values. By contrast, differences in extraction rates (k_0) were observed. In fact, in the case of frozen PP, TPC and TF showed k_0 (16 and 6 c. u./min) and consequently K values (507 ± 22 and 174 ± 9 c. u.) two order of magnitude higher than those of TA (k_0 = 0.084 c. u./min; K = 3.6 ± 0.5 c. u.) (Table 2), suggesting that TA were extracted according to a slower kinetics. Although the similar kinetic values observed in frozen and dried PP for both TPC and TF, the k_0 and k_1 of AA resulted two-folds higher in the case of frozen PP (k_0 = 10 c. u./min; k_1 = 0.054 ± 0.012 min^{-1}), indicating that both stages occurred more rapidly. This possibly suggests that the biocompounds extracted from frozen PP exerted a higher antioxidant activity as compared to those extracted from dried PP but were also more prone to degradation. During solid-liquid extraction, three stages take place: (i) solute phase change, during which the biocompounds pass from the solid phase to the liquid, where they are dissolved through the solid-liquid interface; (ii) diffusion of the solute in the solvent contained in the pores of the solid: the solute is transferred from inside the solid particle to its surface, due to the concentration gradient between the solid-liquid interface and the outer surface of the solid, according to Fick law; (iii) transfer of the solute from the surface of the particles to the sine of the solution, driven by a concentration gradient. Thus, it can be hypothesized that the matrix difference between frozen and dried PP affected the development of these solid-liquid extraction stages. In particular, differently from dried PP, where it is likely that all the 3 stages took place, the extraction from frozen PP is thought to occur mainly based on the second and third stage, being the biocompounds already dissolved in the aqueous fraction, and thus possibly more prone to degradation. Moreover, air-drying is known to cause thermal polymerization of phenolic compounds, leading to the formation of high molecular weight insoluble derivatives (Naczk and Shahidi, 2004). Despite this would reduce their solubility and AA activity, it would also stabilize them against degradation.

3.3. Extraction assisted by pulsed electric fields (PEF)

Extracts from frozen and dried PP were obtained applying PEF at field strengths from 0.8 up to 10 kV/cm and for increasing pulse numbers up to 30, leading to energy values up to 20 and 70 kJ/kg, for frozen and dried PP, respectively. As expected, PEF treatments did not induce significant increase in sample temperature ($p \geq 0.05$). Extraction data showing TPC, TF, TA, VIT C and AA as a function of the applied energy density are reported in Fig. 2.

As compared to the control extract (Table 1), the biocompound content and the AA of extracts from frozen and dried PP were significantly enhanced ($p < 0.05$) by PEF extraction at the lowest energy densities (E_v) values, i.e. in the range 0.02–0.07 and 0.06–0.26 kJ/kg for, respectively (Fig. 2). These E_v values were in fact associated to the maximum concentration of the considered biocompounds and

Table 2
Estimated kinetic parameters of CTT (Eq. (8)) to describe the assisted extraction process for frozen and dried peach pomace. Data shown are a mean ± standard error. TPC: Total phenolic content (mg GAE/100 g dm); TF: Total flavonoid content (mg QE/100 g dm); TA: Total anthocyanin content (mg CGE/100 g dm); AA: Antioxidant scavenging activity (mg TE/100 g dm) measured by DPPH assay; c.u.: concentration units.CTT model: $C_B = K - (K - C_{B0}) \cdot e^{-k_1 t}$

Peach pomace	*Biocompound/Antioxidant activity*	C_{BO} (c.u.)	K (c.u.)	k_1 (min^{-1})	k_0 (c.u./min)[a]	R^2
Frozen	TPC	212 ± 18	507 ± 22	0.032 ± 0.007	16	0.9797
	TF	35 ± 8	174 ± 9	0.034 ± 0.007	6.0	0.981
	TA	0.35 ± 0.24	3.6 ± 0.5	0.023 ± 0.008	0.084	0.9631
	AA	130 ± 5	186 ± 3	0.054 ± 0.012	10.0	0.9692
Dried	TPC	411 ± 17	574 ± 28	0.027 ± 0.011	15	0.9360
	TF	15 ± 8	110 ± 5	0.059 ± 0.014	6.5	0.9695
	AA	94 ± 10	189 ± 17	0.027 ± 0.012	5.2	0.9290

[a] Calculated as $K \times k_1$.

