Improvement of anthocyanins extraction from Hibiscus sabdariffa by coupling solvent and DIC process

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Summary

The aim of this study is to investigate the effect of the "Instantaneous Controlled Pressure Drop" DIC (Détente Instantanée Contrôlée) on the yield of extraction of Total Monomeric Anthocyanins from *Hibiscus sabdariffa* calyces. It was concluded that the DIC has a positive effect on the yield of extraction of anthocyanins from *Hibiscus sabdariffa.* The optimized conditions of DIC were pressure of treatment 0.25 MPa and time of treatment 5s giving an improvement of 145% for TMA.

Mots-clés : Anthocyanins, Extraction, DIC

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 calyces of the flower are consumed worldwide as a cold beverage or a hot drink. These extracts are also used in folk medicine against many complaints that include high blood pressure, liver disease and fever (Wang, Wang et al. 2000). The positive physiological effect of this plant extract could be related to the presence of anthocyanins with potent antioxidant activity.

Anthocyanins are the most important group of water soluble pigments that, after chlorophyll, are visible to the human eyes. They are responsible for many of the attractive colours, from scarlet to blue, of flowers, fruits, leaves and storage organs. The vegetable materials contain generally only a small amount of active solute, but usually with high added value. The extraction and purification of bioactive compounds from natural sources become very important for the utilization of phytochemicals in the preparation of dietary supplements or neutraceuticals, functional food ingredients and additives to food, pharmaceutical and cosmetic products. Extraction of anthocyanins is commonly carried out under cold conditions with methanol or ethanol containing a small amount of acid with the objective of obtaining the flavylium cation form, which is red and stable in a highly acid medium. 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 implies multiple steps. However, the main part of the operation is limited by diffusion, because of the natural structure of the plant which opposes a resistance to the solvent penetration; process is then very slow and would need a big amount of solvent. Similar considerations would intervene when used solvent is supercritical fluid. Some other recent alternative extraction methods (ultrasonification, microwave...) are proposed in order to intensify transfer processes but they remain too costly techniques for scaling up.

For improving technological aptitude of raw material in terms of extraction, one may modify the initial structure by cutting, grinding, etc. In our laboratory, we proposed to carry out studies concerning the effect of structure expansion. In several cases, we proved that the higher the expansion rate, the better the diffusivity constant. So we applied a swelling operation using the well-known process of "Instantaneous

Controlled Pressure Drop" DIC (Détente Instantanée Contrôlée) witch was developed in our laboratory since some years (Allaf et al., 1989, Allaf et al., 1994). Firstly, this process was used for swell-drying various fruit and vegetable food products; it assures a high quality by improving the hydration kinetics and capacity. DIC treatment is based on fundamental studies concerning the thermodynamics of instantaneity (Allaf, 2002). It consists on a thermo-mechanical processing induced by subjecting the product to an abrupt transition from high steam pressure towards vacuum. This process also used for the extraction by instant autovaporization of essential oils and other volatile molecules (Allaf et al., 1998), allows the product to get higher global diffusivity and improves the availability of some compounds in the treated plant.
The aim of this work is to study the impact of DIC treatment on the aqueous anthocyanins extraction, from the calyces of Roselle. A comparison between yield of extraction of anthocyanins from treated and untreated calyces of Roselle will be exposed.

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2. experimental protocol

2.1 raw material and chemicals

Dried calyces of Roselle were obtained from Egypt. The deionized water used for the extraction was prepared with a GFL Deioniser (Germany). Analytical grade Sodium acetate trihydrate and potassium chloride were purchased from Merk (Germany). Delphinidin-3-O-glucoside chloride was obtained from Extrasynthese (Lyon, France).

2.2 Measurement of moisture content

The moisture content of the samples was done using the oven-dry method which uses weight loss to obtain a direct measure of the moisture content of the samples. 2g of each sample was placed in a glass and was dried for 24 hours under an air flux at 105°C. The initial water content of the dried calyces of Roselle was 14.87 % WB.

2.3 DIC process

Experimental set up

The experimental set up was largely described (Allaf et al., 1998; (Louka and Allaf 2004). It is composed of three main elements (figure 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 reactor, and an adequate vacuum pump. The initial vacuum level was maintained at 50 kPa in all the experiments.
- A pneumatic valve (3) that assures the connection/separation between the vacuum tank and the processing vessel. It can be opened in less than 0.2 second, this ensure the abrupt instant pressure drop within the reactor

