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Effect of vacuum freeze-drying on the antioxidant properties of eggplants (*Solanum Melongena L.*)

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Abstract

The Vacuum Freeze Drying (VFD) process is a low temperature drying technique that can be used for food preservation. The aim of this work is to evaluate how VFD operating conditions affect the amount of some nutritional compounds of eggplants (*Solanum Melongena L.*). The product samples were freeze-dried under different pressure and temperature conditions and had their ascorbic acid (AA) concentration, total polyphenol content (TPC) and antioxidant capacity (AC) measured before and after processing. Temperature was found to have a significative effect on primary drying times: considering results obtained at 40 Pa, the duration moved from 8.6 ± 2.5 when the process was carried out at 0°C, to 20.9 ± 7.8 h at -30°C. Chamber pressure had a less significant effect, being drying time, at 0°C, ranging from 6.2 ± 2.3 h, for the test at 20 Pa, to 8.6 ± 2.5, for test done at 40 Pa. Very low temperatures resulted in higher AC retentions, being the AC loss 68.6 ± 1.5% at 0°C and 49.9 ± 3.2% at -30°C (at 10 Pa), while having a negative impact on the AA content, being the AA loss 12.2 ± 1.7% at 0°C and 37.9 ± 3.4% at -30°C (at 10 Pa). Higher pressures were favourable for TPC retention in the dried product: being the TPC loss 47.7 ± 5.5% at 10 Pa and 32.5 ± 8.5% at 40 Pa (at 0°C). A similar trend can be observed for AC, where the AC loss was 68.8 ± 1.5% at 10 Pa and 35.7 ± 12.1% at 40 Pa (at 0°C). Freezing stage had a non-significant effect on the targeted compounds.

Keywords

Vacuum freeze-drying; eggplant; ascorbic acid; polyphenol; antioxidant compounds.

Abbreviations

AA	ascorbic acid
AC	antioxidant capacity
AFD	atmospheric freeze-drying

TPC	total polyphenol content
VFD	vacuum freeze-drying

Introduction

Food products, such as fruits and vegetables, are usually characterized by high-water contents, favouring microbial growth and several biochemical reactions, such as enzymatic browning, oxidation and hydrolytic reactions, isomerization, cyclization and protein denaturation.[1] These reactions may lead to undesirable changes in the product and, thus, should be prevented. For this reason, dehydration is one of the most ancient methods used to preserve food products.[2],[3] However, the drying process may partially or severely affect the properties of foodstuffs, especially when done at high temperatures.

Freeze-drying is a low temperature dehydration process based on the sublimation of ice as the water removal mechanism. This sublimation may take place under vacuum (vacuum freeze-drying, VFD) or atmospheric pressure (atmospheric freeze-drying, AFD), since the driving force is the vapor pressure gradient between the food product and the chamber, rather than the absolute pressure in the system.[4]-[6] When compared to the high temperature drying processes, freeze-drying is more expensive, mainly due to the equipment required and to the duration of the operation.[3] However, higher final product quality is generally achieved by this low temperature drying, thus making this process of particular interest for both the pharmaceutical field, and for some high value food products due to the seasonal availability, perishability and nutritional content.[7]

In freeze-drying drying time is an important concern since it is usually longer than in other dehydration methods.[3] For the VFD process the thermal exchange between the equipment and the product may be affected by the low pressure, particularly the conduction between the heating shelf of the freeze-dryer and the product lying upon it. Whereas, with respect to the AFD process, the rate is usually limited by the diffusion of water vapor in the solid matrix. Besides, it is crucial to understand in which way the operational parameters, namely shelf temperature and chamber pressure, affect the process rate and the final product

quality. Product temperature, chamber pressure, and the resulting drying time, are in fact the variables that may interfere in different ways on nutritional compounds of foods during a freeze-drying process, and thus, should be further investigated.[1]

Different compounds may behave differently to the pressure and temperature used in a given process. Therefore, it is important to first identify the compounds of interest and then choose and design the most suited processing conditions. There is a number of published works on the effects of freeze-drying on the final product quality, being the rehydratability the most studied attribute.[7] For instance, green peas appeared significantly softer when they were rehydrated after the AFD process carried out in a fluidized bed and did not recover their original hardness.[8] Microwave pre-drying combined with VFD resulted in better rehydration capacity for carrot chips than VFD alone.[9] However, microwave assisted VFD of potato chips pre-treated by osmotic dehydration resulted in lower rehydration capacity than untreated samples.[10] Infra-red assisted VFD on tiger prawn, in another study, resulted in an increase of the rehydration ratio.[11] Still, many high valued products are esteemed due to their nutritional properties such as antioxidant capacity and vitamins content.[1] The antioxidant capacity of foods was found to be related with vitamins or phenolic compounds.[12] Phenolic compounds, naturally present in foodstuffs, may in fact lower the risks of a large range of diseases.[13] In addition, vitamin C is believed to be one of the most important hydrophilic antioxidants, being used as a food preservative in a wide variety of food products.[14] Hence, measuring the drop in the concentration of target attributes, i.e. antioxidant capacity (AC), ascorbic acid (AA) and total polyphenols (TPC), can be a practical approach to evaluate the impact of drying processes on the final product nutritional properties.[15]

Besides drying conditions, freezing plays an important role in product quality preservation; furthermore, it influences drying rate. This is due to the fact that the operating parameters of the freezing stage have a direct impact on the product final structure, such as the

connectivity of the porous matrix and its pore size distribution.[16] Food products have generally a more stable structure, less prone to shrinkage if compared to pharmaceutical solutions. However, shrinkage, i.e. a reduction in volume and loss in porosity, may occur also in food products due to capillary forces, temperature gradients and any other event that may produce a stress on the solid structure.[7] Low freezing rates result in big ice crystals, which can disrupt cell walls and membranes leading to irreversible changes in texture. Conversely, fast freezing rates form smaller ice crystals, having usually a lower impact on product quality.[17] Moreover, it is important to remember that a certain amount of water in the food matrix does not freeze, and it remains bounded to the other molecules.[1] In some cases, freeze-dried samples presented no significant changes in the concentration of the compounds of interest, or even showed an increase of these values. Many authors attribute this increase to the effects of the freezing step, since the formation of ice crystals can damage cell walls and, thus, facilitate the extraction of the measured compounds.[18],[19] For instance, muskmelon had no significant content change after VFD for TPC, β-carotene and antioxidant capacity values measured by DPPH and FRAP assays.[18] Lipid peroxide inhibition values were higher for muskmelon after VFD[18] as well as the flavonoids and TPC contents of wheatgrass after VFD.[19]

