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Impact of texturing using instant pressure drop treatment prior to solvent extraction of anthocyanins from Malaysian Roselle (*Hibiscus sabdariffa*)

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ABSTRACT

Because of the natural structure of plants, which oppose resistance to penetration by any liquid, solvent extraction is very slow. The kinetics involves multiple serial steps and generally internal diffusion is the limiting process. To improve the technological aptitude, and intensify solvent extraction, we propose to submit plants to a treatment by thermo-mechanical Instant Controlled Pressure Drop (DIC), in order to ensure the expansion of the plant structure. In this paper, we present the results of a study concerning the effect of DIC treatment on the extraction of Roselle anthocyanins. By identifying the effect of various operative parameters, we found that an appropriate DIC treatment improved both the kinetics and yield of extraction of anthocyanins from Roselle calyces. By studying extraction kinetics, we identified and quantified the effective diffusivity, which ranged from 4.62 to 6.11 $\times 10^{-11}$ m^2 s^{-1} for DIC treated material compared with 4.19 $\times 10^{-11}$ m^2 s^{-1} for raw material.

1. Introduction

Hibiscus sabdariffa, commonly known as Roselle or red Sorrel, is widely grown in Central and West Africa and South-East Asia. The red and fleshy cup-shaped flower calyces are consumed worldwide as a cold beverage or a hot drink (Paul, 1995). These extracts are also used in folk medicine to treat many complaints that include high blood pressure, liver disease and fever (Wang et al., 2000). The positive physiological effect of this plant extract could be related to the presence of anthocyanins with a potent antioxidant activity.

Anthocyanins are one of the most important groups of water soluble pigments visible to the human eye. They are responsible for many of the attractive colors, from scarlet to blue, of flowers, fruits, leaves and storage organs (Brouillard, 1993; Harborne, 1967). Chemically, anthocyanins are flavonoids, and consequently are built on a C15 skeleton with a chromane ring bearing a second aromatic ring B in position 2 (C6–C3–C6), with one or more sugar molecules bonded at different hydroxylated positions on the basic structure. Glycosylation and acylation of the aglycone moieties by different sugars and acids at different positions account for the broad structural diversity of these pigments (Brouillard, 1982; Markakis, 1982). *Hibiscus* anthocyanins were identified as having Delphinidin-3-sambubioside (Dp-3-sam) (70% of the anthocyanins) and Cyanidin-3-sambubioside (Cyn-3-sam) as the major pigments, with Delphinidin-3-glucoside (Dp-3-glu) and Cyanidin-3-glucoside (Cyn-3-glu) as the minor ones (Bridle & Timberlake, 1997; Du & Francis, 1973; Frank et al., 2005).

Plant materials generally contain a small amount of high added value active solute. Extraction and purification of bioactive compounds from natural sources have become very important for the utilization of phytochemicals in the preparation of dietary supplements or neutraceuticals, functional food ingredients and food additives, and pharmaceutical and cosmetic products. The extraction of anthocyanins is commonly carried out under cold conditions using methanol or ethanol containing a small amount of acid in order to obtain the flavylium cation form, which is red and stable in a highly acid medium (Du & Francis, 1973; Mazza & Miniati, 1993; Métivier et al., 1980; Pifferi & Vaccari 1983). Heated water is also used (Tsai et al., 2002). However, acid may cause partial hydrolysis of the acyl moieties in acylated anthocyanins, especially in anthocyanins acylated with dicarboxylic acids such as malonic acid.

The extraction kinetics involves multiple steps. However, the main part of the operation is limited by diffusion because the natural structure of plants opposes a resistance to any penetration by a liquid; thus the process is very slow.

