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Optimisation of Instant Controlled Pressure Drop (DIC)-assisted dehydrofreezing using Response Surface Methodology towards better bioactive compounds retention of quince fruit

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ABSTRACT

Pre-drying prior to freezing may reduce several freezing drawbacks. Nevertheless, drying may cause nutritional quality losses. Instant Controlled Pressure Drop process has been proposed to intensify pre-drying process. This research is dedicated to study the evolution of the main bioactive compounds (total phenolics, flavonoid, and tannins contents) of quince dehydrofrozen fruits. Fresh samples were subjected to air drying at 40 °C and 3 m s^{-1} air velocity down to a final water content of 0.3 g g^{-1} db. Pre-dried samples were Instant Controlled Pressure Drop (DIC) treated under different conditions, i.e. saturated steam pressure (P) and treatment time (t), following a 2-factor/5-level Experimental Design. Treated fruits were frozen at –30 °C then were thawed at 20 °C in order to study the impacts of DIC on phenolic compounds. Response Surface Methodology (RSM) confirmed that pressure was the most influencing parameter in terms of polyphenol, flavonoid, and tannins contents. Finally, DIC pre-treatment allowed the improvement of phenolic content retention compared to untreated DIC samples.

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KEYWORDS

dehydrofreezing, DIC, phenolic compound, quince, RSM

1. INTRODUCTION

Quince fruit (*Cydonia oblonga*) is known for its nutritional attributes, pleasant odour, distinctive taste, and especially as source of bioactive phenolic compounds (Hajji et al., 2020). Despite the fact that fresh quince fruit is not well appreciated due to its pulp hardness, bitterness, and especially astringency, in its processed form, it is in high demand for consumption (Silva et al., 2005).

Unfortunately, postharvest life of quince is relatively short due to its high water content and its susceptibility to bruises and fungal attacks, which may affects its bioactive compounds.

Even though freezing is the most commonly used method to extend the shelf life of fruits and preserve their bioactive compounds, drawbacks such as ice crystals formation leads to nutritional quality loss with thawing water exudate. Moreover, irreversible structural damage of high-water content products with low cellular elasticity is observed after freezing treatments. Dehydrofreezing process, which involves partial dehydration before freezing, may be an alternative, reducing freezing time and diminishing tissue damage thanks to partial water removing prior to freezing (Hajji et al., 2019, 2020).

In this work, coupling DIC with dehydrofreezing operation was studied. Instant Controlled Pressure Drop (DIC) treatment was coupled to air drying prior to freezing process in order to improve the quality of frozen products. DIC treatment is an intensification strategy of air drying, thus defining swell-drying process. DIC is used as a texturing process to eradicate the shrinkage phenomenon related to drying (Mounir et al., 2014).

Moreover, microbial control, which is not achieved during air drying and subsequent freezing, may be achieved under DIC treatment mainly through well controlled heat treatment and mechanical stress on organisms due to the instantaneous pressure drop leading to their explosion (Mounir et al., 2014).

No work has been reported in the literature on the effects of DIC treatment on the nutritional quality of dehydrofrozen treated fruits. Hence, the main objective of this study is to evaluate and assess the effects of different DIC experimental operating conditions on bioactive phenolic compounds of partially dehydrated quince fruit after freezing/thawing processes, in terms of total phenolic, flavonoid, and tannins contents.

2. MATERIALS AND METHODS

2.1. Sample preparation

Pieces of quince fruit (*C. oblonga.* var. Pineapple) were selected randomly from a local market from Tunis (Tunisia). Fruit was peeled, cored, and the hard pieces around the core were removed. Quince were sorted, washed with tap water, and cut with kitchen knife into parallelepipeds ($5 \times 2 \times 0.5\ cm^3$).

2.2. Water content determination

AOAC official method 934.04 at 105 °C for 24 h (AOAC, 1990) was used to determine the water content of fresh, pre-dried, and DIC treated samples of quince. The initial water content of fresh quince was about 6 g g^{-1} db (db: dry basis).