Previous studies regarding the effect of freeze-drying on the nutritional properties of food products yielded different results according to the product and drying conditions applied.[1] Apples freeze-dried with and without the use of vacuum micro-wave assisted drying had ascorbic acid retentions varying from 58.6% to 82.6%.[20] When using AFD, TPC, AC and AA content reduction varied according to the shape and operating conditions used.[21] Still regarding apples, ascorbic acid decreased of 70%, TPC decreased of 39% and antioxidant activity, measured by the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method, decreased of 6% after AFD with respect to VFD, as shown in another study.[22] Blueberry cultivars after VFD showed an increase in anthocyanin and phenolic concentration, but the increase was, in most cases, not

significant. In addition, the total antioxidant capacity, measured by means of 2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid assay, after VFD was not significant.[23] The antioxidant properties of porcine placenta hydrolysates after vacuum freeze-drying did not differ significantly from the values found in the samples before drying. Results were similar both using DPPH, superoxide and hydroxyl radical scavenging activity and lecithin liposome antioxidant activity assays. These results show the superior quality that can be achieved through freeze-drying methods.[24]

Eggplants are an important vegetable product in Asian and Mediterranean diets while they present a very limited shelf life for freshness.[25] Eggplants (*Solanum melongena*) are a very interesting case study since they exhibits a high antioxidant capacity and even more sophisticated drying methods such as vacuum drying[25] and atmospheric freeze-drying[26] were previously studied, for instance. In addition, eggplants have a soft porous structure which requires shorter freeze-drying times, thus standing as a good candidate for obtaining commercial freeze-dried products.[25],[27] Still, it is important to be careful regarding product variability. Many studies have found variations in nutritional compounds such as vitamin C, phenolic compounds and antioxidant capacity, for eggplants according to the cultivar[28][29], agricultural practices[30],[31] and harvest season[27].

The objective of this study is to evaluate the effect of vacuum freeze-drying operating conditions, as well as the freezing step, on some targeted nutritional properties of eggplants (*Solanum melongena L.*). The operating parameters investigated are shelf temperature and chamber pressure, while the targeted bioactive compounds are vitamin C, total phenolic content and antioxidant capacity. Results of this study will thus be able to assess if freeze-drying is a valuable technology for this challenging case study, and which are the operating conditions to maximize plant productivity (i.e. minimize drying duration) and component retention.

Materials and methods

The freeze-drying experiments were carried out in a REVO® Freeze Dryer (Millrock Technology, Kingston, USA), which is a pilot-scale equipment with about 1 m^2 shelf area. It is equipped with an external condenser, which operates at approximately -80°C (having a maximum condensing capacity of 30 kg).

The ratio between the measurement of the capacitance gauge (Baratron type 626A, MKS Instruments, Andover, MA, USA) and the thermal conductivity gauge (Pirani type PSG- 101-S, Inficon, Bad Ragaz, Switzerland) was used to assess the effect of the process variables on the primary drying duration: it presents a sharp decreasing trend as the drying process is close to be completed.[32] The on-set and the off-set of the decreasing part of the curve defines the range of variability of drying time, considering that the drying conditions, and thus the drying duration, are non-uniform in the batch. Accordingly, to define a representative time for the end of primary drying, the midpoint of this part of the curve, between the on-set and off-set points, was regarded as the average primary drying duration.

The eggplant (*Solanum melongena L.*) samples used in this study were of the black beauty variety and were purchased in the morning of each experiment day from the same local market in the region of Turin (Italy). The samples were then cut using a household tool to get cubes from the inside of the eggplants of approximately 9 mm side.

To avoid enzymatic browning of the phenols, and the degradation of other bioactive compounds, all samples were treated for 5 minutes with a sodium metabisulfite solution at 2% w/v (Honeywell Fluka™, ≥ 98%) similar to what was described by Akyildiz et al.[33] For simplicity, this paper will refer to the fresh pre-treated samples as “fresh” samples, but it should be noted that all comparisons between before and after drying were made based on the fresh pre-treated samples before and after drying. This pre-step was performed also by Colucci et al.[26] when investigating the effect of atmospheric freeze-drying on eggplant cubes. By

preventing enzymatic browning, phenol content variations can be more consistently compared within the experiment as well as to the results measured in other studies where other drying methods are used, as long as they follow the same sample preparation steps. This is especially relevant when comparing VFD to other drying methods in the presence of oxygen, such as AFD, since polyphenol oxidase enzyme activity, for instance, requires oxygen. To enrich this study, a comparison between the results from the application of VFD and AFD to dry eggplants was done. This way, the same pre-steps done in that study Colucci et al.[26] were chosen for consistent comparisons of results.

For each tested condition, all cubes were lined up on the freeze-drier shelf approximately 1 cm apart from each other. In each test approximately 50 eggplant cubes were processed to ensure enough material for samples extraction, necessary for the quality evaluation. After placing the samples in the freeze-drier, shelf temperature was reduced to -40°C and kept at this value for 4 hours to ensure complete freezing of the samples. To monitor temperature evolution in each batch, ten T-type thermocouples (Tersid, Milano, Italy) were used. Half of them were carefully placed inside the product, approximately 1 mm from the top of the sample, and half were placed approximately 1 mm from the bottom. This way, sample temperatures near the top (T_{top}) and bottom (T_{bottom}) were monitored throughout the whole process. The values of temperature and pressure in the drying stage were then set according to the design of experiments.

Design of experiments

First, an experiment was carried out to evaluate the effects of the freezing step on the selected nutritional properties. The product was frozen under the same conditions used for the drying tests, i.e. -40°C for 4 hours. The nutritional properties were measured before the product started thawing, and the extraction was done following the same protocol as for the dried samples.

described further ahead. The effect of freezing was evaluated with four repetitions, and the average compound concentrations found for the frozen product were compared to those of the corresponding fresh pre-treated product.

