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Research Article

Dehydrofreezing: An Integrated Process Towards Better Bioactive Compounds Retention in Strawberry Fruits

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Abstract

Dehydrofreezing process involves partially removing water before freezing. This treatment has been proposed in order to lessen the negative impacts of conventional or even accelerated freezing, especially on color quality and phytochemical compounds of high-water content fruits, such as strawberries. Indeed, in such cases, conventional freezing and thawing processes result in severe damage of the integrity of product's cell structure due to the formation of ice crystals, which induce bioactive compounds losses.

Fresh samples and samples previously dehydrated up to different water contents (1, and 0.3 g/g db) were frozen at constant temperature of -30 °C and thawed at room temperature. The effects of water content (W) were investigated in terms of phenolic compounds retention and color examination, after thawing process. The obtained results show that lower sample water content implied equally: higher total polyphenol content (TPC), total flavonoids content (TFC) and higher total anthocyanins content (TAC). Moreover, the impact of moisture level on color characteristics and browning index was significant and very important with drying intensity. Therefore, partial removal of water is a promising solution to reduce the negative impacts of freezing on strawberry quality with total independence of freezing process and to improve phytochemical compounds retention.

Keywords: Strawberry Fruit; Pre-drying; Freezing; Phenolic Compounds; Color

Abbreviation

TPC: Total Polyphenol Content; TFC: Total Flavonoid Content; TAC: Total Anthocyanin Content; BI: Browning Index

Introduction

Strawberries fruits are known for their nutritional attributes, pleasant odor, distinctive taste and especially as source of bioactive phenolic compounds [1]. Indeed, these bioactive compounds are of prominent importance; they constitute an integral part of the sensory attributes and contribute directly to characterize each type of fruits. Moreover, they are known to have hypoglycemic, anti-inflammatory, anti-carcinogenic, antimicrobial, anti-allergic, and anti-ulcerative properties, and act as a tonic for heart and brain [2,3]. However, strawberries postharvest life is relatively short

since they are highly perishable fruits due to their high-water content and susceptibility to quality deterioration. At the same time, these fruits are highly susceptible to bruises and fungal attacks which may affect their bioactive compounds.

Up to now, freezing technology is the most commonly used method to extend fruits and vegetables shelf life and preserve their bioactive compounds. However, the development of ice crystals during freezing process causes substantial nutritional quality degradation after thawing, through water exudation and organoleptic degradation. Moreover, in the case of perishable fruits and vegetables with high water content and low cellular structure elasticity, such as strawberry fruits, freezing treatment causes irreversible structural damage due to the difference of volume of water before and after freezing. Moreover, leaching phenomena upon thawing contribute to a further loss of solutes, micronutrients, and biocompounds. Indeed, according to the findings of Yanat and Baysal [4], there was 50% loss of total phenolics in cherries for 6 months of storage at -23°C.

Dehydrofreezing process which is defined as a preservation process that involves partial dehydration before freezing may be considered as a potential solution to be used in order to reduce freezing time and to diminish tissue damage by removing part of water from material product prior to freezing [5,6]. Despite these benefits, researches on studying the impacts of dehydrofreezing on nutritional quality preservation are still relatively scarce. Hence, the main objective of this research work is to evaluate the effects of pre-drying operating conditions on bioactive phenolic compounds of partially dehydrated strawberries fruits after freezing/thawing processes, in terms of total phenolic content, flavonoid content, total anthocyanins content and color.

Materials and Methods

All the materials and methods that are used to complete the study should be mentioned.

Sample preparation

Strawberries (Fragaria var. Charlotte) were purchased from a supermarket in La Rochelle, (France). They were transported to the laboratory and stored at 4ºC until the next day. Strawberries fruits were sorted, washed with tap water and cut with kitchen knife into discs, with 5 ± 0.38 mm as thickness. The initial water content was determined according to AOAC official method 934.06 [7]. The initial water content of fresh strawberry samples was 9.79 ± 0.81 g H_2O/g db.