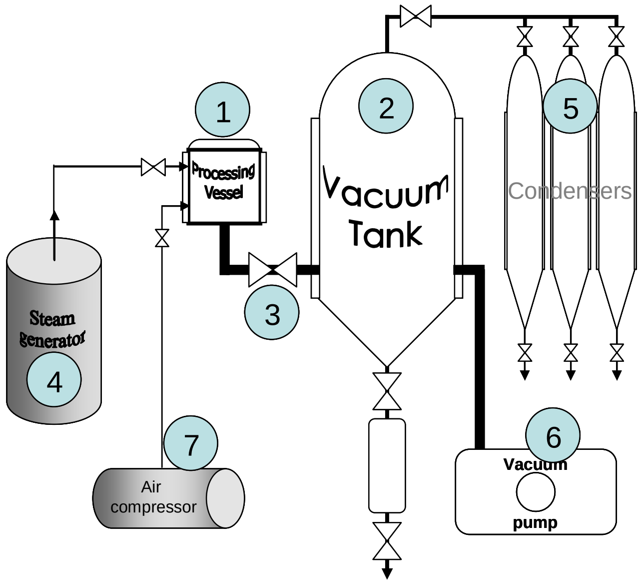

Figure 1*: Schematic diagram of the DIC reactor. 1: Processing vessel; 2: Vacuum tank; 3: Quick motion valve; 4: Steam generator; 5: condensers 6: Vacuum pump; 7: Air compressor.*

2.3.1 DIC treatment

Dried calyces of Roselle are firstly placed in the DIC treatment vessel; then we establish a first vacuum stage in this vessel in order to reduce the resistance toward the steam diffusion as heating fluid through the plant material and consequently improve heat transfer. After closing the pneumatic valve of vacuum, steam under pressure was injected in the reactor and maintained for the treatment time. The thermal treatment is followed by an abrupt pressure drop towards vacuum. The resulting autovaporization induces an "instant" cooling of the treated material. After the treatment, the calyces of Roselle were recovered and ready for extraction. The water content of each sample was determined before extraction.

2.4 Experimental design

In order to reduce the experimental points needed for carrying out the effects of the main operative parameters (steam pressure P and processing time t), we used a 2 variable central composite rotatable experimental design. This design needs 11 experiments with 3 repetitions for the central point. The experiments were run in random in order to minimize the effects of unexpected variability in the observed responses due to extraneous factors. The table 1 lists the independent variables and their level.

Table 1: level of independent variables used in developing experimental data

Level	-α	-1	0	1	-α
Processing pressure (bar)	1	1.4	2.5	3.6	4
Processing time (s)	5	13	33	52	60

α (axial distance)= $\sqrt[4]{N}$, N is the number of experiments of orthogonal design, i.e of the factorial design. In our 2 parameter case, α= 1.4142

The surface responses were obtained by using the analysis design procedure of Statgraphics plus for Windows (1994-4.1 version). Table 2 shows the factorial design matrix, with coded variables.

Table 2: Experimental data of the composite central design

Experiment n°	Processing pressure (bar)	Processing time (s)
1	3.6	52
2	3.6	13
3	1.4	52
4	1.4	13
5	2.5	60
6	2.5	5
7	4.0	33
8	1.0	33
9	2.5	33
10	2.5	33
11	2.5	33

2.5 Extraction of anthocyanins

The extraction of anthocyanins from the dried calyces of Roselle, with water as solvent 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 solvent. The content was heated to 100±2°C and then a pre-weighed amount of dried calyces of Roselle (2g) was added at time t=0. The flask temperature was controlled with a thermometer. At 10 min, 3 ml of the solution were taken from 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

UV-Vis spectrophotometer at 520 nm was used to determine the absorbance of acidified aqueous (1%HCl) solution of Dp-3-glu with concentration of 5, 10, 25 and 50 mg/l. Plotting concentration (mol/l) against absorbance, the molar absorption coefficient of Dp-3-glu was 27481 mol.l^{-1}.cm^{-1}.

2.6.2 Quantification of total monomeric anthocyanins

Total monomeric anthocyanins content of Roselle dried calyces was determined using the pH differential method described by Guisti et Wrolstad, 2001. A Hélios UV/Visible spectrophotometer and 1 cm path length glass cells were used for spectral measurements at 520 and 700 nm respectively against distilled water as blank. For this purpose, aliquots of Roselle extract were brought to pH 1.0 and 4.5 and allowed to equilibrate for 20 min. The absorbance of each equilibrated solution was then measured at the wavelength of maximum absorption λ_{max} and 700 nm for haze correction. The difference in absorbance values at pH 1.0 and 4.5 was directly proportional to 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 molar absorptivity of 27481 L/cm-mg. The pH measurements were done using a Denver Instrument company Model 15 pH-meter calibrated with pH 4, 7 and 10 buffers.

3 Results and discussion

3.3 Yield of extraction

This study aimed to identify the impact of the DIC treatment on the kinetics and total yield of extraction of Total Monomeric Anthocyanins (TMA) from the calyces of Roselle. The experimental response in term of TMA is summarized in table 3. By using DIC as pre-treatment, the extraction of TMA has been improved from 104 up to 145% compared to untreated calyces. The calyces treated by DIC at (Steam pressure P: 0.25 MPa; thermal treatment time: 5 s) gives the highest value of extracted TMA yield, which reaches up to 14.60 mg/ g DM, since the untreated calyces, gave only about 10.01 mg/g DM.