Thereafter, to evaluate the effects of shelf temperature and chamber pressure in vacuum freeze-drying of eggplants, two sets of experiments were carried out. The first set had the goal to assess the effect of the temperature, while the second set evaluated the combined effect of temperature and pressure on drying times and targeted nutritional compounds loss after drying. For the first, pressure was set at 10 Pa, using primary drying temperatures of -30°C, -15°C and 0°C. For the second set, three pressure levels, 10 Pa, 20 Pa and 40 Pa were tested at -30°C and 0°C. After primary drying, all batches had the same secondary drying step: chamber pressure value was the same of the primary drying stage, while shelf temperature was risen to 20°C for 2 hours. Each test, for a given temperature and pressure condition, was done with three repetitions.

Quality assessment procedure

Since samples were pre-treated with sodium metabisulfite, the concentration reduction of the targeted compounds was evaluated by comparing the initial concentration (c_0) of the components in the fresh samples after pre-treatment to that after freeze-drying (c_d) or after freezing, in case of the freezing test. The component loss percentage was calculated as described in Equation 1:

$$\%\ Reduction = \frac{c_0 - c_d}{c_0} \times 100\% \quad (1)$$

To minimize the effect of the natural product variability, for each test done, part of the fresh pre-treated eggplant samples had its targeted compounds measured to be compared to the values obtained after drying. These bioactive compound content measurements for the fresh pre-treated samples were done with two extracts, and the average values found with the analytical

methods explained ahead were regarded as the fresh sample contents for each batch.

The extraction of the bioactive compounds from the eggplants was done with ethanol (Honeywell Fluka™, 96% v/v) at room temperature (20°C). Each extraction was performed by following the same procedure, with 0.5 ± 0.05 g of dried product for freeze-dried samples, or 8 ± 0.15 g of fresh eggplant for fresh pre-treated samples, which represented approximately 15 cubes for each extraction. These cubes were smashed, with a mortar and pistil, with 15 mL of ethanol, then homogenized with an Ultra-Turrax® (IKA T-25) for 3 minutes at 9500 rpm, and, finally, put into a magnetic stirrer for 20 minutes. The final extract was obtained by light vacuum filtration, performed with a vacuum pump (BUCHI V-700) and glass microfiber filter (GFFC grade, 1.2 µm). The final volume of the extract was adjusted with ethanol in a 25 mL volumetric flask. During the extraction, the product was protected from light degradation by covering the container with an aluminium foil, and the analytical assays to evaluate the final product nutritional properties were carried right away after extraction.

Ascorbic acid content

The method used to measure the ascorbic acid content was a colorimetric test, based on Jagota and Dani[34], using the following reactants:

- Folin-Ciocalteu (Sigma-Aldrich, 2 M) reagent, diluted in distilled water (1:10 v/v);
- Trichloroacetic acid (Sigma-Aldrich, 6.1 N), at 7.5% by volume.

At first, 1 mL of extract and 1 mL of trichloroacetic acid were vigorously mixed in a cylinder and then left to rest for 5 minutes in a fridge at 4 ± 1°C. Then, the solution was filtered with a 0.45 µL nylon syringe filter (SFNY, 0.45 µm). Both for the measures on the fresh and on the dried product it was not necessary diluting the extract. For the colorimetric reading, 0.2 mL of this filtered extract were placed in a 4.5 mL spectrophotometer cuvette with 0.2 mL of Folin-Ciocalteu reagent and 2 mL of water. After 10 minutes resting in the dark, the absorbance was

read in a spectrophotometer (JENWAY, 6850 UV/Vis) at 720 nm, at its peak absorbance. For each sample extract, three cuvettes were used for the spectrophotometric measurement, and the average value of these readings was regarded as the reading for that extract repetition.

The amount of vitamin C was quantified through a calibration curve previously obtained with known solutions of ascorbic acid and water, in the range of 25 – 600 $mg_{AA}L^{-1}$. The results were reported as equivalent milligrams of ascorbic acid (mg_{AA}) per gram of sample on wet basis, and the percentage reduction was calculated in comparison to the fresh product (Equation 1).

Total phenolic content

The method used to measure the total phenolic content was based on Gao et al.[35], with some modifications as used by Colucci et al.[26]. The reactants used in this case were the following:

- Folin-Ciocalteu (Sigma-Aldrich, 2 M) reagent, diluted in distilled water (1:10 v/v);
- Sodium carbonate (Chem Lab NV, 99.8%), at 20% w/v.

In order to be in the adequate range for measurement, the extract was firstly diluted with ethanol (Honeywell Fluka™, 96% v/v). The extracts obtained from the fresh eggplants were diluted 1:9 v/v, while those obtained from the dried product 1:4 v/v. In a 4.5 mL spectrophotometer cuvette 0.1 mL of the diluted extract was placed, with 0.2 mL of Folin-Ciocalteu reagent and 2 mL of water. After three minutes in the dark, 1 mL of sodium carbonate was added to each cuvette. After 1 h of incubation in the dark, at room temperature, the absorbance was read at 765 nm. Also, in this case, for each sample extract, three cuvettes were used for the spectrophotometric measurement, and the average value of these readings was regarded as the reading for that extract repetition.

The calibration curve to measure the content was obtained with gallic acid (Sigma-Aldrich, 98%) and ethanol (Honeywell Fluka™, 96% v/v) in the range of 20 – 120 mg_{GA} L^{-1}.

The results were thus reported as milligrams of gallic acid (mg_{GA}) per gram on wet basis (g_w) and the percentage reduction was then calculated in comparison to the fresh product (Equation 1).

Antioxidant capacity

To determine the antioxidant capacity of the extracts, the assay used was the Ferric Reducing Antioxidant Power (FRAP) and the reactants used were the following:

- Iron (III) chloride.6aq (LabChem NV, 99%);
- Glacial acetic acid (LabChem NV, 99-100%);
- Sodium Acetate (Honeywell Fluka™, ≥ 99%);
- 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (Sigma-Aldrich, 99%);
- HCl 40 mM (Sigma-Aldrich, 37%).