To intensify this type of operation, a solvent is sometimes chosen in order to improve diffusion. Similar considerations would need to be taken into account even with a supercritical fluid as a solvent. To improve the technological aptitude of raw material for extraction, the initial structure may be modified by cutting, grinding, etc. In our laboratory, we proposed to carry out studies concerning the effect of structure expansion. In several cases, we

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proved that the higher the expansion rate, the greater the diffusivity constant. So, we applied a swelling operation using the well-known "Instant Controlled Pressure-Drop" process (DIC®: Détente Instantanée Contrôlée), which was developed in our laboratory and has been used for some years (Allaf & Louka, 1994; Allaf & Vidal, 1989). First, this process was used to swell-dry various fruits and vegetables; it ensures a high quality by improving both the kinetics and the capacity for dehydration and rehydration. DIC treatment is based on fundamental studies concerning the thermodynamics of instantaneity (Allaf, 2002). It consists of a thermo-mechanical processing induced by subjecting the product to an abrupt transition from a high steam pressure to close to a vacuum. This process is also used for the extraction of essential oils and other volatile molecules by instant autovaporization (Allaf & Louka, 1999); this gives the plant (seeds, fruits, vegetables) a higher overall diffusivity and improves the availability of some of its compounds.

The aim of this work was to study the impact of texturing by DIC treatment on the aqueous anthocyanin extraction from Roselle calyces, in terms of comparative kinetics and yield for treated and untreated Roselle calyces.

2. Experimental protocol

2.1. Chemicals and standards

The dried Roselle calyces used in this study were from Malaysia. The deionised water used for the extraction was prepared with a GFL Deioniser (Germany). The solvents used for extraction (ethanol, methanol) were purchased from Carlo Erba. Analytical grade sodium acetate trihydrate, potassium chloride and formic acid were purchased from Merk (Germany). Acetonitrile and water were of HPLC grade. Cyanidin-3-O-glucoside chloride and Delphinidin-3-O-glucoside chloride were obtained from Extrasynthese (Lyon, France).

2.2. Measurement of moisture content

The moisture content of the samples was determined using the oven-dry method. 2 g of each sample was placed in a glass and was dried for 24 h at 105 °C. The initial water content of the dried calyces of Roselle was 12.56% Dry Basis (DB).

2.3. DIC process

2.3.1. Experimental set up

The experimental set up was largely described previously (Allaf & Vidal, 1989; Louka & Allaf, 2004) It is composed of three main elements (Fig. 1):

- The processing vessel (1) where we place and treat the samples.
- The vacuum system, which consists mainly of a vacuum tank (2) with a volume 130 times greater than the processing vessel, and an adequate vacuum pump. The initial vacuum level was maintained at 5 kPa in all the experiments.
- A pneumatic valve (3) that ensures the connection/separation between the vacuum tank and the processing vessel. It is capable of producing the abrupt pressure drop within the reactor in less than 0.2 s ($\Delta P/\Delta t > 0.5$ MPa s^{-1}).
- Fig. 1.

2.3.2. DIC treatment

Dried Roselle calyces are first placed in the DIC treatment vessel where a first vacuum stage is established in order to reduce the resistance vis-à-vis the steam diffusion, which acts as the heating fluid through the plant material, and consequently improve heat transfer. After closing the pneumatic valve, high pressure steam is injected into the reactor and maintained during the treatment. The thermal treatment is followed by an abrupt pressure drop towards a vacuum. The resulting autovaporisation induces an "instant" cooling of the solid material. After the treatment, the Roselle calyces were recovered and were ready for extraction.

2.4. Experimental design

To reduce the experimental points needed to carry out studies concerning the effects of the main operative parameters (steam pressure P and processing time t), we used a 2 variable central composite rotatable experimental design. In this case, this design needs 11 experiments with 3 repetitions for the central point. The experiments were run at random in order to minimize the effects of unexpected variability in the observed responses due to extraneous factors.