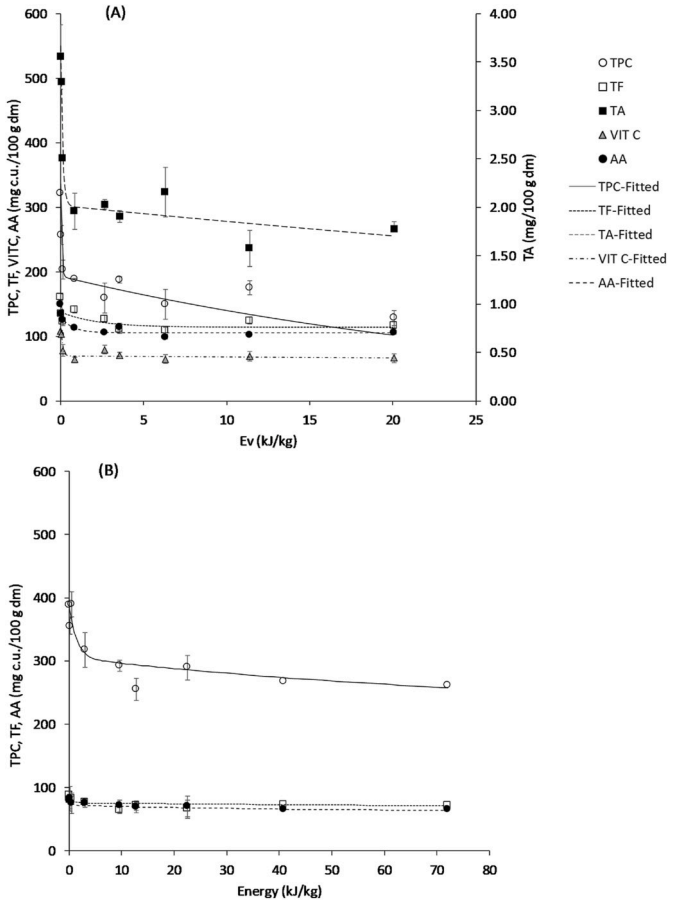

Fig. 2. Evolution of antioxidant biocompounds concentration assisted by PEF extraction process for frozen (A) and dried (B) peach pomace. Data shown are a mean ± standard deviation. TPC: Total phenolic content (mg GAE/100 g dm); TF: Total flavonoid content (mg QE/100 g dm); TA: Total anthocyanin content (mg CGE/100 g dm); Vit C: Total vitamin C content; AA: Antioxidant scavenging activity (mg TE/100 g dm) measured by DPPH assay; c. u.: concentration units.

antioxidant activity (C_{max}). These results can be explained based on the capacity of PEF to electroporate the PP vegetable tissue, leading to a faster solubilization of compounds in the extraction solvent (Donsì et al., 2010). By contrast, an increase in PEF E_v beyond these threshold values led to extracts with progressively lower biocompound concentration and AA, until reaching a plateau value (Fig. 2). For example, the application of an E_v of 0.02 kJ/kg to the frozen PP resulted in TPC, TF, TA, VIT C and AA values about 1.6, 5.1, 11.8, 1.8 and 1.1 times higher than those of the corresponding control extract (Table 1), while an increase of E_V up to 70 kJ/kg resulted in extraction values comparable or even lower than those of the control extract. The degradation of both anthocyanins and vitamin C has been reported to increase with the increase of PEF treatment intensity in previous studies. Odriozola-Serrano et al. (2008) noticed a significant decrease in VIT C content of tomato juice with the increase of both PEF treatment time and electric field strength. Similar results were also observed by Zhang et al. (2007) on the degradation of cyanidin-3-glucoside in a model system. By contrast, it must be noted that these results were not expected in the case of other phenolic compounds (TPC and TF), since most of literature studies relevant to PEF extraction of bioactive compounds from waste materials found that PEF extractive efficacy progressively increases with the overall PEF energy, due to the progressive tissue disintegration, (Donsì et al., 2010; Knorr and Angersbach, 1998; Kumari et al., 2018). This difference with the literature suggests that, in the present study, the TPC and TF biocompounds of the PP were more sensitive to PEF degradation. To our knowledge, no data are available on the effect of PEF on an already strongly damaged tissue. In most studies aiming at extracting biocompounds from vegetable discards, the waste material is freshly prepared in laboratory conditions, or freeze-dried, which would guarantee a low cellular damage. By contrast, in the present work, the considered waste material was either frozen or air-dried and ground to flour. It can be inferred that these waste treatments altered the stability of biocompounds by changing their compartmentalization in the cellular tissue. Freezing and drying, in fact, strongly damage cells, favouring the release of intracellular compounds, possibly making them more sensitive to PEF conditions (Karam et al., 2016; Xu et al., 2017). This hypothesis is supported by literature studies showing that the increase in PEF-induced bioactive release is commonly associated to a more pronounced degradation of the extracted biocompounds upon further storage (Leong et al., 2016).