The assay is based on a mixture of equal volumes (1:1:1) of the following three solutions: an acetate buffer 0.3 M, pH 3.6 (0.155 g of sodium acetate and 0.8 mL of acetic acid in distilled water), a 20 mmol L^{-1} ferric chloride solution (0.2717 g in 50 mL of water), and a TPTZ solution (0.064 g in 20 mL of HCl at 40 mM). These three reactants were prepared in the same day of the tests and stored at room temperature, protected from light. The extract from the fresh eggplant was diluted 1:19 v/v, and that from the dried product 1:7 v/v. 1.5 mL cuvettes for the spectrophotometric analysis were filled with 30 µL of diluted extract, 30 µL of water, and 900 µL of the FRAP mixture, then incubated in a 37°C thermal bath for 30 minutes before reading the absorbance at 595 nm. Again, in this case, for each sample extract, three cuvettes were used for the spectrophotometric measurement, and the average value of these readings was regarded as the reading for that extract repetition.

The calibration curve to measure the antioxidant capacity was obtained with Trolox (Sigma-Aldrich, 97%) and ethanol (Honeywell Fluka™, 96% v/v) in the range of 60 – 200

$mg_T L^{-1}$. The results were thus reported as milligrams of Trolox (mg_T) per gram on wet basis, and the percentage reduction was calculated in comparison to the fresh product (Equation 1).

Statistical Analysis

To evaluate the effect of VFD shelf temperature and chamber pressure on AA, TPC and AC loss after drying, statistical analysis was done. As above-mentioned, each tested condition of pressure and temperature had three repetitions. The AA, TPC and AC contents of each extract after drying were compared to the average contents of the correspondent fresh pre-treated product and the percentage content reductions concerning each extract were computed. Thereby, each tested condition had three content percentage reductions computed for each attribute measured, AA, TPC and AC. The three content loss measurements of each specific targeted compound (AA, TPC or AC), for one tested condition, are defined as a *group* of results and their averages and standard deviations are reported in the results section. The same was done for the freezing tests, but with 4 repetitions.

For the analysis of the effect of shelf temperature, since three temperature levels were used with a constant pressure of 10 Pa, a one-way analysis of variance (ANOVA) was performed to verify if the content losses observed were different between *groups*. In this case, it would imply that shelf temperature might influence the losses observed and also the drying time.

For the second set of experiments, since both effects of temperature (T) and pressure (P) should be evaluated, a two-way ANOVA was done. This allowed to compare the *group* mean values for AA, TPC and AC loss, as well as for the drying time, considering the influence of both tested parameters plus their interaction (T x P), i.e. the combined effects of both.

For this study, a confidence level (α level) of 0.05 was chosen to test the null hypothesis (H_0), i.e. that the *group* mean values do not differ significantly from each other: if the

probability of obtaining the observed results (*p*-value), considering H_0 true, is lower than 0.05, then H_0 should be rejected, indicating that the values from at least one *group* differ from the values of the other *groups*. When the ANOVA gives a significant result (p-value < 0.05), this indicates that at least one *group* differs from the other *groups*. Withal, it does not indicate which *group* differs from one another. This way, to compare all mean values found after obtaining a significant ANOVA result, a multiple comparison test can be done.

Multiple comparison tests usually compare means in pairs, for that reason they are called pairwise tests. The first pairwise comparison test was developed by Fisher in 1935[36] and it is also known as least significance difference (LSD) test. Although very commonly used when doing a multiple comparison, this test does not correct the α level for each comparison. By doing so, it increases Type I errors, i.e., assuming the *group* mean values are different when they are not. This means that doing LSD tests, in this case, there is a higher probability of considering that the AA, TPC and AC values measured for each tested condition are significantly different from each other, when in fact they are not. Other pairwise tests where developed considering these characteristics of α level and Type I errors. The Tukey pairwise test[37] adjusts the α level within each test and, thus, it is more conservative when comparing differences between mean values and might be a suitable approach for the present study.[38]

As abovementioned, the factors tested were shelf temperature and chamber pressure while the mean values evaluated were the AA, TPC and AC percentage losses and the primary drying times. When the ANOVA resulted in significant differences among the means, a Tukey pairwise test was done to compare them. Statistically similar means share the same coded letter, while statistically different ones have different coded letters. A mean value might be similar to multiple mean values simultaneously, while being different from just one other mean value, for instance. Therefore, it is interesting to do these multiple comparison tests after finding significant ANOVA results, to understand where these differences actually exist.

To compare the reductions between the fresh and frozen product, a Student t-test between the two groups was done. The ANOVA, Tukey pairwise and t-tests were done using MiniTab® 17, always considering a 95% confidence interval.

Results and discussion

The role of freezing

For fresh pre-treated eggplants, the average concentration of the targeted compounds measured were: 0.060 ± 0.02 mg_{AA}/g_w for ascorbic acid, 0.143 ± 0.05 mg_{GA}/g_w for TPC and 0.465 ± 0.04 mg_T/g_w for the antioxidant activity. Hanson et al.[28] found ascorbic acid values ranging from 56 ± 14 mg_{AA}/100g to 129 ± 9 mg_{AA}/100g dry weight basis, which corresponds approximately to values from 0.03 mg_{AA}/g_w to 0.08 mg_{AA}/g_w. Ninfali et al.[39] reported TPC values expressed as 54.7 ± 6.0 mg of caffeic acid equivalents per 100 g in fresh weight basis for the black beauty variety. Finally, the antioxidant capacity found by Kaur et al.[40] for eggplant variaties was from 0.72 ± 0.4 $\mu mol_{Trolox}/g_w$ to 8.11 ± 1.2 $\mu mol_{Trolox}/g_{w,}$ which corresponds roughly to 0.2 mg_T/g_w to 2 mg_T/g_w. It can thus be concluded that the concentration of AA, TPC and the AC of the fresh product considered in this study are in good agreement with the values reported in the literature (taking also into account the role of product variability).

Freezing has been reported to affect product quality in different studies and, therefore, it was interesting to investigate the effect of this step on the targeted compounds. The average contents found for the targeted compounds in the fresh and frozen product are presented in Figure 1. Comparing the content values found for the fresh pre-treated product to those obtained after freezing through a t-test, the averages are not significantly different. For AA, the p-value found was 0.702, for TPC 0.807 and for AC 0.256. The results for the pre-treated frozen product were 0.061 ± 0.005 mg_{AA}/g_w for AA, 0.133 ± 0.004 mg_{GA}/g_w for TPC and 0.524 ± 0.029 mg_T/g_w for AC.