Partial convective air drying

Air-drying was used in this study, as a pretreatment to freezing process, in order to partially remove water in strawberries tissues. Partially drying experiments were run in a laboratory-scale dryer. Drying was automatically performed using pre-selected parameters of airflow temperature 40°C and air velocity 3m/s. These operating conditions were chosen following series of experimental studies on drying treatment before freezing process carried out and applied to several food matrices such as strawberry [8], quince [6], and apple [9].

Freezing Process

All strawberry samples at different water content (W) levels i.e. fresh (9 g/g db) and partially dried (at 1 and 0.3g/g db) were frozen in a freezer (Whirlpool Model AFG 363/G, Italy) with an air temperature of -30°C.

Thawing process

Just before starting the different quality analyses, conventionally frozen and dehydrofrozen, strawberries fruit pieces were finally placed for thawing step. The treated fruits containing temperature probes already positioned at their centers (a type K thermocouple probe NEDA16046F22006P, diameter 1 mm, range -200 to +200°C, accuracy ± 1°C), connected to an automatic recorder (Dostmann electronic 5020-0309, Germany) were removed from the chest freezer operating at -30°C, to be thawed at room temperature until the final temperature reached the room temperature (20°C).

Quality assessment

Determination of bioactive compounds

Extraction process

For extraction, 0.5g of each strawberry sample was placed in a 30 ml centrifuge tube within10 ml of acidified methanol (1.0% HCl in methanol, v/v). The mixture was agitated, at room temperature for 2 hours, in darkness. The sample suspensions were centrifuged at 6000 rpm for10 min at 4°C and the supernatants were stored at -20°C until analysis [10].

Total Phenolic Content TPC

The phenolic content in strawberry extracts was determined by the Folin-Ciocalteu reagent using Gallic acid as the reference compound [10]. After incubation for 30 min at room temperature, absorption was measured at 765 nm in a JENWAY 6715 UV/V. Gallic acid was used as a standard and the total phenolic content was expressed as gallic acid equivalents (GAE) per 100gram dry basis (g GAE/100 g db) of frozen-thawed fruit, for three replications, through the calibration curve with Gallic acid in the range of 0-500 µg/ml [5].

Total flavonoid content TFC

Total flavonoid content (TFC) of Strawberry fruit was measured using a colorimetric method based on complex aluminum-flavonoid formation [11], followed with the measurement of absorbance at 510 nm against the blank. The outcome data were expressed as mg/g of quercetin equivalents in milligrams per gram (mg QE/g) of dry extract.

Total anthocyanin concentration

0.2 ml of strawberry extract was diluted with 1.8 ml acidified methanol (1.0% HCl in methanol) and absorbance was taken at 250 nm. An acid pH was used to move the anthocyanins to the flavy liumion form, which exhibits coloration, thus being able to quantify them by spectrophotometry. Total anthocyanin concentration (TAC) was calculated as pelargonidin-3-glucoside according to Eq.1 similarly to [10]:

$$TAC = \frac{A}{\varepsilon} * \frac{V}{1000} * MW * \frac{1}{m} * 10\text{^}6 \quad \text{---------(1)}$$

Where:

TAC: Concentration of total anthocyanins content per sample was expressed as g pelargonidin-3-glucoside equivalent per 100 g of dry basis (g eq.Pe-3-Gl/100g db),

A: The absorbance reading,

ε: The molar absorptivity (pelargonidin-3-glucoside=15.600l/(mol cm)),

V: Total volume of anthocyanins extract l,

MW: The molar weight of pelargonidin-3-glucoside=433.2g/mol

m: The sample weight, g.

Color examination

The color of frozen, and dehydrofrozen at different moisture content level strawberries samples was measured with a Konica Minolta CR-300 colorimeter. In this study, response color coordinates L, a, and b were adopted in order to evaluate the effect of partial water removal prior to freezing process on strawberry optical quality.