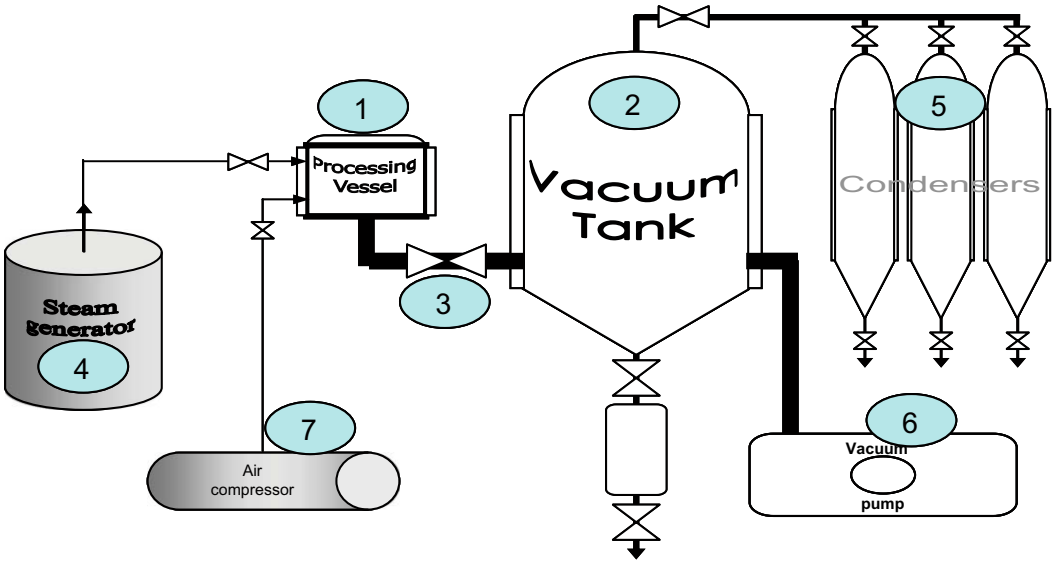

Fig. 1. Schematic diagram of the DIC reactor. 1: Processing vessel; 2: Vacuum tank; 3: Quick motion valve; 4: Steam generator; 5: condensers 6: Vacuum pump; 7: Air compressor.

The responses were obtained by using the analysis design procedure of Statgraphics plus for Windows (1994-4.1 version). Table 1 shows the factorial design matrix, with real parameters.

2.5. Extraction of anthocyanins

Water was used as a solvent and the extraction was performed with stirring in a batch extractor. The batch extraction system used in this study was composed of a 250 ml round bottomed flask with a three-necked top, a magnetic stirrer and a boiler. The batch extractor was first filled with 200 ml of solvent, the contents heated to 100 °C and then a pre-weighed amount of dried Roselle calyces (2 g) was added at time $t = 0$. The flask temperature was controlled with a thermometer. At specific time intervals, samples of 3 ml were taken from the solution in the batch extractor and filtered with a 0.45 μm syringe filter before analysis.

2.6. Determination of total anthocyanin content

2.6.1. Calibration curve

A UV–Vis spectrophotometer at 520 nm was used to determine the absorbance of an acidified aqueous (1% HCl) solution of Dp-3-glu with concentrations of 5, 10, 25 and 50 mg/l. By plotting concentration (mol/l) against absorbance, the molar absorption coefficient of Dp-3-glu was calculated to be 27481 l mol^{-1} cm^{-1} (Giusti M., 2001).

2.6.2. Quantification of total monomeric Anthocyanins

The Total Monomeric Anthocyanin content (TMA) of dried Roselle calyces was determined using the pH differential method described by Giusti and Wrolstad (2001). A Helios β UV–Vis spectrophotometer and 1 cm path length glass cells were used for spectral measurements at 520 and 700 nm respectively, using distilled water as a blank. For this purpose, aliquots of Roselle extract were brought to pH 1.0 and 4.5; 20 min later, the absorbance of each equilibrated solution was measured at the maximum absorption wavelength; λ_{max} = 520 nm and 700 nm for haze correction. The difference in absorbance values at pH 1.0 and 4.5 was directly proportional to the Total Monomeric Anthocyanin concentration, which was calculated based on Delphinidin-3-glucoside (Dp-3-glu), with a molecular weight of 465.2 g/mol and a molar absorption coefficient of 27481 l mol^{-1} cm^{-1}. The pH measurements were carried out using a Denver Instrument company Model 15 pH-meter calibrated with buffers at pH 4, 7 and 10.

2.7. High performance liquid chromatography; sample preparation

2.7.1. Calibration curve

Reference standard solutions for Dp-3-glu and Cyn-3-glu were prepared first followed by solutions of Dp-3-glu and Cyn-3-glu in 1% HCl in water at a concentration ranging between 5 and 100 mg/l. A linear regression analysis was carried out on the data of the peak area versus concentration. A linear calibration was obtained which was more than 99.5% accurate for the two standards.