Vitamin C and phenolic compounds can be degraded by enzymatic activity in the presence of oxygen by ascorbate oxidase and polyphenol oxidases, besides other metabolic routes.[17],[41] Still, it has been suggested that a partial reduction of polyphenol oxidase activity may happen due to an increase in H^+ concentration during freezing, which changes pH values turning the enzyme unstable.[41] Additionally, in this study, eggplants were treated with a 2% m/v sodium metabisulfite solution which has a bleaching effect, reducing enzymatic browning during processing.[42],[43] This could have played an important role in preventing losses in content of the targeted bioactive compounds by the freezing step. Thus, for this product, given the pre-treatment and freezing conditions, freezing did not have a relevant effect on the contents of targeted nutritional compounds.

Influence of the operating parameters on drying time and product temperature

The heating shelf temperature and the chamber pressure may influence drying time, i.e. the time required for complete ice removal. Longer times favour the occurrence of biochemical and enzymatic reactions, which can lead to a loss of the studied compounds. Additionally, product temperature is a consequence of these parameters, and it may affect chemical reactions involving the target compounds within the product. As such, these parameters must be evaluated to properly understand their effect on product quality.

The primary drying step duration for the first set of experiments, regarding only temperature effects, is shown in Figure 2. The results for the second set, considering both temperature and pressure effects, are presentend in Figure 3. As abovementioned, these values were calculated as the midpoint of the pressure ratio curve.

For the first set of experiments all batches were dried at 10 Pa and the increase in the temperature of the heating shelf clearly decreased the required drying time, as the duration for -30°C was 15.3 ± 6 h, while for -15°C was 9.9 ± 3.5 h and for 0°C just 7.0 ± 2.1 h, as it can be

seen in Figure 2. However, through the one-way ANOVA on the effect of the tested shelf temperatures under 10 Pa, this effect was not significant (p-value = 0.123), mainly due to the (quite) large uncertainty range of the obtained values (it has to be highlighted that it is not possible to measure the true value of drying time, but it is inferred from the pressure ratio curve, that is the standard approach to assess drying time and its variability in the batch).[32]

For the second set of experiments, for tests carried out at -30°C the drying times varied from 14 ± 5.7 h for the test at 20 Pa to 20.9 ± 7.8 h for the test at 40 Pa. For the batches run at 0°C, drying times varied from 6.2 ± 2.3 h for the test at 20 Pa to 8.6 ± 2.5 for test done at 40 Pa. This indicates that pressure might have a mild effect on process duration as higher pressure values, for both tested temperatures, slightly increased primary drying time. This can be observed in Figure 3, were for the two temperatures tested, the higher pressure used resulted in the longest drying time. However, through the two-way ANOVA, pressure appeared to be not significant ($p = 0.271$), neither the interactive effect between temperature and pressure ($p = 0.694$). Observing the coded results for the Tukey pairwise test in Figure 3, this is clearly confirmed, since average drying times for the same shelf temperature set, but different pressures, share a common letter, meaning that the averages are statistically similar to each other.

On the other hand, for this second set of experiments, the effect of the temperature seemed more relevant since average durations at 0°C are about half compared to the ones at -30°C at any pressure. At 20 Pa, for instance, the duration at -30°C was 14 ± 5.7 while at 0°C it was 6.2 ± 2.3, the other values for 10 Pa and 40 Pa were listed above and follow the same trend. Indeed, shelf temperature effect on primary average drying time was significant through this two-way ANOVA ($p = 0.002$). Although, through the Tukey results represented by the different coded letters in Figure 3, this significative difference found comes from the average drying time for the -30°C and 40 Pa tests compared to the 0°C at 10 Pa and 20 Pa tests, indicating that some

combined pressure effect might also have an influence on the results for this set of experiments.

Figure 4 reports the sample bottom and surface temperature profiles observed for all batches: graphs A and B corresponds to the tests at 40 Pa, C and D to the tests at 20 Pa and E and F to the tests at 10 Pa. The two main heat-transfer mechanisms involved in the freeze-drying process are conduction through the space between the product and the shelves and radiation from the chamber walls to the product surface. Thus, shelf temperature is directly related to product temperature. After the initial transient, for the test carried out at -30°C and 10 Pa, the bottom and top temperatures were approximately -22.1°C and -21.9°C respectively. For the test at -30°C and 20 Pa, values of -24.7°C and 22.9°C were obtained for bottom and top positions. At the same temperature but at 40 Pa these temperatures were -24.7°C and -23.8°C respectively. Whereas for shelf temperatures of 0°C, at 10 Pa the bottom and top temperatures were 2.7°C and 2.8°C respectively, after the initial transient. At 20 Pa, 2.0°C and 2.8°C, bottom and top temperatures respectively, and at 40 Pa, 1.8°C bottom and 3.1°C top temperatures were detected (after the initial transient). Finally, for the test run at -15°C and 10 Pa, final bottom temperate was approximately -10.6°C while on the top it was -8.8°C. From these results, it appears that, as expected, the final product temperature is always slightly above the shelf temperature used. This means that the system was also heated by other heating mechanisms, in this case, radiation from the chamber walls, which is supported by the fact that top temperatures are also slightly higher than bottom temperatures. With respect to the velocity of temperature rise, it appears that at 10 Pa the slope of temperature increase is lower than that at 20 Pa and at 40 Pa. This is due to the fact that the higher the chamber pressure, the higher the heat transfer coefficient between the shelf and the product and, thus, the heat transfer rate to the product. For a given value of chamber pressure, the higher the temperature of the heating shelf, the higher the rate of temperature change, as it can be expected, due to the increase of the driving force for heat exchange.

Influence of operating parameters on antioxidant compounds

The operating parameters of the freeze-drying process, namely shelf temperature and chamber pressure, may influence the nutritional properties of the eggplants. Differences on antioxidant compound loss were found by Herbig and Renard[44] when operating at diverse temperature ranges, suggesting that different degradation mechanisms might be in place according to the operating conditions.

