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Studio delle variazioni di costituenti fenolici e polifenolici e del potere antiossidante di estratti ottenuti da frutti sottoposti a trattamenti osmotici e surgelazione

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Title
Study of the variation of the phenolic and polyphenolic content and of the antioxidant capacity of extracts obtained from osmotically pre-treated and frozen fruits

Key words
Fruits, phenols, osmodehydratation, freezing, sensory evaluation

Parole chiave
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Summary
Over last years consumers are paying more and more attention to a diet rich in fruits and vegetables that assure a proportionate intake of antioxidant compounds such as carotenoids, tocopherols, ascorbic acid, flavonoids and other phenolic compounds. Several scientific studies proved the efficacy of these minor compounds of foods in contrasting or slowing down degenerative and cardiovascular diseases. Freezing is a useful technology for preservation of nutritional compounds of food over long periods; but thawing causes a dramatic decrement of organoleptic and nutritional characteristic due to the high content of water in fruits. Several technologies, based upon osmotic processes (OD, PVOD, VI, ICF, etc.) could be used as pre-treatment, in order to improve fruit performance in freezing/thawing processes. This work reports results on the preservation of antioxidant capacity (based on ABTS radical cation test) and phenolic content (determined by HPLC) of some fruits (apples, nectarines and strawberries) osmotically pre-treated and subsequently frozen. Results show that these technologies could preserve phenolic content of fruits along with the storage period and during thawing for direct consumption.

Riassunto
E' ormai sempre più diffusa tra i consumatori una particolare attenzione e sensibilità riguardo all'importanza di un regime alimentare ricco in prodotti di origine vegetale che assicuri un adeguato apporto di composti antiossidanti, quali caroteni e carotenoidi, tocoferoli, acido ascorbico, flavonoidi ed altre molecole a struttura fenolica. Molti studi scientifici hanno infatti dimostrato l'efficacia di questi costituenti minori degli alimenti nel contrastare o rallentare l'insorgenza di numerose patologie cardiovascolari e degenerative. La surgelazione è una tecnologia di conservazione che consente di preservare le caratteristiche nutrizionali degli alimenti molto a lungo; d'altro canto essa si presta meno per la conservazione dei frutti, a causa del loro elevato contenuto di acqua che in fase di scongelamento determina un decremento organolettico e nutrizionale rilevante. Per migliorare le performance dei frutti in surgelazione/decongelamento, sono in fase di applicazione negli ultimi anni diverse tecnologie osmotiche (OD, PVOD, VI,

ICF, etc.) durante il pre-trattamento di frutti e vegetali. Nel presente lavoro si riportano alcuni risultati ottenuti mediante l'utilizzo di processi osmotici su frutti surgelati (mele, pesche nettarine e fragole) in merito alla conservazione del profilo fenolico, valutato mediante HPLC, nonché alla capacità antiossidante (test spettrofotometrici basati sull'impiego del radicale catione ABTS) degli stessi frutti. I risultati ottenuti dimostrano come l'utilizzo di tali tecnologie siano in grado di preservare il contenuto fenolico dei frutti durante la conservazione e le seguenti fasi di decongelamento e consumo.

Abbreviations:
2,2'-azinobis(3-ethylbenzothiaziline-6-sulfonate) (ABTS); dry matter (DM); immersion chilling freezing (ICF); osmotic dehydration (OD); pulsed vacuum osmotic dehydration (PVOD); solid gain (SG); titratable acidity (TA); vacuum impregnation (VI); water loss (WL); weight reduction (WR).