2.7.2. HPLC-PDA

HPLC analysis was carried out on a 1100 Agilent High Performance Liquid Chromatography system (HPLC) equipped with a quaternary pump, an autosampler and a photodiode array detector (DAD) using a 250 × 4.6 mm Kromasil (5 μm) column (Chromoptic) and a security guard cartridge (Phenomenex). The column temperature was maintained at 40 °C to obtain the best resolution and a stability of the retention time. Compounds were separated by gradient elution using formic acid: water (10:90, v/v) (solvent A) and HPLC grade acetonitrile (solvent B) with the following gradient: 0 min, 5% B; 20 min, 24% B followed by a post run of 2 min. The solvent flow rate was 1 ml/min. Detection was performed at 520 nm. The injection volume was 20 μl.

2.7.3. HPLC–MS

High performance liquid chromatography (HPLC) (Agilent) coupled to photodiode array (PDA) detection and electrospray ion trap mass spectrometry (ESI-MS/MS) on a triple quadrupole instrument was used for the identification of the anthocyanins in the extracts prepared from Roselle calyces. The MS parameters were as follows: positive mode; skimmer 1; dry gas (N_2) temperature, 350 °C; flow, 15 l/min; nebuliser, 60 psi; scan range m/z 100–800; the separations were performed on a 250 × 4.6 mm, 5 × m Kromasil C18 column (Chromoptic). The mobile phase was: solvent A (water: acetic acid; 99:1, v/v) and solvent B acetonitrile at a flow rate of 0.5 ml/min and a linear gradient of A/B from 95:5 to 76:24 in 20 min, and then back to the initial conditions.

2.8. Determination of anthocyanin diffusivity

A specific fundamental study of the kinetics of solvent extraction was defined by Ben Amor and Allaf (2007). This study provided different solutions for the diffusion equation, depending on the initial and boundary conditions. Using Fick's second law, a

Table 1
Experimental and calculated data of the composite central design; Values of Y_0, Y_∞, k_{obs}, and D_{eff}; TMA, Dp-3-sam and Cyn-3-sam amount, and relative percent of each individual anthocyanin of total anthocyanins determined by HPLC.

Run	1	2	3	4	5	6	7	8	9	10	11	RM
Pressure (MPa)	0.18	0.18	0.09	0.09	0.14	0.14	0.2	0.07	0.14	0.14	0.14	–
Processing time (s)	26	9	26	9	30	5	18	18	18	18	18	–
TMA[a]	10.61	11.72	11.59	10.33	11.67	10.32	8.96	9.64	10.61	10.02	10.94	8.82 ± 0.23
Improvement rate of TMA yields (%)[b]	122	135	133	119	134	124	103	111	122	124	126	100
Dp-3-sam[a]	5.74	6.39	5.22	6.23	5.03	5.47	5.44	4.36	5.44	4.99	5.29	5.39
Cym-3-sam[a]	257	256	2.18	2.02	2.07	2.01	2.14	1.63	2 37	1.93	2.06	2.03
Dp-3-sam (%)[c]	69	71	71	76	71	73	72	73	70	72	72	73
Cyn-3-sam (%)[c]	31	29	29	24	29	27	28	27	30	28	29	27
Y_0	1.99	2.7	2.91	1.5	2.42	1.68	2.44	1.93	1.36	2.24	1.74	1.74
Y_∞	10.76	11.91	11.75	10.48	11.83	10.9	9.08	9.82	10.76	10.96	11.09	8.82 ± 0.23
Rate of yield Improvement (%)	122	135	133	119	134	124	103	111	112	124	126	100
k_∞ (10^{-2} s^{-1})	41.6	37.5	45.8	39.5	40.5	46.5	38	43	38	44.8	49.6	34
dp (10^{-3} m)	0.135	0.135	0.135	0.135	0.135	0.135	0.135	0.135	0.135	0.135	0.135	0.135
D_{eff}(10^{-11} m2 s^{-1})	5.13	4.62	5.64	4.87	4.99	5.73	4.68	5.3	4.68	5.52	6.11	4.19