2.3. Hot air drying (HAD)

Quince slices were dried in a hot air drier (Memmert: Universal Oven UNB Model 800, Schwabach, Germany) at 40 °C, with an air flow velocity of 3m s^{-1} up to 0.3 g g^{-1} db of water content. After 6 h of drying, samples were stored in a refrigerator at 4 °C for 24 h within air-tight bags in order to homogenise water content. Subsequently, quince slices were treated in a DIC-reactor according to the experimental design presented in Tables 1 and 2.

2.4. DIC treatment

Partially dried quince samples were treated by DIC equipment (ABCAR-DIC Process, La Rochelle, France) as described by Mounir and Allaf (2017).

Table 1. Coded and real ranges of independent variables used in Response Surface Methodology (RSM)

Factor	$-\alpha$	−1	0	1	$+\alpha$
Processing pressure (MPa)	0.2	0.229	0.3	0.371	0.4
Processing time (s)	10	11	15	19	20

With $\alpha = \sqrt[4]{2K} = 1.4142$ as the axial distance; fork = 2 as the number of orthogonal design parameters.

Table 2. Trials in the control sample and from the experimental design for DIC process

Run CS*	Saturated steam absolute pressure *P* (MPa) /	Processing temperature T (°C) /	Processing time *t* (s) /
DIC1	0.3	133.6	15
DIC2	0.4	143.7	15
DIC3	0.3	133.6	20
DIC4	0.3	133.6	15
DIC5	0.37	140.9	19
DIC6	0.37	140.9	11
DIC7	0.3	133.6	15
DIC8	0.23	124.7	11
DIC9	0.23	124.7	19
DIC10	0.3	133.6	15
DIC11	0.2	120.2	15
DIC12	0.3	133.6	10
DIC13	0.3	133.6	15

ACP: Average Centre Point of DIC1, DIC4, DIC7, DIC10, and DIC13.
* CS: Control sample dehydrofrozen fruit at 0.3 g g^{-1} db, without DIC.

2.5. DIC experimental design

The operating parameters of DIC treatment were only the saturated steam pressure (MPa) and the thermal holding time (s), while the initial water content (W) of samples was maintained constant at 0.3 g g^{-1} db. Hence, in order to study the effects of these parameters on the various response parameters (total phenolic content, TPC; flavonoid content, TFC; and tannins content, TC), a central composite rotatable design was used (Statgraphics, Centurion XV, USA) (Table 1). For 2 factors, the design resulted in 13 trials: 4 (2^2) factorial points, 4 (2×2) axial points to form a central composite design, and 5 centre points for replications (Table 2). The ranges and the centre point (Table 1) were defined after preliminary trials. The 13 trials were run in random in order to minimise the effects of the unexpected variability on observed responses due to the external uncontrolled factors. Statistical treatment was executed using the analysis design procedure Statgraphics Plus software for Windows (1994, version 4.1, Levallois-Perret, France). An analysis of variance (ANOVA) is performed to determine the significant differences between the independent variables ($P < 0.05$) for each response parameter. A Pareto chart is used to identify the impact of the variables on the response under consideration, and a vertical line indicates the parameters that are statistically significant at the 95% confidence level. The response surface methodology provides an empirical regression model that fits the experimental data through corrected R^2 correlation values, which allow the optimisation of the operating parameters.

2.6. Active compounds analyses

Frozen and dehydrofrozen quince sample extracts were obtained by methanol extraction and stored at 4 °C (Hajji et al., 2020). Total polyphenol content (TPC) was determined by the Folin–Ciocalteu method using gallic acid as the reference compound, and results were expressed in grams of gallic acid equivalents per 100 g dry basis (Hajji et al., 2020). The total flavonoid content (TFC) was measured by a colorimetric method based on the formation of the aluminium-flavonoid complex, and results are expressed as g quercetin equivalents (QE) per 100 g dry basis, as described by Ben Haj Said et al. (2013). Tannins content (TC) were measured according to the colorimetric method of Folin–Denis and expressed in grams of tannic acid equivalent (TAE) per 100 g of dry matter as described Schofield et al. (2001). Analyses were run in triplicates.