Table 1 lists the one-way and two-way ANOVA results for freeze-drying pressure and temperature effects on the selected quality properties of eggplants. With respect to the analysis on the effect of the operating temperature, the one-way ANOVA indicated that this parameter is significant for all compounds: AA, TPC and AC. For the two-way ANOVA, evaluating temperature and pressure, temperature alone had a significative effect on AA and TPC but did not have a significative effect on AC. However, the combined effect of pressure and temperature were found to be significant in this case for AC and for AA, but not for TPC. Pressure alone, on the other hand, was significant for TPC and AC, but not for AA. As mentioned, for the AC content loss values measured, pressure seemed to have an expressive effect from the two-way ANOVA results, while temperature seemed to be only relevant on the one-way ANOVA. The pressure effect over the AC content losses measured might have increased its variability in each temperature level tested and mitigated the observable effects of shelf temperature for the two-way ANOVA under the tested conditions of this set of experiments. These combined effects between pressure and temperature might help to explain these differences found in the statistical tests. The presented results were obtained assuming a 95% confidence interval and will be further discussed ahead. Deeper understanding of how temperature and pressure affect drying time and targeted nutritional compounds is required for process design and optimization.

The AA, TPC and, by extension, AC content drop is influenced by light, oxygen, temperature, viscosity and pH of the medium and is also catalysed by metal ions, particularly Cu^{2+}, Fe^{2+}, and Zn^{2+}.[45],[46] This study was conducted in a closed chamber (and, thus, no meaningful light effect is expected) and under vacuum, which means that the oxygen partial pressure during drying is very low. In addition, samples were placed over a stainless-steel surface, and the metal could catalyse reactions, favouring losses in nutritional content. Indeed, in the dark, AA degradation major cause was found to be chemical oxidation, favoured by metal ions in solution.[46] The losses measured for AA, TPC and AC in the first group of tests are shown in Figure 5. Further ahead, Figure 6 shows the combined effect of temperature and pressure in freeze-drying on compound loss. Both results will be evaluated and discussed ahead, as they complement each other.

Ascorbic acid is heat sensitive, thus, increasing temperature should increase its loss in the final product. However, under the tested conditions, increasing temperature seems to considerably minimize AA content loss as the percentual decrease at 10 Pa at -30°C was 37.9 ± 3.4%, while at -15°C it was 26.9 ± 6.6% and at 0°C it was even smaller having a 12.2 ± 1.7% reduction. This is markedly evidenced by the Tukey pairwise results in Figure 5 since all mean values differ from each other (different coded letters), showing how strong was the temperature influence on AA content in this study. This effect of higher temperatures leading to lower AA loss can be seen on both groups of experiments. It should be noted that all tested operative conditions are at sub-zero temperatures and low oxygen pressure; thus, under such circumstances, the main degradation pathways must be anaerobic. The rate constants for anaerobic degradation of ascorbic acid are two to three orders of magnitude lower than those for the oxidative reaction.[45],[47] This way, longer process durations might be a key-factor in anaerobic ascorbic acid degradation, since they could grant enough time for these reactions to take place. Longer processing times at lower temperatures could favour degradation reactions

on the rubbery-state water fraction of the food product. For the second set of experiments, the observed AA losses are graphically represented in Figure 6. At 20 Pa, the AA losses were 27.8 ± 10.9% at -30°C and 27.5 ± 8.3% at 0°C, while at 40 Pa the losses were 34.3 ± 4.7% at -30°C and 14.5 ± 5.2% at 0°C, the losses at 10 Pa were presented above. As expected from the two-way ANOVA results listed in Table 1, pressure alone did not seem to have a meaningful effect, and only its combined effect with temperature was found significant. In Figure 6, this can also be verified from the Tukey pairwise test, since within the same shelf temperature (-30°C or 0°C), all AA mean losses at different pressures where statistically similar (they share a common letter, “a” for the -30°C tests and “b” for the 0°C tests).

From the statistical analysis, temperature and pressure separately were found to have a significant effect on TPC content loss but not their combined effect. Evaluating the effect on temperature alone at 10 Pa, in Figure 5, TPC losses do not seem to follow a linear trend as the smallest loss, 36.0 ± 2.3%, was observed for the intermediate shelf temperature tested, -15°C. Furthermore, comparing the results only between -30°C and 0°C presented in Figure 6, for all tested pressures, TPC losses at lower shelf temperature seem slightly higher. For tests at -30°C and 10, 20 and 40 Pa they were respectively 55.2 ± 5.4%, 62.8 ± 6.9% and 49.8 ± 5.6%, while these loses at 0°C, at the same pressures, TPC losses were respectively 47.7 ± 5.5%, 42.6 ± 7.6% and 32.5 ± 8.5%. Although, from the Tukey comparisons, many mean values are not significantly different from each other, as they share common coded letters. Still, longer processing times under lower temperatures could lead to higher TPC losses.

Evaluating pressure effects, for both tested temperatures, the highest pressure resulted in a smaller TPC content decrease. This effect of increase in pressure leading to smaller losses is more pronounced for the tests at 0°C, as they seem to follow a linear trend. A possible explanation could lie on the vapor pressure change with respect to temperature, which can increase dramatically with the increase of temperature, favouring compound volatilization. For

very low temperatures such as -30°C, the vapor pressure should be small, and chamber pressure variation might have a milder effect on volatilization. Whereas at 0°C vapor pressure is higher, thus allowing a more evident effect on volatilizations from the changes in chamber pressure.

The FRAP assay, used to determine the AC percentual losses, is based on the ferric reducing ability of the compounds in the sample while the reduction potential of the Fe(II)/(III) is 0.70 V.[53][48] Accordingly, only antioxidant functional groups showing a redox potential above this threshold limit will be measured by the FRAP assay.[49][49] The redox values reported for ascorbic acid, for instance, appear to be below this value[50] while many phenolic groups present values above this limit.[51] This means that the FRAP assay probably comprehends the antioxidant capacity from part of the phenolic compounds in a sample plus other antioxidant molecules, while it should not be much affected by the ascorbic acid content. In fact, high correlations between the TPC and AC contents were reported in different freeze-drying studies[1],[52] also specifically for AC values measured by the FRAP assay.[53],[54] This way, the FRAP assay measures the antioxidant activity of a large group of molecules with different properties. Expectedly, the behaviour of such a large group of different molecules according to the operating conditions can be tricky to describe.