[a] mg/g DM.
[b] TMA of trailed material/TMA of untreated material) × 100.
[c] Reported to total anthocyanins by HPLC.

number of mathematical solutions may be proposed: series or error functions for slow diffusion times, or trigonometric series or Bessel functions for faster diffusion times. While seeking to find a solution to all of the extraction processes (as a continuous liquid phase) within the solid matrix, we adopted the solution suggested by Crank according to the geometry of the solid matrix (Crank 1975):

$$\frac{X - X_r}{X_m - X_o - X_r} = \sum A_i \exp(-q_i^2 \tau) \quad (1)$$

where X, X_m and X_r are the amounts of anthocyanins (mg/g MS) in the solid matrix at time t, at initial time ($t = 0$) and at a very long time ($t \to \infty$) respectively; X_0 is calculated as the amount of anthocyanins in the solid matrix (mg/g MS), which corresponds to the quantity available on the surface and extracted from it in a very short time (evaluated by extrapolation as at $t = 0$). By texturing, breaking the cell wall, improving porosity, the values of X_m and X_o vary and may be considered as the most important response parameters characterizing DIC treatment,. A_i et q_i: coefficients given according to the geometry of the solid matrix; τ: Fick's number = $D_{eff} \times t/d_p^2$; D_{eff}: Effective diffusivity (m^2/s); d_p: the characteristic length (m), which was, in our case, the half thickness of the sample.

Having to start from this fundamental analysis, we expressed the amount X of anthocyanins in the solid, according to the extracted quantity Y, which is measurable. Eq. (1) then becomes:

$$\frac{Y_\infty - Y}{Y_\infty - Y_0} = \sum_{=1}^{\infty} A_i \exp(-q_i^2 \tau) \quad (2)$$

where Y is the amount of anthocyanins extracted at time t, Y_∞ the value of Y at $t \to \infty$ which is the Y value at equilibrium, Y_0 is the amount of anthocyanins extracted at $t \to 0$ from the surface sample, mainly by convection

In the case of an infinite plate, Eq. (2) becomes:

$$y = \frac{Y_\infty - Y}{Y_\infty - Y_0} = \sum_{1}^{\infty} \frac{8}{(2i-1)^2 \pi^2} e^{-\frac{(2i-1)^2 \pi^2 D_{eff} t}{4dp^2}} \quad (3)$$

where y is the anthocyanins ratio; D_{eff} is the effective diffusivity ($m^2\ s^{-1}$) and l is the characteristic length (m), the half thickness of the sample.

Eq. (3) could be expressed by limiting it to the first term; the kinetics curves of the aqueous anthocyanin extraction follow an exponential pattern according to the equation:

$$\frac{Y_\infty - Y}{Y_\infty - Y_0} = A\ exp(-kt) \quad (4)$$

By using the logarithmic representation, a straight line could be defined whose slope is:

$$k = \frac{\pi^2 D_{eff}}{4dp^2} \quad (5)$$

from which D_{eff} could be determined.

3. Results and discussion

3.1. Extraction kinetics

3.1.1. Spectrophotometric investigation

We determined the Total Monomeric Anthocyanins extracted (TMA) using the spectroscopic pH differential method (Giusti & Wrolstad, 2001). For the untreated materials, all the analyses were performed in triplicate.

In order to carry out the study on the extraction kinetics of anthocyanins from untreated Roselle calyces using boiling water, the Total Monomeric Anthocyanins extracted (TMA) was plotted as a function of time. The plot shows that the TMA rises and asymptotically approaches a limiting equilibrium concentration (Y_∞). The shape of this curve closely resembles those already published for similar infusions (Jaganyi & Price, 1999).

It was shown that the maximum Total Monomeric Anthocyanins extracted from untreated Roselle calyces was about 8.82 ± 0.23 mg/g of dried matter (DM). The comparison between the extraction kinetics of anthocyanins from the untreated samples and in the different DIC experiments shows that there is an improvement in the extraction kinetics in all the DIC experiments and that the maximum TMA extracted from dried Roselle calyces was obtained within a short time (between 3 and 6 min) compared to untreated calyces (about 10 min). Fig. 2 shows the comparison between the extraction kinetics of TMA from untreated calyces and DIC treated calyces from experiments 2, 3 and 5. It is clear from the results of experiment 2 that the same amount was obtained in 3 min as that obtained in 10 min with untreated calyces.