3. RESULTS AND DISCUSSION

3.1. Effect of DIC on TPC

The TPC of dehydrofrozen quince control sample and DIC-assisted dehydrofrozen ones are gathered in Table 3. The positive impact of DIC on TPC retention appears clearly. Moreover, unlike other heat treatments such as conventional pre-drying (Zhao et al., 2016) or cooking treatment (Chuah et al., 2008), which show important reductions of TPC, DIC process, as a high-temperature and short-time treatment, allowed improving TPC retention, showing that degradation of polyphenols is related not only to applied heat treatment but also to processing time. According to Téllez-Pérez et al. (2013), polyphenol oxidase enzyme may be inactivated during the DIC process, avoiding the degradation of polyphenols. Besides, high temperatures (i.e. >90 °C) would cause the formation of phenolic compounds because of the availability of

Table 3. TPC, TFC, and TC in dehydrofrozen and DIC-assisted dehydrofrozen quince

Operational parameters	Pressure P (MPa)	Time t (s)	Bioactive compounds		
			TPC	TFC	TC
CS*	–	–	0.44 ± 0.01	0.65 ± 0.02	3.18 ± 0.1
DIC 2	0.40	15	0.70 ± 0.01	1.08 ± 0.2	1.1 ± 0.04
DIC 3	0.30	20	0.62 ± 0.16	0.99 ± 0.09	1.62 ± 0.2
DIC 5	0.37	19	0.69 ± 0.22	1.01 ± 0.01	1.14 ± 0.8
DIC 6	0.37	11	0.67 ± 0.01	0.99 ± 0.03	1.22 ± 0.1
DIC 8	0.23	11	0.47 ± 0.02	0.74 ± 0.06	2.13 ± 0.1
DIC 9	0.23	19	0.48 ± 0.01	0.76 ± 0.04	2.13 ± 0.2
DIC 11	0.19	15	0.45 ± 0.06	0.72 ± 0.06	2.91 ± 0.06
DIC 12	0.30	10	0.55 ± 0.06	0.86 ± 0.03	1.74 ± 0.1
ACP**	0.30	15	0.60 ± 0.02	0.90 ± 0.01	1.98 ± 0.06

*CS: Control sample dehydrofrozen fruit at 0.3 g g^{-1} db, without DIC.
**ACP: Average Centre Point.
TPC: total phenolic content, TFC: total flavonoid content; TC: tannins content.

precursors of phenolic molecules by non-enzymatic inter-conversion between phenolic molecules (Dutra et al., 2008).

Figure 1 (A, B) shows the effect of saturated steam pressure and thermal holding time on TPC of dehydrofrozen treated-DIC quince samples under different operating conditions. It is inferred that after freezing/thawing, steam pressure (P) appears as the most significant DIC operative parameter on quince TPC. Indeed, the highest TPC value, 0.70 g GAE/100 g db, was verified for samples treated at 4 MPa as saturated steam pressure for 15 s (Fig. 1). Thus, DIC is an effective treatment for the intensification of quince phenolic compounds as measured after thawing operation. This can be attributed to the mechanical effect induced by the dropping pressure towards a vacuum, resulting in cell walls broken-down and thus, the formation of some vacuoles and pores within the product thanks to water auto vaporisation. Such new structures