Introduction

Over last years consumers are paying more and more attention to a diet rich in fruits and vegetables that assure a proportionate intake of antioxidant compounds such as carotenoids, tocopherols, ascorbic acid, flavonoids and other phenolic compounds. In fact, fresh fruits consumption is associated with a reduced risk of cancer and cardiovascular disease and protects the human body against the cellular oxidation reaction (1-3).
After harvest and before consumption, vegetables may be stored for variable periods of time and may be processed and prepared under a wide range of conditions. The nutritional value of fresh fruits, that present an elevated antioxidant capacity and phenolic content, tends to decrease quickly with storage time and freshness loss. Moreover the antioxidant capacity of processed and canned fruits decreases drastically during the technological processes, in particular for heat application.
Freezing of fruits is one of the most common ways for the maintainance of the quality of these products. The demand for storing raw material, using industrial freezing, has increased (4, 5). The freezing process itself has little or no effect on phenolic compounds content (6). It has been specified, in recent studies, that the most important nutritional changes in frozen foods are due to the storage temperature and times (7). Frozen fruits consumption take place after the fruits thawing: thawing causes cell disruption (due to ice crystal damages during freezing and storage) and other important modifications of fruits quality (organoleptic and nutritional losses) as, first of all, phenolic oxidation by enzymatic reactions.
In recent years many studies have pointed out the importance of pretreatment before the freezing process to limit ice crystal damages. Osmotic treatments are used before freezing to produce several kinds of food products that can be stored for long periods; foods treated in this way retain, after thawing, good texture, color, and flavor (8, 9). One of the recent evolution of osmotic processes is vacuum impregnation (10).
This paper report results of new and previous investigations in fruit antioxidant content in osmo-dehydrated frozen fruits:
Experiment 1. The first work studied the effects of freezing and osmotic processes on the phenolic fraction of strawberries (11).
Experiment 2. The second work investigated the changes in phenolic content and the antioxidant capacity of extract of apples from two cultivars submitted to an osmotic

process, freezed and stored at -18°C for 7 days before analysis (12).
Experiment 3. Finally, this work reports results of phenolic fraction and antioxidant capacity of osmotically treated frozen nectarines after thawing (13).

Experimental

Strawberries samples

Strawberries of *cv*. Alba were bought at a local market. From an initial weight of 24 kg of strawberries, at pink state of ripeness, fruits without damages and ranging from 23 to 27 g were selected obtaining a final global sample of 12 kg. After stalk removing, the fruits were accurately mixed up and the sample was divided in three subsamples of 4 kg each: fresh strawberries (FR3 and FR4), fresh frozen strawberries (TQ) and vacuum impregnated + osmo dehydrated strawberries (VI).
Fresh strawberries: whole fruits were washed in tap water and drained and then immediately analyzed as described below.
Fresh frozen strawberries: whole fruits were washed in tap water and drained and immediately frozen in a freezing chamber with direct contact with dry ice. Fruits at -79.8°C were stored in a conventional freezer at -18°C and stored for 3 days at the end of which they were analyzed as described below.
Vacuum impregnated + osmodheydrated strawberries: whole fruits were washed in tap water, drained and put in a vacuum chamber connected to a vacuum pump. Then, sucrose syrup (50%) was added and fruits were kept submerged by using a grid. The syrup/fruit ratio was 5/1 and syrup temperature was kept constant during all the process (30°C). A first vacuum step was applied at 100 mbar and for 5 min. This osmotic dehydration continued for 3 h and 55 min. Fruit agitation were realized manually at regular intervals of time. At the end of the process, fruits were drained from residual syrup and quickly frozen as described before and stored for 3 days at -18°C in a coventional freezer.
Each analysis (described in the following) was carried out on subsamples of 19 strawberries (about 250 g). For fresh strawberries 8 subsamples were analyzed to verify the representativeness of subsample size. In the Results and Discussion section we reported results of two of the 8 subsamples analyzed (FR3 and FR4), that was the couple of subsamples characterized for the greatest statistical differences in the phenolic content, thus making the data discussion more robust.

Apples samples

For details about samples and analyses carried out on experiment 2 presented in this paper we refer to Blanda et al. (12).

Nectarines samples

For details about samples and analyses carried out in experiment 3 presented in this paper we remind to Blanda et al. (13).
All analyses (dry matter, soluble solids, tritratable acidity, mass transfer determination, sensory evaluations analyses, total *o*-diphenol, ABTS•+ assay, and HPLC-DAD phenolic determination) carried out on the samples are extensively described in Blanda et al. 2008b and 2008c (12, 13).
As regard phenolic extraction of strawberry samples and HPLC-DAD-MS (with electrospray interface) phenolic determination, we adapted the method described by Gil et al. (14).