Based on these considerations, the extraction model reported in section 2.7.2 was proposed. According to this mechanism, the degradation of biocompounds upon PEF treatments beyond a threshold E_v value, can be modelled using the two-terms degradation model described by Eq. (10), which adequately fitted experimental data, as indicated by the high R^2 (Table 3).

The proposed degradation model assumes that the PP biocompounds can be classified into two major classes, showing different sensitivity to degradation. In particular, the more labile compounds are identified by the degradation constant $k_1^{'}$ (ranged from 0.22 to 9.2 kg/kJ), which resulted much higher than that of the second class of biocompounds, which are the more resistant and are identified by the constant $k_2^{'}$ (ranged from 0.001 to 0.012 kg/kJ) (Table 3). It can be noted that the $k_2^{'}$ values obtained in frozen and dried PP resulted comparable; by contrast, $k_1^{'}$ values resulted higher for frozen matrix, indicating a more rapid degradation kinetics. As already observed in the case of CTT, this can be explained based on the fact that the biocompounds present in frozen and

Table 3
Estimated kinetic parameters of PEF (Eq. (10)) to describe the assisted extraction process for frozen and dried peach pomace. Data shown are a mean ± standard error. TPC: Total phenolic content (mg GAE/100 g dm); TF: Total flavonoid content (mg QE/100 g dm); TA: Total anthocyanin content (mg CGE/100 g dm); VIT C: Vitamin C (mg/100 g dm); AA: Antioxidant scavenging activity (mg TE/100 g dm) measured by DPPH assay; c.u.: concentration units.PEF model: $C_B = C_{eq} + (C_{B0} - C_{eq}) \cdot (e^{-k_1^{'}E_V} + e^{-k_2^{'}E_V})$

Peach waste	*Biocompound/Antioxidant activity*	C_{B0} (c.u.)	C_{eq} (c.u.)	$k_1^{'}$ (kg/kJ)	$k_2^{'}$ (kg/kJ)	R^2
Frozen	TPC	371 ± 32	182 ± 10	15 ± 4	0.012 ± 0.006	0.9516
	TF	176 ± 17	117 ± 6	17 ± 9	0.0010 ± 0.0095	0.8603
	TA	3.9 ± 0.3	2.01 ± 0.13	8.2 ± 3.0	0.008 ± 0.007	0.9422
	VIT C	119 ± 11	70 ± 5	9.2 ± 4.5	0.003 ± 0.009	0.8788
	AA	156 ± 8	109 ± 3	8.5 ± 3.4	0.007 ± 0.008	0.9300
Dried	TPC	386 ± 13	278 ± 18	0.31 ± 0.19	0.002 ± 0.004	0.9221
	TF	86 ± 3	68 ± 5	0.31 ± 0.29	0.0010 ± 0.0061	0.7616
	AA	80 ± 2	70 ± 3	0.22 ± 0.19	0.008 ± 0.008	0.9156

dried PP show differences in terms of both solvation and solubility. In particular, the higher solvation and solubility of biocompounds in frozen PP would account for their higher sensitivity to degradation.

3.4. CTT and PEF extraction efficacy

The C_{max} values were identified by ANOVA in the case of CTT (see section 3.2) and by the model C_{B0} value in the case of PEF (Table 3). These values were used to calculate the CTT and PEF extraction efficacy according to Eq. (5) (Table 4). A CTT of at least 40–50 min was needed for reaching the C_{max} in both frozen and dried PP and, beyond this time, no further significant increase was observed. The TF extraction efficacy was 367 ± 11 and 790 ± 12 for frozen and dried PP, respectively, which resulted much higher as compared to the extraction efficacy relevant to TPC (frozen: 96 ± 10; dried: 26 ± 7) and AA (frozen: 41.1 ± 1.3; dried: 48 ± 6) in both matrices. The extraction efficacy of TA from frozen PP resulted particularly high (809 ± 30) while VIT C was not effectively extracted by CTT, giving an extraction efficacy of 0.25 ± 0.03.