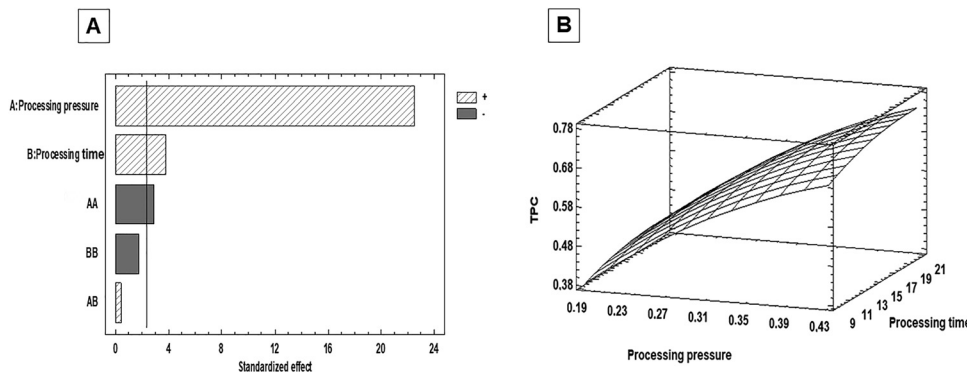

Fig. 1. Effects of DIC operating parameters; saturated steam pressure, P MPa, and processing time, t sec, on the total phenol content, TPC g GAE/100 g db of dehydrofrozen quince A) Pareto Chart and B) Response Surface.
TPC: Total phenol content; GAE: Gallic acid equivalent

can increase the availability of the bound phenols and facilitate, subsequently, their extraction (Alonzo-Macías et al., 2013), while pre-drying and conventional freezing do not affect phenol-cell wall association (Silva et al., 2020). Albitar et al. (2011) proved that the increase in phenol availability is also dependent on the initial water content.

An empirical polynomial equation model was obtained with the statistical package Statgraphics. It represents the quantitative effect of process variables and their interactions on the measured response (TPC). The values of the coefficients of P and t were related to the effect of these variables on the response. A positive value represented an effect that augments TPC value, while a negative value indicated an antagonistic effect. This empirical model is listed in Table 4 (Eq. 1), with P and t being DIC saturated steam pressure and treatment time, respectively.

RSM optimisation was used to show the impact of the operative factors in terms of quince TPC. Thus, it was possible to optimise DIC operating parameters based on the maximum TPC retention (1.02 g GAE/100 g db), after thawing operation. These optimised conditions were 0.4 MPa and 20.65 s for DIC saturated steam pressure (P) and DIC treatment time (t), respectively, for dehydrofrozen quince.

3.2. Effect of DIC treatment on TFC

Flavonoids are the most common group of phenolic compounds. They are particularly important antioxidants because of their high redox potential, which allows acting as reducing agents, hydrogen donors, and scavengers of singlet oxygen (Mkaouar et al., 2018).

Results of TFC of dehydrofrozen and DIC-assisted dehydrofrozen quince samples after thawing are shown in Table 3.

After DIC, the TFC amount increased significantly ($P < 0.05$) to reach a value of about two times higher than that of the control samples. Mkaouar et al. (2018) explain that the impact of DIC treatment on fruit microstructure facilitates solvent extraction and thus, enhances bioactive molecules availability.

DIC operating parameters impact on TFC is shown in Fig. 2 (A, B). Similar behaviour described for TPC was observed for TFC. The saturated steam pressure had a significant effect on the TFC compared to the thermal holding time. Hence, the higher the saturated steam pressure, the higher the flavonoid content.

The highest value of TFC was recorded for treated samples at saturated steam pressure of 0.4 MPa for 15 s (Table 3). Thermal degradation of air-dried products correlates with long-time operation and shrinkage phenomenon, which occurs during drying processes. Indeed, shrinkage leads to a decrease of water diffusivity (Allaf et al., 2014). Contrariwise, DIC texturing can greatly increase diffusivity, reduce drying time, preserve natural nutritive value, and improve the

Table 4. Empirical model equations of different phenolic compounds for quince fruit