In the first group of experiments (Figure 5), higher temperatures seem to increase AC degradation, which differs from the behaviour observed for AA and TPC, which is curious since, as mentioned above, AC was found to be correlated to TPC as TPC compounds have antioxidant activity. The temperature influence on AC can be supported by the Tukey pairwise results in Figure 5, as the average loss at 0°C, 68.6 ± 1.5%, was significantly different from the other two values, 49.9 ± 3.2% and 57.8 ± 5.9% at -30°C and -15°C respectively. Observing the results for the second set of experiments presented in Figure 6, lower temperature also seems to result in slightly smaller decrease, however temperature alone was found to not have a significant effect in this set. Pressure, nonetheless, appears to have a more expressive effect in

AC content loss, with higher pressures resulting in lower AC content reductions as observed for TPC. At both temperatures, the lowest decreases observed, 41.9 ± 5.0% and 35.1 ± 12.1% for -30°C and 0°C respectively, were at 40 Pa. As pointed out by the Tukey comparison, this was more marked for the 0°C tests since the average result at 40 Pa was significantly different from the average losses under 10 and 20 Pa. Again, this result could be due to vapor pressure changes according to the temperature, as described in the paragraph above.

Except from the AA results, TPC and AC findings are similar from what Colucci et al.[26] found when investigating the effect of the atmospheric freeze-drying process on eggplant cubes. They evaluated the effects of ultrasound assisted atmospheric freeze-drying on eggplant quality properties and drying time for process optimization. They found no significant effect on the measured nutritional properties when applying ultrasound. However, they did find significant impairment on the product caused by air-velocity and drying temperature, according also to sample size. To properly compare AFD and VFD findings, AFD results from the study done by Colucci et al.[26] being referred in this study are only those from batches under comparable conditions. Signifying, the AFD dataset being compared does not include any kind of assisted drying technology and the air velocity of 2 m/s was chosen since it gave the best retentions for the measured compounds in that study. Nonetheless, it is important to point out that because of process characteristics and the different experimental designs adopted, the AFD temperatures used are different from the VFD ones. In addition, a blast chiller was used for freezing the AFD samples, which means a higher freezing rate compared to the freezing method used in the present study. Still, it can be interesting to compare the effects of both processes on the targeted bioactive compounds of eggplants.

Firstly, AFD and VFD can be compared in terms of drying time which also influences the nutritional qualities of the product. AFD drying times were 15.31 ± 0.95 h for -10°C air temperature, 10.98 ± 1.5 h for -7.5°C and 14.38 ± 0.82 h for -5°C.[26] The average drying times

obtained for the VFD process, presented in Figure 2 and Figure 3, point out that even for the lower temperature of -15°C, the VFD drying time was shorter. It should be noted that both processes are different and have distinct sublimation rate controlling mechanisms. Still, from a practical point of view, VFD drying times tended to be shorter. Nonetheless, it is important to add that even though VFD might require shorter times than AFD, Wolff and Gibert[55] estimated that AFD could save 38% of cold requirements and 34% of heat requirements, thus reducing energy costs compared to VFD. Moreover, as shown by Colucci et al.[26] drying time may be reduced by using ultrasound assistance, without significantly affecting the change in the concentration of the antioxidant compounds, that is the main focus of our research. AFD ascorbic acid percentage reduction was 46 ± 9% for -10°C air temperature, 63 ± 10% for -7.5°C and 61 ± 11% for -5°C. Total phenolic content reduction was 58 ± 17%, 71 ± 19% and 65 ± 16% respectively for the same air temperatures. Finally, antioxidant capacity reduction, also by the FRAP assay, was 34 ± 23%, 59 ± 16% and 53 ± 20% for the same temperatures (in all cases air velocity was equal to 2 m s^{-1} without any assisting drying technology).[26] The AA average percentage reductions found for VFD, presented in Figure 5 and Figure 6 and discussed above, are considerably lower than those found for AFD. In AFD, a dried air flux runs over the product and could be considered as one of the major AA concentration drop responsible observed since it favours aerobic AA degradation pathways. On the other hand, during VFD the product was subjected to vacuum, so AA degradation pathways must have been anaerobic. As abovementioned,, the rate constants for anaerobic degradation of ascorbic acid are two to three orders of magnitude lower than those for the oxidative reaction.[45],[47] Marfil et al.[56] found correlations between increased exposition to air and increased vitamin C loss, suggesting the use of inert gas during processing to reduce degradation reactions.

For the phenolic compounds, VFD again seems to result in less average content losses than AFD, but with less dramatic differences than those observed for AA results. Reyes et al.[15]

found about 39% decrease in total polyphenols content and 70% decrease in ascorbic acid content of blueberries in AFD with respect to VFD. This suggests that higher degradations might be observed in AFD processed foods, which could be attributed to the presence of oxygen and, when applicable, longer process durations.

AC average content loss values after AFD and VFD were not drastically different from each other. Mahn et al.[57] investigated broccoli freeze-drying under AFD and VFD. Anti-radical power reduction after VFD was 65.5% while after AFD it was 80.1%. For TPC reduction, after VFD about 48.7% was lost, while AFD had 41.9% reduction without significant difference between both. They also found higher air temperatures during AFD to have a negative effect on TPC and anti-radical power contents of broccoli, however, this effect was statistically significant only for the later. The smaller differences observed between AFD and VFD with respect to TPC and AC content drops after drying might be related to the pre-treatment used in both studies. The pre-treatment with sodium metabisulfite prevents enzymatic browning which is intensified by the presence of oxygen in a process. More dramatic differences in TPC and AC content loss might be observed between AFD and VFD if neither process uses this pre-treatment step prior to drying. This did not affect ascorbic acid content loss since its main degradation pathways seem to be non-enzymatic.