Regarding PEF extraction efficacy, the minimum E_V was identified as the condition allowing the C_{max} (corresponding to the C_{B0} from Table 3) to be obtained, which were used to estimate the extraction efficacy (Table 4). In the case of dried PP, although PEF treatments resulted in extracts presenting TPC and AA values not significantly different from those of control extract ($p \geq 0.05$), as indicated by the extraction efficacies (−7 ± 5 and −17 ± 8, respectively), they were quite effective in the extraction of TF (extraction efficacy of 621 ± 51). In the case of frozen PP, PEF resulted in a good extraction efficacy of TPC (57.3 ± 0.4), TF (409 ± 47), VIT C (77 ± 3), AA (14 ± 5), but especially of TA (1080 ± 110).

As compared to CTT extraction efficacy, PEF treatments resulted more efficient ($p < 0.05$) in the extraction of TF, TA and VIT C from frozen PP. In addition, it must be underlined that PEF treatments allowed obtaining C_{max} extraction efficacy higher than those of CTT in a much shorter time. In this sense, the minimum E_V intensity was delivered by 4-pulse treatments, corresponding to 16 μs of actual treatment. In this regard, PEF treatments have been largely shown to present a higher efficiency as compared to traditional extraction (Donsì et al., 2010). In this sense, PEF treatments have been successfully applied for the valorisation of vegetable waste streams into extracts presenting a commercial interest, such as pigments, antioxidants, and flavours. For example, PEF treatments at 1–5 kV/cm have been applied to tomato juice waste leading to an extraction efficacy around 45% of β-carotene (Andreou et al., 2020; Pataro et al., 2019). Similarly, Peiró et al. (2019), and Frontuto et al. (2019) found that PEF treatments at field strength lower than 10 kV/cm enhance polyphenol extraction from lemon residues and potato peels by 300% and 10%, respectively.

Table 4
Extraction efficacy of the conventional thermal treatment (CTT) and pulsed electric fields (PEF) assisted extraction process for frozen and dried peach pomace. Data shown are a mean ± standard error. TPC: Total phenolic content (mg GAE/100 g dm); TF: Total flavonoid content (mg QE/100 g dm); TA: Total anthocyanin content (mg CGE/100 g dm); VIT C: Vitamin C (mg/100 g dm); AA: Antioxidant scavenging activity (mg TE/100 g dm) measured by DPPH assay.

Peach pomace	*Biocompound/ Antioxidant activity*	Extraction efficacy (%)	
		CTT	PEF
Frozen	TPC	96 ± 10	57 .3 ± 0.4
	TF	367 ± 11	409 ± 47
	TA	809 ± 30	1080 ± 110
	VIT C	0.25 ± 0.03	77 ± 3
	AA	41.1 ± 1.3	14 ± 5
Dried	TPC	26 ± 7	−7 ± 5
	TF	790 ± 12	621 ± 51
	AA	48 ± 6	−17 ± 8

4. Conclusions

The results obtained in this study allow concluding (under the experimental conditions in this work) that the recovery of biocompounds from peach pomace, assisted by conventional thermal treatment (CTT) or pulsed electric fields (PEF) can be adequately explained by the proposed kinetic mechanistic and empirical models, respectively, which result to be feasible tools to understand the involved phenomena and predict the assisted extraction results.

Process requirements should be accurately evaluated in order to select the more advantageous extraction procedure. In this regard, although drying would allow reducing waste volumes and avoiding cold-chain storage, freezing better preserves the biocompounds originally present in the peach pomace. Additionally, it must be underlined that PEF treatments would require extraction times in the order of μs, which is much shorter than the time required for thermal extraction (40 min), reaching similar extraction efficiencies. Thus, in the optic of developing an industrial process for peach waste valorisation, PEF would allow a significant reduction in extraction time. Hence, under the considered experimental conditions, PEF E_V values between 0.02 and 0.06 kJ/kg were shown to allow reaching the maximum biocompound extraction from peach pomace. Such values resulted extremely lower than the E_V estimation for CTT (89 kJ/kg). Nevertheless, the process should be properly optimized in order to avoid a fast degradation of the extracted biocompounds. In particular, it seems that the characteristics of the vegetable matrix before extraction play a key-role in determining PEF extraction efficacy.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

S. Plazzotta: Investigation, Visualization, Formal analysis, Writing - original draft, Writing - review & editing. **R. Ibarz:** Conceptualization, Methodology, Supervision, Visualization, Formal analysis, Writing - original draft, Writing - review & editing. **L. Manzocco:** Supervision, Writing - review & editing. **O. Martín-Belloso:** Supervision, Funding acquisition, Project administration, Writing - review & editing.

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