Empirical model	$R^{2\%}$	Eq.
$TPC = -0.1727 + 2.7744{*}P + 0.0166{*}t - 2.6036{*}P^2 + 0.0088{*}P{*}t - 0.0005{*}t^2$	97.77	1
$TFC = 0.1386 + 2.8924{*}P + 0.0011{*}t - 1.8597{*}P^2 + 0.0001{*}\ t^2$	93.36	2
$TC = 0.7212 - 2.4557{*}P + 0.3812{*}t - 7.1911{*}P^2 - 0.0704{*}P{*}t - 0.0122{*}t^2$	97.52	3

TPC: total phenolic content, TFC: total flavonoid content; TC: tannins content, P: DIC Saturated Steam Pressure, t: DIC treatment time

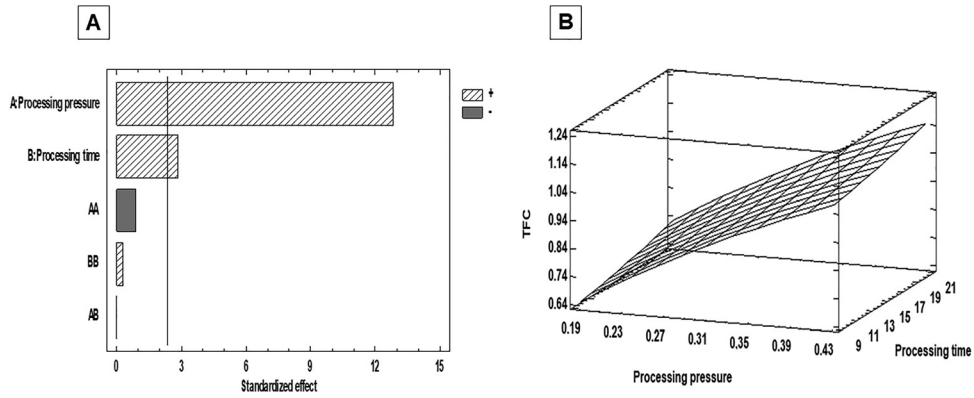

Fig. 2. Effects of DIC operating parameters; pressure, *P* MPa, and time, *t* sec, on the total flavonoid content, TFC g QE/100 g db of dehydrofrozen quince: A) Pareto Chart and B) Response Surface
TFC: Total flavonoid content, QE: quercetin equivalent

bioactive compounds availability. Indeed, Mounir et al. (2011) reported that the amount of TFC in DIC-treated apple compared to untreated fresh ones increased by up to 700%. Furthermore, studies on onions proved that instantaneity of the pressure drop toward a vacuum is an important factor influencing flavonoid availability (Mounir et al., 2011). TFC increase of DIC-treated dehydrofrozen samples maybe related to the stabilisation of quince flavonoid compounds during the DIC treatment. At this respect, Adamczak et al. (2009) claimed that drying temperatures ranging between 120 and 150 °C stabilise better flavonoid compounds than 40–60 °C.

Empirical models estimated for quince (Eq. 2) are listed in Table 4. Good fits were achieved and most of the responses' variability was explained by the model; the coefficient of multiple determinations (R^2) being 96.12%.

RSM optimisation was used to show the impact of the operative factors in terms of quince flavonoid content. Thus, it was possible to optimise DIC operating parameters based on the maximum TFC retention (1.10 QE/100 g db), after thawing operation. These optimised conditions obtained were $P = 0.4$ MPa and $t = 20.65$ s. Indeed, the impact of DIC operating parameters of saturated steam pressure *P* and processing time *t* (as independent parameters) on TFC of final products were evaluated by implementing a specific two-parameter/five-level central composite rotatable design of experiments DoE. The results were statistically analysed using response surface methodology. DoE and response surface methodology were so powerful, relevant, and convenient tools that they allowed identifying the impact of the processing parameters on this quality attribute and determining the optimised operating parameters of the swell drying process versus quality attributes of dehydrofrozen quince. Fifteen seconds of DIC treatment applied on quince sample showed the maximum TPC value 1.08 ± 0.2 as shown in Table 3. However, 20.65 s is the optimised value of DIC duration that was estimated by DoE. The DoE shows the combinations of the levels of the factors that maximize TFC; for these operating conditions, the optimum TFC to reach is 1.10 and the optimal conditions were $P = 0.4$ MPa and $t = 20.65$.