L, a, and b coordinates show the degree of brightness, the degree of redness (a) or greenness (-a), and the degree of yellowness (b) or blueness (-b), respectively.

Color was measured in Hunter L, a, b units, and as Browning index (BI), defined as following:

$$BI = (100 * (x - 0{,}31)) \,/\, 0{,}17 \text{ -------- } (2)$$

With: $x = \frac{a+1.75*L}{5{,}645*L+a-3{,}012*b}$

Statistical analysis

Since the operating parameter is the initial water content (W) of strawberry samples, one-factorial analysis of variance (ANOVA) was carried out to estimate the least significant differences (LSD) among the media of phenolic compounds: TPC, TFC and TAC and color properties at a confidence level of 95% ($p<0.05$). The statistical estimation was done using Statgraphics Plus software for Windows (1994, version4.1, Levallois-Perret, France).

Results and Discussion

The impacts of initial water content levels (W) on phenolic compounds (TPC, TFC, and TAC), and color properties of conventionally frozen and dehydrofrozen strawberry fruit were investigated.

Strawberry phenolic compounds

Total polyphenol content TPC

The results of total polyphenol contents (TPC) of frozen and dehydrofrozen strawberries with different water contents, after thawing at 20°C are given in table 1.

Data are expressed as the mean ± standard deviation. Values for the same line, having the same letter (a, b and c) for TPC, TFC, and TAC, respectively are not significantly different at a confidence level of 95%.

Phenolic compounds	Water content (g H_2O/g db)		
	9*	1	0.3
TPC (g AGE/g 100g db)	2.77 ± 0.18a	1.89 ± 0.08b	1.27 ± 0.29c
TFC (g QE/100g db)	0.68 ± 0.3a	0.41 ± 0.05b	0.37 ± 0.05c
TAC (Pe-3-Gl/100g db)	0.29 ± 0.01a	0.36 ± 0.03b	0.34 ± 0.04b

Table 1: Impact of Conventional Freezing and Dehydrofreezing processes on phytochemical compounds of Strawberry fruit after thawing at 20°C.

*Frozen and thawed raw material.

Fresh strawberry fruit var Charlotte presents a total initial polyphenol content of 4.18 ± 0.16 g Gallic acid/100 g dry strawberry sample. This level of phenols for unprocessed strawberry is in the range of those reported by several other authors [12].

Partial air drying implied a significant reduction of total polyphenol content (TPC). Indeed, TPC of partially dried strawberry samples were 2.48 and 1.54 g AGE/100g db for water contents of 1 and 0.3g H_2O/g db, respectively. TPC losses as compared with the fresh samples varied from 41 to 63%. Similar tendencies were found on strawberries dried at different temperatures from 40°C to 60°C; Indeed, López-Ortiz, Méndez-Lagunas [13] proved that increasing temperature and drying time negatively affected the TPC concentration during drying of strawberries samples. The decrease in TPC can be also associated with degradation of enzymes, cell structure and molecular deformation.

Similarly, as it is shown in Table 1, the conventional freezing process of fresh strawberry fruit with 9 g H_2O/g db water content, caused a significant reduction in TPC. TPC losses were important for strawberry samples without any pre-dehydration stage and decreased for partially dried ones after the freezing/thawing process. Indeed, TPC losses caused by freezing and thawing processes are about 50, 24, and18% for samples with initial water contents of 9, 1, and 0.3g H_2O/g db, respectively. These experimental results are in agreement with those of other researchers, who claimed that polyphenol content reduction, during freezing could be linked to polyphenol oxidase (PPO) linked to the cell wall, released with cellular disturbance of the fruit [14]. Besides, the degradation of polyphenolic compounds is greater during the thawing step and their interaction with oxidative enzyme is most active in samples with higher water content fruit. In parallel, the intense texture damage after the freezing/thawing process for such high-water level fruit leads to extremely high water exudation and, consequently, more leaching of water-soluble nutrients [5].