Results and discussion

Table 1 shows results of the pH, tritratable acidity, dry matter and rifractometric index in fresh (FR3, FR4), frozen (TQ) and vacuum impregnated + osmodehydrated + frozen (VI) strawberries. It has been also reported the mass transfer occurred during the VI process. It could be seen the relatively high water loss extent occurred in 4 h processes on whole fruits. This partial water removal accounts for bet-

Table 1 - pH, tritratable acidity, dry matter, rifractometric index and mass transfers of strawberry samples. The symbol % indicates percentage of initial sample weights

Samples	pH	TA (meq O_2)	DM (%)	°BX (%)	WR (%)	SG (%)	WL (%)
FR3	3.43ab	10.80a	6.16b	5.65b	/	/	/
FR4	3.45ab	10.76a	6.15b	5.65b	/	/	/
TQ	3.41ab	10.80a	6.21b	5.66b	/	/	/
VI	3.41ab	9.90b	10.92a	8.15a	8.24	3.86	12.10

Letters (a, b, ab) indicate statistical significant differences (HSD Tuckey $p<0.05$). Abbreviation used: TA (titratable acidity); DM (dry matter); WR (weight reduction); SG (solid gain); WL (water loss); FR3 and FR4 (fresh strawberries); TQ (fresh frozen strawberries); VI (vacuum impregnated + osmo dehydrated strawberries)

ter freezing/thawing performance of fruit and higher texture retention and organoleptic quality retention (data not shown). As mentioned above, fresh strawberries were processed at a pink stage of ripeness as it could be evinced from the high acidity/refractometric index ratio.

In table 2 are reported results of HPLC-DAD-MS phenolic determination. As it can be observed, the amount of the 19 phenolic compounds quantified agrees with the results obtained by M. Kosar et al for strawberries at an early (pink) maturation stage (15). In general, the TQ sample shows a decrease (although not always statistically significant) of the phenolic compounds content with different extent for each compound. The total phenolic compounds content shows a reduction that is significative only if compared with FR4 sample (about -20%) and it is not significative if compared with FR3 sample. In the VI process there is a further decrease of phenolics compounds respect to TQ samples, but this difference (-10.8%) it is not statistically different. Anyway we can affirm that the global process (freezing + VI process) causes a phenolic decrease in strawberries of about 21-29%. This decrease would be higher (27-34%) in absolute terms, considering that VI strawberries underwent an 8.24% weight reduction that causes a partial concentration of chemical compounds. The causes of phenolic decrease due to freezing and VI process are not well known. Certainly, freezing causes cell breakdown thus putting enzymes in contact with substrates. Although this phenomenon is accelerated during the thawing out process in a bland way, it could occur at the end of freezing and during the days of storage as well. As regard the VI process, due to the temperature applied and to the use of syrup, phenolic depletion could be due to oxidation and hydrolysis reactions and to leaching of chemical compounds in the solution.

In a second work (12) we used dextrose + sucrose syrup with high ascorbic acid (AA) content during the vacuum impregnation of apple slices from two varieties, in order to prevent phenolic oxidation during the process and during the 7 days storage. Obviously, the antioxidant capacity of the phenolic extracts obtained from the pre-treated + frozen extract were higher than the control samples, while the HPLC-DAD phenolic profile was significantly lower of about 12% and 13% in Stark Delicious and Granny Smith variety. We supposed that the decrease occurred during the process was due to phenolic leaching into the treating solution and to hydrolysis reactions. The VI process last for 30 min but it was sufficient to obtain apple slices with high sensory quality. In fact, during the QDA sensory analysis of sample, judges rejected to taste thawed control samples considering them not eatable while they tasted VI samples and quantified sensory attributes.

Table 2 - HPLC-DAD-MSD (with electrospray ionization source) contents of phenolic compounds (mg/100 g) quantified in fresh, frozen and pre-treated + frozen strawberries. Maximum of absorbance and the most abundant mass fragment in positive or negative modality are reported