Table 3: TMA from calyces of Roselle

Run	Steam Pressure (MPa)	Processing time (s)	TMA mg/g DM	TMA (%)[a]
1	0.36	52	6.62	66
2	0.36	13	10.49	104
3	0.14	52	12.06	120
4	0.14	13	12.73	126
5	0.25	60	9.11	90
6	0.25	5	14.60	145
7	0.40	33	5.16	51
8	0.10	33	14.43	143
9	0.25	33	10.50	104
10	0.25	33	9.59	95
11	0.25	33	10.51	104
Untreated material	-	-	10.09	-

[a]: (TMA of treated material/TMA of untreated material)*100

3.3.1 Statistical analyses

The results concerning the evolution of TMA yield versus operative parameters of DIC allow us to get the following second order polynomial model:

$$\text{TMA yield}=13.0156+2.34406*P-0.0272833*t-0.705038*P^2-0.037296*P*t+0.000639539t^2.$$

The coefficient of regression we got is R^2=85%; it shows that a high proportion of variability of TMA extraction versus the effects of the DIC parameters (steam pressure and thermal treatment time) is reported by this polynomial model, which is thus a relevant model; indeed, (Joglekar and May 1987) suggested that for a good fit of a model, R^2 should be at least 0.8.

3.3.2 Effect of DIC parameters

Pareto chart, main effects plot of DIC parameters and response surface for TMA extraction are shown in figure 2-4. The effect of a parameter is considered as statistically significant if the histogram cross the vertical line, translating the threshold of significance of 5%. So, according to the Figure 2, and in the field of variation of the process parameters, the simple effect of pressure and time were statistically significant on the TMA content. Figure 3, shows that the higher the steam pressure, the lower the TMA content. Similar results were obtained with the time which has a negative effect on the TMA content.

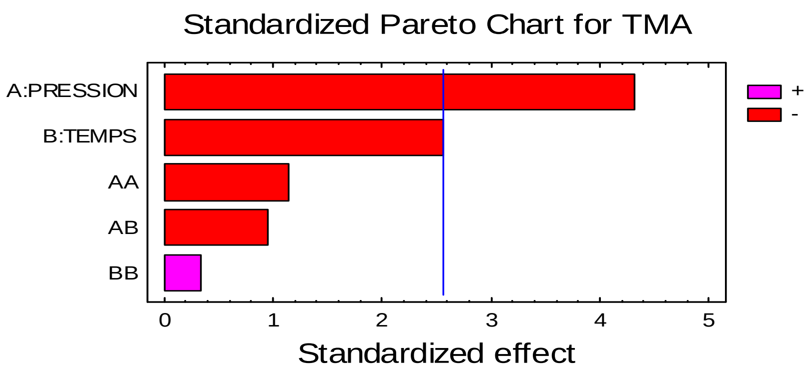

Figure 2: Standardized Pareto chart for TMA

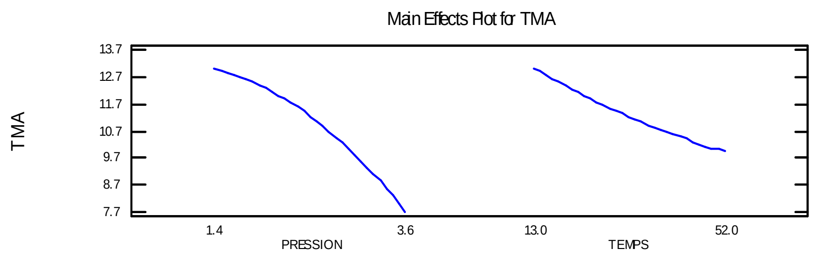

Figure 3: Main effects plot for TMA

For better illustrating the variation of the TMA according to a couple of operational parameters, witch are pressure and time of treatment, we use the response surface.

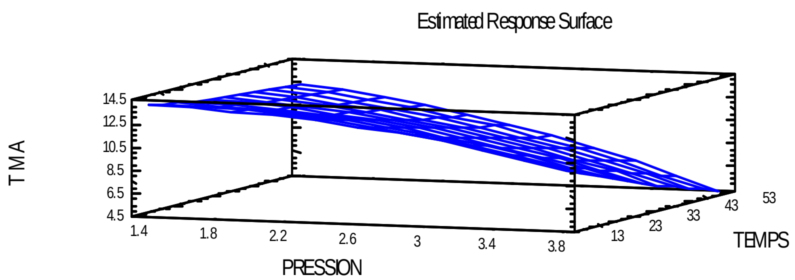

Figure 4: Estimated response surface for TMA

4 Conclusion

In conclusion, the data obtained in the present work have demonstrated that the DIC used as a pretreatment in a solvent extraction process has a positive impact on the yield of extraction of anthocyanins from the dried calyces of Roselle. Between the different combinations of the parameters of DIC, the experiment 6 (P: 0.25 MPa; T: 5 s) have proved to be the best treatment giving an improvement of 145 % for TMA.

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