Moreover, several researchers studied the effect of the dehydrofreezing process on polyphenols content, on different fruit and vegetables such as apples [15], and quince fruit [5]. These authors reported that dehydrofreezing induced as light depletion of polyphenolic compounds in dehydrofrozen samples compared to conventionally frozen ones, which proved a higher polyphenols retention, respect to conventional freezing. This support that partial dehydration is useful to relieve the loss of polyphenols in frozen/thawed samples.

Total flavonoid content TFC

Among the phytochemical substances present in fruit, flavonoids are considered as the main components of functional foods that act on health through the prevention of cardiovascular disease, oxidative stress, cancer, and inflammation. The total flavonoid content of fresh strawberries was found to be 1.08 ± 0.08 g EQ/100 g db. These values are in good agreement with the data between 0.4 and 0.98 g EQ/100 g in nine different strawberry genotypes [16]. The amount of phenolic compounds in fresh fruit depends on the harvest period, environmental characteristics, cultivar variability, and fruit maturity [17].

As described in table 1, the conventional freezing process has a significant loss effect in total flavonoid content ($p < 0.05$). TFC losses after the freezing and thawing processes are about 37%. This significant reduction of TFC is related to the initial high-water content in a similar way to the results obtained for TPC.

Partial air-drying results in a significant decrease of total flavonoid content (TFC). Indeed, TFC of partially dried strawberry samples with different water contents of 1 and 0.3g H_2O/g db were 0.62 and 0.41 g QE/100 g db, respectively. TFC losses during drying as compared with fresh strawberry samples varied from 43 to 62%.

It is worth highlighting, from Table 1, that the dehydrofreezing process is a relevant solution to sustain freezing major problems, in terms of phytochemical retention. Compared to fresh material,

TFC losses were in the range of 34% and 12% for dehydrofrozen strawberry samples at 1 and 0.3 g H_2O/g db, respectively. Indeed, partial dehydration prior to freezing reduces TFC losses. Again, the level of these reductions can be correlated with the level of thawed water exudates and the preservation of cell walls [5]. The low water activity of the strawberry sample after the drying process would lead to a reduction in enzymatic browning reactions and preservation of the quality of the strawberry [18].

In fact, the degradation of flavonoid in frozen fruit is most likely related to the presence of native enzymes, in particular polyphenol oxidase, which would lead to the degradation of flavonoid into phenolic acids or simple phenolic compounds as a result of degradation of the cell structure [19]. Moreover, Flavonoid losses promoted by hot air drying could be explained by their possible oxidation. Indeed, prolonged thermal treatment may be responsible for a significant loss of natural antioxidants, as most of these compounds are relatively unstable.

Combining convective air-drying and freezing processes, leads to better TFC retention. Indeed, TFC losses are less important for lower initial water content levels (W < 1g H_2O/g db). These findings explain the cryoprotecting effect of the dehydrofreezing process in the phytochemical content of frozen/thawed fruit. This effect may be correlated to the lower freezable water content, which plays a key role in the decrease of the series of reactions leading to the brownness of fruit tissues.

Finally, dehydrofreezing could better maintain phenolic compounds such as flavonoid presumably thanks to the preservation of the cellular structure after freezing, the less generation of native enzymes such as polyphenol oxidase, and the decrease in enzymatic browning in damaged tissues.

Total anthocyanins content

The group of phenolic compounds in strawberry which has received the most attention is the anthocyanins, a major cluster of water-soluble pigment from the flavonoid group. It directly contributes to the coloration of the strawberries and is responsible for their bright red color. Anthocyanins were claimed to possess diverse biological properties and therefore are considered as secondary metabolites with a potential nutritional value [1].