The value of Y obtained by extrapolation at $t \to 0$ and the 10 min Y value were used as Y_o and Y_∞ respectively to model the extraction process and to estimate the observed rate constant (k_{obs}). Therefore, the corrected values of Y_∞, Y_o and Y and the TMA at time t were fitted from Eq. (3) by getting the first approximation order into Eq. (6):

$$\mathrm{Ln}\frac{Y_\infty - Y}{Y_\infty - Y_0} = -k_{obs} t \quad (6)$$

The kobs values obtained from the different runs under various DIC conditions are shown in Table 1. These values (Y_o, Y_∞ and k_{obs}) are different for the non DIC treated material and the materials that underwent diverse DIC treatments; this is due to the increase in availability of anthocyanins and the modification of porosity by DIC treatment.

For all DIC treated material, the values of Y_∞, which expresses the yield of the overall extraction process, were systematically higher than that obtained with untreated material, and in some cases were 135% higher. Similar observations can be made with the quantity of Y_0 for almost all DIC treated materials, showing that DIC treatment results in more readily available soluble compounds at the surface of natural plants at $t \to 0$. Finally, the k_{obs} values were systematically higher than those obtained with untreated material.

The observations concerning Y_0 correlate perfectly with those described by some authors (Jaganyi & Mdletshe, 2000; Jaganyi & Wheeler, 2003; Price & Spitzer, 1994; Spiro & Lam, 1995) who postulated that the value of the compound content intercepted at $t = 0$ was affected by the loss of soluble molecules from the material structure and its uptake of water at the beginning of the infusion process, before the diffusion processes start. Therefore, Y_0 serves

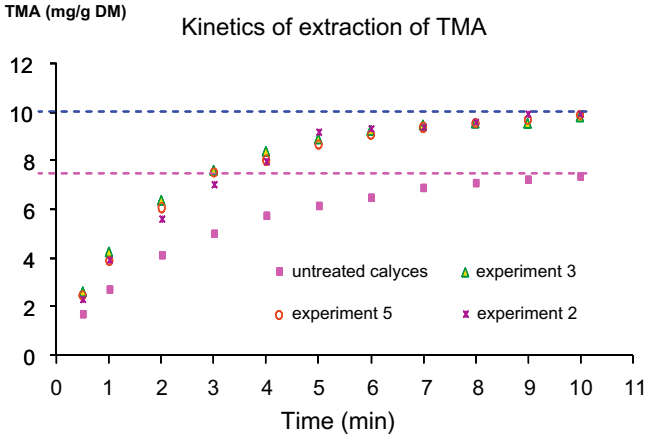

Fig. 2. Kinetics of extraction of Total Monomeric Anthocyanins (TMA) from untreated and Instant Pressure Drop (DIC) treated Roselle.

as one of the most important indicators of the technological aptitude of a product to be treated for extraction, and not as an indicator of the quality of the data and the deviation from the model employed, as previously suggested (Price & Spitzer, 1994).

To get a better understanding of the impact of texturing by DIC in terms of the overall diffusion as the limiting process of the extraction kinetics, we tried to determine the D_{eff} values. Indeed, it's generally assumed that, for extraction from plant matrixes, as soon as external resistance is negligible, the diffusion of a solvent into the plant structure and, possibly the diffusion of soluble molecules in the solvent must be at their slowest and so constitute the limiting process. Assuming that D_{eff} is constant with concentration, we could use Eq. (6) connecting k_{obs} with D_{eff}. Table 1 shows the D_{eff} values for the raw material and DIC treated materials.

As seen in Table 1, the effective diffusivity systematically increased with DIC treatment compared to the raw material, obviously because of the porosity and expansion generated through texturing by DIC treatment.

3.1.2. HPLC analysis

3.1.2.1. Optimization of HPLC separation. In this investigation, the anthocyanins were also determined by the HPLC method. HPLC has many advantages because it allows anthocyanins to be determined separately, and quantified using Cyn-3-glu and Dp-3-glu as standards.