Compound (retention time)			FR3	FR4	TQ	VI
Cyanidin 3-glucoside (19.21 min)	abs (nm)	280, 520	0.089 ab	0.103 a	0.076 ab	0.063 b
	m/z +	449				
Pelargonidin 3-glucoside (21.18 min)	abs (nm)	280, 505	3.764 ab	4.455 a	3.062 b	2.771b
	m/z +	433.1				
Pelargonidin 3-rutinoside (22.60 min)	abs (nm)	275, 505	0.209 ab	0.250 a	0.172 ab	0.143 b
	m/z +	579				
Unknown anthocyanin (25.58 min)	abs (nm)	520, 285	0.015 a	0.016 a	0.009 b	0.010 b
	m/z +	ND				
Unknown anthocyanin (28.17 min)	abs (nm)	504	0.528 a	0.631 a	0.355 b	0.333 b
	m/z +	519.3				
Pelargonidin 3-acetylglucoside (33.43 min)	abs (nm)	504	0.031 b	0.046 a	0.027 b	0.028 b
	m/z +	475				
Galloyl derivative (27.54 min)	abs (nm)	284	2.322 a	2.502 a	2.738 a	2.322 a
	m/z +/-	333, 355				
Caffeoyl glucose (9.56 min)	abs (nm)	250, 300sh, 330	0.062 a	0.066 a	0.090 a	0.072 a
	m/z -	341.1				
p-coumaryl glucoside (14.60 min)	abs (nm)	236, 316	0.403 b	0.533 a	0.564 a	0.370 b
	m/z +	325.1				
Unknown compound (15.35 min)	abs (nm)	246, 296sh, 330	0.025 a	0.030 a	0.020 b	0.017 b
	m/z +	ND				
Unknown compound (15.91 min)	abs (nm)	236, 316	0.067 ab	0.077 a	0.057 ab	0.044 b
	m/z +	ND				
Unknown compound (16.83 min)	abs (nm)	244, 328	0.052 a	0.055 a	0.047 ab	0.043 ab
	m/z +	ND				
Ellagic derivative (31.06 min)	abs (nm)	254, 302, 260	0.129 a	0.117 a	0.092 b	0.086 b
	m/z +	ND				
Quercetin 3-glucuronide + glucoside (32.62 min)	abs (nm)	254, 300sh, 356	0.654 a	0.523 a	0.235 b	0.334 ab
	m/z -	463, 477				
Ellagic acid (33.64 min)	abs (nm)	254, 300sh, 360	0.257 a	0.195 a	0.118 b	0.169 ab
	m/z -	301				
Unknown compound (34.77 min)	abs (nm)	258, 356	0.035 a	0.039 a	0.022 b	0.022 b
	m/z +	ND				
Kaempferol 3-glucuronide (37.20 min)	abs (nm)	265, 300sh, 350	0.179 a	0.157 a	0.096 b	0.103 b
	m/z -	461				
Kaempferol derivative (39.06 min)	abs (nm)	265, 350	0.080 a	0.083 a	0.051 b	0.053 b
	m/z +	ND				
Kaempferol derivative (41.11 min)	abs (nm)	265, 350	0.019 ab	0.023 a	0.014 b	0.015 b
	m/z +	ND				
Total polyphenols			8.920 ab	9.898 a	7.846 ab	6.995 b

Letters (a, b, ab) indicates statistical significant differences (HSD Tuckey $p<0.05$). Abbreviation used: abs (maximum of absorbance with sh that indicates maximum of absorbance of shoulder spectra); m/z (mass of most abundant ion/s in positive or negative mode); ND (not detected); FR3 and FR4 (fresh strawberries); TQ (fresh frozen strawberries); VI (vacuum impregnated + osmo dehydrated strawberries)

In a third research (13) we submitted nectarine slices to two different osmotic pre-treatments (soaked and vacuum impregnated for 15 min in a fructose syrup containing other solutes among which 2% AA) and freezing. We analyzed the antioxidant capacity (ABTS test), *o*-diphenols (spectrophotometric), chlorogenic and neochlorogenic acids (HPLC-DAD) and hedonic sensory values of fresh slices versus not treated, soaked and vacuum impregnated frozen slices after thawing out for 4 hours at controlled temperature. It was very interesting to observe that the ascorbic acid prevent phenolic oxidation during thawing and accounted for higher hedonic value of treated samples. In particular the VI slices were impregnated with higher syrup quantities thus presenting higher antioxidant capacity, *o*-diphenolic and chlorogenic and neochlorogenic acid content than any other sample. In conclusion, osmotic processes with vacuum applications used as pre-treatments to freezing lead to a discrete water removal from whole or sliced fruits, thus permitting less damages to fruit cell structure during freezing/thawing processes and improved organoleptic values with respect to not treated fruits. Moreover, through VI it is possible to introduce, in a relative short time, small quantities of sugars and others chemical compounds with different purposes. This chemical-physical and sensory "gain" requires a moderate "duty" of fruit phenolic amount with respect to exclusively frozen strawberries.

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