Fresh strawberries have a total anthocyanins content of 0.55 ± 0.05 g Pe-3-Gl/100 g db. These values corroborate those reported in the literature with a TAC for fresh strawberries varying between 0.32 and 0.56 g/100 g db [20].

The pre-drying process results in a significant loss (19% to 27%) of anthocyanins, largely due to their very high sensitivity to temperature and the oxygen. Pelargonidine 3-O-glucoside, which is the predominant anthocyanins in strawberries with 90% of the total anthocyanins, is less stable during drying than other anthocyanins. This result is consistent with other findings and several research studies on the effects of drying on the anthocyanins content of strawberries [21].

In recent years, several studies have been carried out to evaluate the effect of different drying methods (thermo-degradation/thermo-protection) on different groups of anthocyanins compounds. Losses of anthocyanins have been reported at rates ranging from 45-75% during drying at elevated temperatures of 60, 70, or 80°C. These experimental results suggest that the duration and intensity of heat treatment have a strong impact on anthocyanins retention. Therefore, although anthocyanins are lost because of the high temperature and the retention of the pigment in a smaller volume, the drying conditions in terms of temperature and presence of oxygen may promote the activity of polyphenol oxidase, resulting in the browning that characterizes many dehydrated food materials. Indeed, the presence of oxygen, is the key factor contributing to the significant change of thermodynamic parameters in anthocyanin degradation and may be regarded as a catalyst for anthocyanin degradation. In oxygen-rich conditions, as the hot air-drying process, the reactivity of anthocyanin degradation is higher and the system can react faster to generate the activated complex [22].

As shown in Table 1, freezing/thawing processes significantly decreased ($p < 0.05$) the TAC when performed on a sample with high initial moisture content. Indeed, the more the freezing/thawing processes adversely affect the structure, the more the retention of TAC decreases. After the strawberries were thawed at 20 °C, a decrease of 47% was observed.

Torreggiani, Forni [23] reported a significant loss of strawberry anthocyanins at -10°C and -20°C, respectively. This reduction is due to numerous factors such as temperature, light, oxygen, ascorbic acid, sugars, and enzymes [24]. Scibisz and Mitek [25] reported also that the quantity of anthocyanins in frozen fruit depended on fruit species and thawing method.

Dehydrofreezing process presented interesting results for TAC retention compared to conventional freezing treatment. Partial water removal before freezing allowed a significant increase in the retention of total anthocyanins content in comparison to TAC of conventional frozen ones. As explained by Gobbi, Bertolo [26] this phenomenon could be due to both enzymatic inactivation and greater extractability resulting from increasing skin permeability after the heat treatments.

Color evaluation

Data on the color characteristics L, a and b and color derivative parameter i.e. browning index (BI) of fresh, frozen and dehydro-frozen strawberries fruits after thawing process, are plotted and depicted in figure 1 (a; b; c; and d).

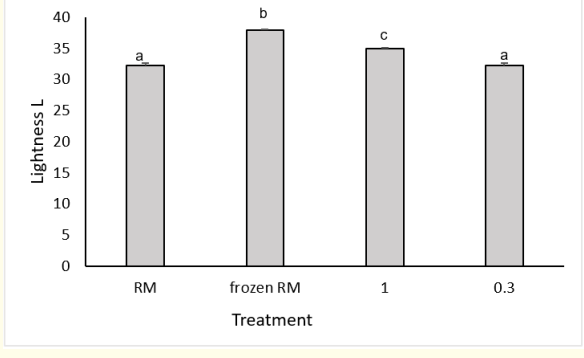

Figure 1a: Effect of freezing and dehydrofreezing processes on lightness coordinate L of RM, and partially dried strawberries samples (1, and 0.3 g H_2O/g db).