3.1.2.2. Linearity of the HPLC method. The selection of the HPLC conditions was guided by the requirement to obtain chromatograms with better resolution of adjacent peaks within a short time of analysis. Mixtures of acetonitrile-water/formic acid and acetonitrile-water/phosphoric acid were tested. Under the optimal chromatographic conditions used in this study, the two calibration curves exhibited good linear regression (0.9998 and 1, respectively).

3.1.2.3. Identification of anthocyanins. Four anthocyanins were identified in previous studies carried out on the calyces of *H. sabdariffa* (Du & Francis, 1973; Karawya et al., 1975; Tsai et al., 2002) including two major species: Dp-3-sam and Cyn-3-sam, which represent more than 85% of the total anthocyanins.

The HPLC profile of the aqueous extract of Roselle had four anthocyanin peaks, which had absorption maxima in the range 510–530 nm. The major anthocyanin peaks were identified by their HPLC elution order, UV–Vis and mass spectrometric characteristics from a comparison with the data in the literature (Fig. 3).

The HPLC–MS chromatogram of peak 1 shows the presence of three main fragment ions at (*m/z*) 597, and 465, and a fragment with 303, which corresponds to the molecular ion of the Delphinidin aglycone resulting from the loss of a hexose (162). The visible maximum absorbance wavelength (λ_{max}) at 526 nm supported the identity of the anthocyanidin. These mass spectra indicated that peak 1 was a Delphinidin diglycoside.

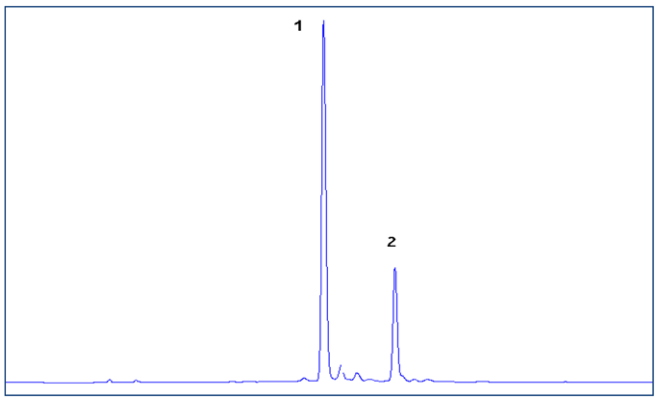

Fig. 3. Chromatogram obtained by HPLC-PDA, of the aqueous extract of anthocyanins from Roselle.

A mass number of *m/z* 581 was found in the LC/MS spectrum of peak 2, and mass numbers of 449 and 287 were obtained in the MS/MS spectrum. It was expected that the *m/z* 287 would correspond to the molecular ion of the cyanidin aglycone and these mass spectra indicated that peak 2 was a cyanidin diglycoside. According to these mass spectra, UV–Vis spectra and published data, peaks 1 and 2 were identified as Delphinidin-3-sambubioside and Cyanidin-3-sambubioside respectively.

3.1.2.4. Extraction kinetics of individual anthocyanins. The concentrations of different individual Roselle anthocyanins were determined by HPLC by comparing them to standards: Dp-3-sam was quantified using Dp-3-glu as a standard and Cyn-3-sam with Cyn-3-glu as a standard. Total anthocyanins obtained with the HPLC method were determined as a sum of the two individual anthocyanins.

The extraction kinetics of Roselle anthocyanins (Dp-3-sam and Cyn-3-sam) from the untreated Roselle calyces are presented in Fig. 4.

It was shown that the extraction kinetics of each individual anthocyanin follows the same trend as the TMA. In addition, Dp-3-sam seems to be the major Roselle anthocyanin (6.2 mg/g DM), followed by Cyn-3-sam (2.4 mg/g DM). By comparing the extraction of these two molecules from untreated and DIC-treated Roselle calyces, an improvement can be seen in the kinetics and the yield of extraction in just about all the experiments. Fig. 4 shows a comparison between the extraction kinetics of the major anthocyanins Dp-3-sam and Cyn-3-sam, respectively, from untreated material and in DIC experiments 2 and 4. It is clear that DIC treatment improved the kinetics and the yield of extraction of these two molecules. As for TMA, the amount of Dp-3-sam that was obtained within 3 min for DIC treated Roselle took 5 min to obtain with untreated calyces.