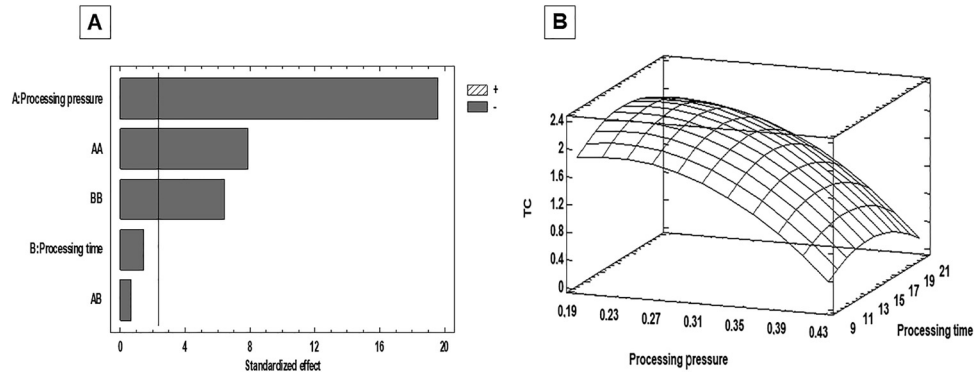

Fig. 3. Effects of DIC operating parameters; pressure, *P* MPa and time, *t* sec, on the total tannins content, TAE g/100 g db of dehydrofrozen quince samples: A) Pareto Chart and B) Response Surface
TC: tannins content.

3.3. Effect of DIC treatment on TC of dehydrofrozen quince fruit

Table 3 shows that tannins contents of the DIC-assisted dehydrofrozen quince samples changed greatly with DIC treatment conditions, with the processing steam pressure being the most responsible parameter (Fig. 3A); the higher the pressure, the lower the TC. Besides, while time impact is non-significant, the interaction of factors showed that high values of pressure imply a negative impact of time treatment on the TC (Fig. 3B). The empirical regression model in Equation 3, cited in Table 4, allowed to determine the optimal operating conditions (*P*: 0.19 MPa; *t*: 14.97s) maximizing the response (2.69 g TAE/100 g db).

Tannins losses for DIC treated samples might be due to the thermal stress caused by this treatment, which leads to the degradation of tannins. Indeed, DIC is an extraction process based on the phenomenon of auto-vaporisation of volatile compounds. The raw material is subjected, for a short time, to high-pressure saturated steam, while the temperature is between 100 and 175 °C (Allaf et al., 2012). Obiang-Obounou and Ryu (2013) stated that temperature extrusion-cooking (120 °C) can effectively reduce amount of tannin in chestnuts by 78%. While high temperature treating *Radix astragali*, Xiao et al. (2008) also observed that tannin content decreased by up to 50%.

4. CONCLUSIONS

DIC was introduced as a texturing process between partial air-drying and freezing steps. It was performed at various operating parameters following an adequate Response Surfaces Methodology with saturated steam pressure (*P*) and thermal treatment time (*t*). Its optimisation confirmed that processing steam pressure was the main DIC operative parameter influencing the availability of bioactive compounds. Under optimal DIC conditions (0.4 MPa for 20.65 s), quince was richer in phenolic and flavonoids compounds compared to untreated dehydrofrozen samples. Saturated steam pressure and processing time allow obtaining the best bioactive compounds preserving DIC treatment.

Interestingly, DIC used as pre-drying intensifying process greatly reduced quince tannins content, which may be beneficial and appreciated by consumers as it reduces the astringency and undesirable properties due to tannins.

Conflict of interest: All authors declare no conflict of interest.

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