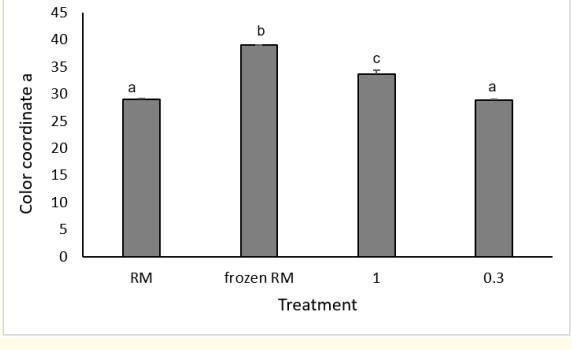

Figure 1b: Effect of freezing and dehydrofreezing processes on color coordinate a of RM, and partially dried strawberries samples (1, and 0.3 g H_2O/g db).

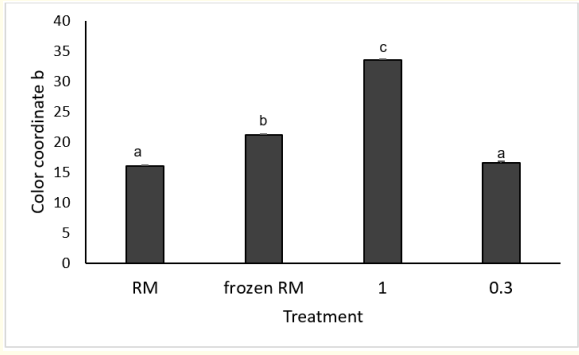

Figure 1c: Effect of freezing and dehydrofreezing processes on color coordinate b of RM, and partially dried strawberries samples (1, and 0.3 g H_2O/g db).

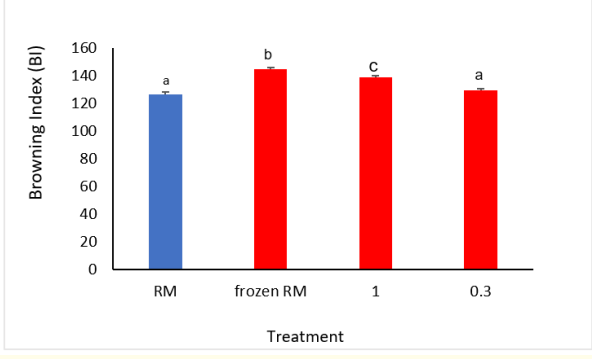

Figure 1d: Effect of freezing and dehydrofreezing processes on Browning index BI of RM, and partially dried strawberries samples (1, and 0.3 g H_2O/g db).

Data are recorded as the mean ± standard deviation. Values having the same lowercase letter (a, b, and c), for different color parameters L, a, b and BI of fresh raw material (RM), frozen RM and dehydrofrozen samples (1 and 0.3 g/g db) are not significantly different at a confidence level of 95%.

As illustrated in Figure 1 (a, b, and c), the measurement of L, a, and b coordinates for fresh samples (RM: 9 g H_2O/g db) showed a low L value ("L" value: 32.22 ± 0.36) for lightness, a positive a value (28.97 ± 0.15) for red color, and a positive b value (16.05 ± 0.13) for yellow color. Freezing and thawing processes, have caused significant changes on all color settings (L, a, and b): these changes

could be attributed to the diffusion of the phenolic pigments from the center of the strawberry fruit to the outermost cell layers due to the damaged walls (the crystallization and fruit volume increase) during freezing, and to the cell sap leaching during thawing, as described and obtained on several fruits and vegetables such as strawberries [27] and quince fruits [6].

Interestingly, among color parameters, an increase in "a" (greenness/redness) value was a characteristic color change which led to a higher value (BI) in frozen/thawed strawberry (Figure 1-d) compared to fresh sample. These Higher "a" values were attributed to pigment diffusion from the center of the fruit to the outmost cell layers because of disrupted cell walls. Moreover, Compared to RM samples, conventionally frozen strawberries exhibited markedly higher "L" values, which might be a result of small ice crystal formation on the fruit surface, as previously reported by Zaritzky, Añón [28].