3.2. Extraction yield

The aim of this part of the study was to use the experimental design to examine the impact of DIC on the extraction yield of TMA and for each individual anthocyanin. The experimental responses in terms of the TMA and the quantity of Dp-3-sam and Cyn-3-sam, together with the overall extraction yield of these two molecules for the different combinations in the experimental design, are summarized in Table 1.

Table 1 shows that the main anthocyanins in the Roselle extract were Dp-3-sam (69–76%) followed by Cyn-3-sam (24–31%). The same results were reported previously (Bridle & Timberlake, 1997; Frank et al., 2005).

Using DIC texturing, we could improve the TMA extracted from Roselle by up to 135%, with the content varying from 8.96 mg/g DM with raw material to 11.72 mg/g DM with optimized DIC-treated material. The contents of Dp-3-sam and Cyn-3-sam varied slightly between the two cases. By improving its availability, DIC allows a higher TMA to be obtained from Roselle; however, it seems to maintain the same relative percentage of Dp-3-sam and Cyn-3-sam.

3.2.1. Impact of DIC parameters on anthocyanin extraction

3.2.1.1. Statistical analyses. The effect of DIC parameters (pressure and processing time) on TMA, Dp-3-sam and Cyn-3-sam extraction is correlated with the coefficient of the second order polynomials and their regression coefficient R^2. Despite the suggestion that a

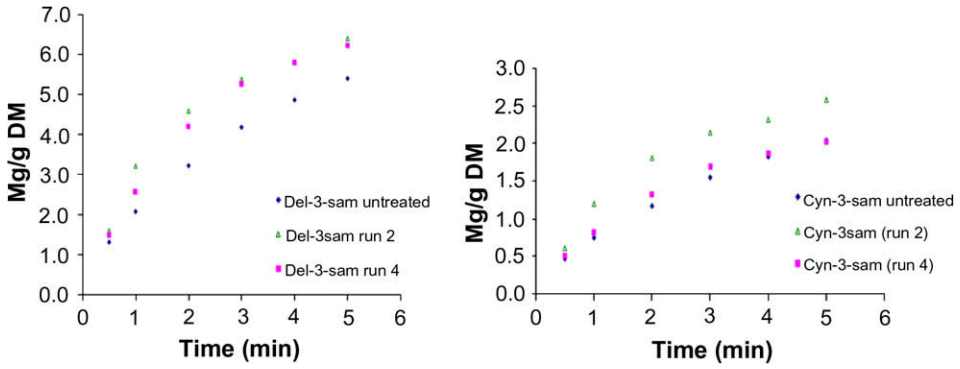

Fig. 4. Comparison of kinetics of extraction of Dp-3-sam and Cyn-3-sam from untreated material and Instant Pressure Drop (DIC) treated Roselle calyces according to run 2 and 4.

good fit of a model implies an R^2 greater than 0.8 (Joeglekar and May, 1987), we can postulate the validity of the TMA model with $R^2 = 0.7521$, while the variations of Dp-3-sam and Cyn-3-sam must be random with $R^2 = 0.46$ and 0.47, respectively. This conclusion confirms earlier observations that DIC acts mainly on the overall TMA yields without significant changes to the composition of Dp-3-sam and Cyn-3-sam.

4. Conclusion

In conclusion, the data obtained in the present work have demonstrated that DIC treatment has a great impact on the kinetics and the extraction yield of anthocyanins from the dried calyces of Roselle. Thanks to an appropriate experimental design, we could identify the optimized DIC operative parameters (a steam processing pressure of 0.18 MPa and a thermal treatment time of18 s) giving an improvement up to 135% in TMA yields. The Dp-3-sam and Cyn-3-sam composition of TMA seems to be constant, for both raw material and DIC treated material. In terms of the kinetics, the effective diffusivity for extracting anthocyanins may reach $6.11 \times 10^{-11}\ m^2\ s^{-1}$ for DIC treated material compared to $4.19\ 10^{-11}\ m^2\ s^{-1}$ for raw material.

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