Based on the data obtained from the dehydrofreezing process, a significant decrease of thawing impact on color coordinates has been noticed. Figures 1(a, and b) highlighted the effect of the freezing/thawing process on lightness and redness decreases with water content deceleration and remained insignificant for samples pre-dried at 0.3g H_2O/g db. Similar studies of various fruits and vegetables have highlighted the protective role of dehydrofreezing on the optical quality of thawed products such as) and mango [29] and quinces [6]. This can be attributed to the lower water activity of strawberries samples after drying process which caused the reduction of enzymatic browning reactions [30].

As expected, calculated browning index BI values of strawberry fruit after thawing (Figure 1-d), induced by freezing and dehydrofreezing processes, strongly agree with color parameters values cited above in Figure 1 (a, b and c). It decreased significantly ($p < 0.05$), with the decrease of water content levels. For frozen RM, 1, and 0.3 g H_2O/g db water content samples, it manifested initial values of 144.93 ± 1.31, 138.83 ± 1.12, and 129.70 ± 1.12 for 9 (frozen RM), 1, and 0.3, respectively. The combination of both methods of convective air-drying and freezing has led to a better preservation of color. Indeed, the lower the partial water content issued from drying process, the lower the strawberry color change issued from freezing/thawing. These findings explain the cryoprotecting effect of dehydrofreezing process in frozen/thawed fruit color. This effect may be due to lower freezable water content, which plays a role in the decrease of the number of reactions leading to the brownness of the fruit tissues [6].

The color change of fruits is generally because of the destruction of anthocyanins causing rise in polymeric color. Since, monomeric anthocyanins convert into polymeric form, frozen products of plant origin undergo various modifications in color during freezing/thawing process, owing to changes in the naturally occurring pigments, such as anthocyanins. or by enzymatic browning, when color change is related to fruits with red hue, this is because of anthocyanin (water soluble) destruction by enzyme-induced oxidation of the polyphenols during processing and storage. Additionally, Alterations in color owing to enzymatic browning are caused by the oxidation of phenols in the presence of oxygen in products with a and so on. Polyphenol oxidases act as catalysts for this reaction, leading to the formation of quinones that condense in the form of brown or reddish-brown compounds.

Previous analysis of phenolic compounds especially, for anthocyanins content of dehydrofrozen strawberry fruit at different water content levels (Table 1) provided compelling evidence that convective airflow drying prior to freezing guarantees pigments retention and thus, attenuates color changes after thawing step. These facts are well consolidated in color coordinates and in browning index analysis of dehydrofrozen strawberries samples. Indeed, it has been stated in several previous studies on color stability of frozen products that ice-crystal formation increases during the storage period, resulting in the breakdown of cellular tissues and pigment losses [5,27]. On the other hand, the pre-dried products were sufficiently dehydrated, preventing the formation of large ice crystals; therefore, ice-crystals formation in these samples did not proceed to the breaking point. This allows a better pigment retention and thus ensures a preservation of frozen product color.

Conclusion

The results obtained in this work showed that strawberries dehydrofreezing exhibited significant effects on phenolic compounds retention. Indeed, convective pre-drying process allowed the freezing/thawing operation to preserve the nutritional values of strawberries fruit, in terms of TPC, TFC and TAC. Compared with untreated frozen strawberries, dehydrofreezing globally preserves the bioactive molecules for treated samples with lower initial moisture content (<1 g/g db). Thus, adequate drying of fruit before

freezing may be a relevant tool to maintain the stability of fruit nutritional quality.

This research work is a contribution to dehydrofreezing process investigation. It provides applied information on the coupling of air drying and conventional freezing processes impact on fruit quality. Hence, further studies on dehydrofreezing technology are needed to better monitoring and explaining the water dynamics of strawberries during dehydrofreezing.

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Conflict of Interest

The authors declare no conflict of interest.

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