Conclusions

In this work the vacuum freeze-drying of eggplant cubes was studied in order to better define the effects of the process conditions on the drying time and on the antioxidant properties of eggplants. Lower shelf temperatures were found to increase AA loss, having values ranging from 12.2 ± 1.7% at 0°C to 37.9 ± 3.4% at -30°C both under 10 Pa. For TPC, shelf temperature had a lower effect with values ranging for instance under 20 Pa from 42.6 ± 7.6% at 0°C to 62.8% ± 6.9% at -30°C. Higher shelf temperatures seemed to slightly increase AC content

losses when comparing results obtained under 10 Pa, with values ranging from 49.9 ± 3.2% at -30°C to 68.6 ± 1.5% at 0°C. However, when evaluating the content losses under all pressures tested, the temperature effect seems less relevant. For instance, calculating an average loss value considering all pressures of 10, 20 and 40 Pa tested, this result ranges from 48.6 ± 7.6% at -30°C to 53.7 ± 16.2% at 0°C. From a process perspective, lower temperatures required longer drying times, as an illustration, primary drying required 6.2 ± 2.3 h at 0°C while it required 15.3 ± 6.0 h at -30°C both under 10 Pa. These longer drying times can favour degradation reactions resulting in loss on measured compounds. Pressure had a mild effect on drying time: at -30°C and 10 Pa, primary drying required 15.3 ± 6 h while at the same temperature but under 40 Pa, it required 20.9 ± 7.8 h. AA content loss had a very small effect, if any, from the pressure used, with values ranging from 12.2 ± 1.7% under 10 Pa to 14.5 ± 5.2% under 40 Pa, both at 0°C shelf temperature. However, higher pressures resulted in significantly lower TPC and AC content losses. To serve as an example using the results at -30°C, TPC losses varied from 49.8 ± 5.6% under 40 Pa to 55.2 ± 5.4% under 10 Pa, while for AC these losses varied from 41.9 ± 5.0% under 40 Pa to 49.9 ± 3.2% under 10 Pa. The freezing step was found to have a small impact on the targeted bioactive compound losses under the tested conditions.

The results suggest that an optimization of the process towards the reduction of drying time may be favourable. Temperature is the main factor influencing drying time and, operating at 0°C resulted in the best results for AA and TPC contents, while having minor effects on AC contents. Care should be taken to ensure the operating temperature will not negatively affect other quality properties such as colour and texture. In addition, higher pressures appear to not affect significantly drying time, while having a positive impact on the retentions of TPC and AC. This suggests that process optimization could also be favoured by operating under slightly higher pressures in VFD.

Comparing VFD to AFD, ascorbic acid had a lower reduction in content in VFD,

maximum loss observed of 37.9 ± 3.4% at -30°C and 10 Pa while the minimum loss observed in AFD was 45.9 ± 8.7% at -10°C. The differences in reduction found for TPC and AC were not as relevant. AA higher loss in AFD might be related to the higher oxygen pressure compared to vacuum freeze-drying. VFD drying times tended to be shorter than AFD ones, unless assisting methods like ultrasounds are used, even if published results in presence of ultrasounds and, thus, with lower drying times, does not show any effect on the tested compounds.

Therefore, VFD stands out as an excellent method to preserve nutritional properties when drying eggplants. Further studies should incorporate the effect of VFD operating conditions on the energy consumption for further process optimization.

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List of Tables

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Table 1

Factor	AA		TPC		AC	
	two-way	one-way	two-way	one-way	two-way	one-way
Temperature	0.000	0.001	0.000	0.007	0.156	0.003
Pressure	0.668		0.022		0.001	
Temperature x Pressure	0.014		0.265		0.027	

Figure 1

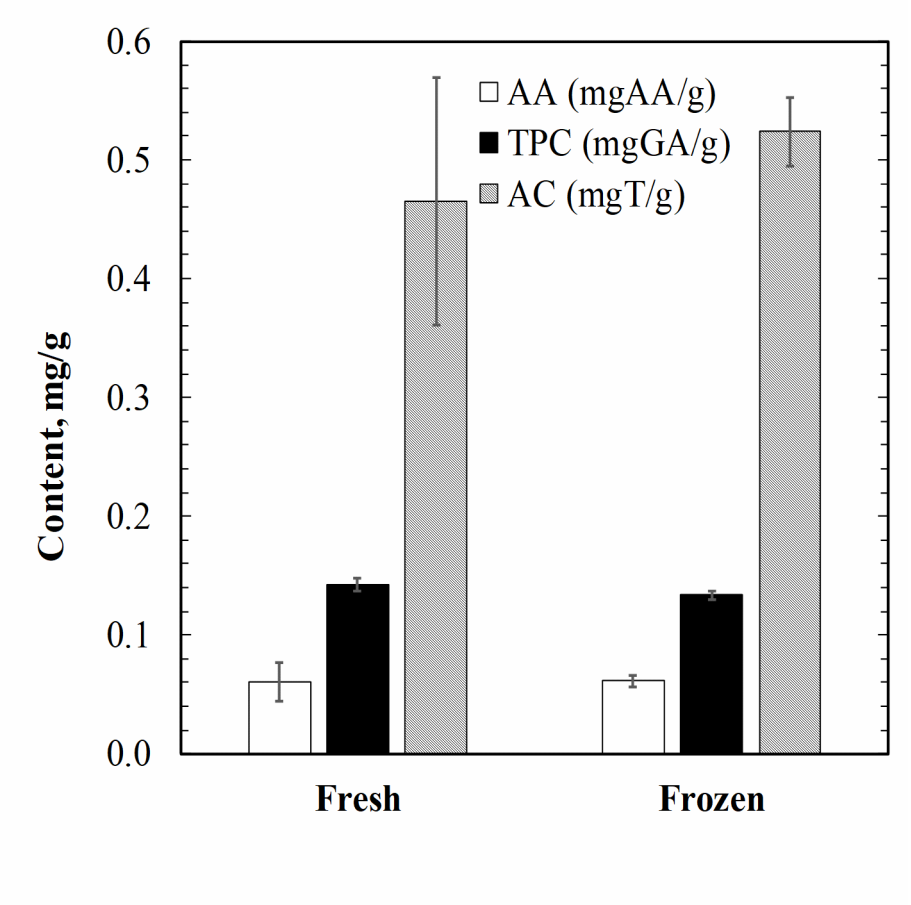

0.6
0.5
0.4
0.3
0.2
0.1
0.0
Content, mg/g
AA (mgAA/g)
TPC (mgGA/g)
AC (mgT/g)
Fresh
Frozen

Figure 2

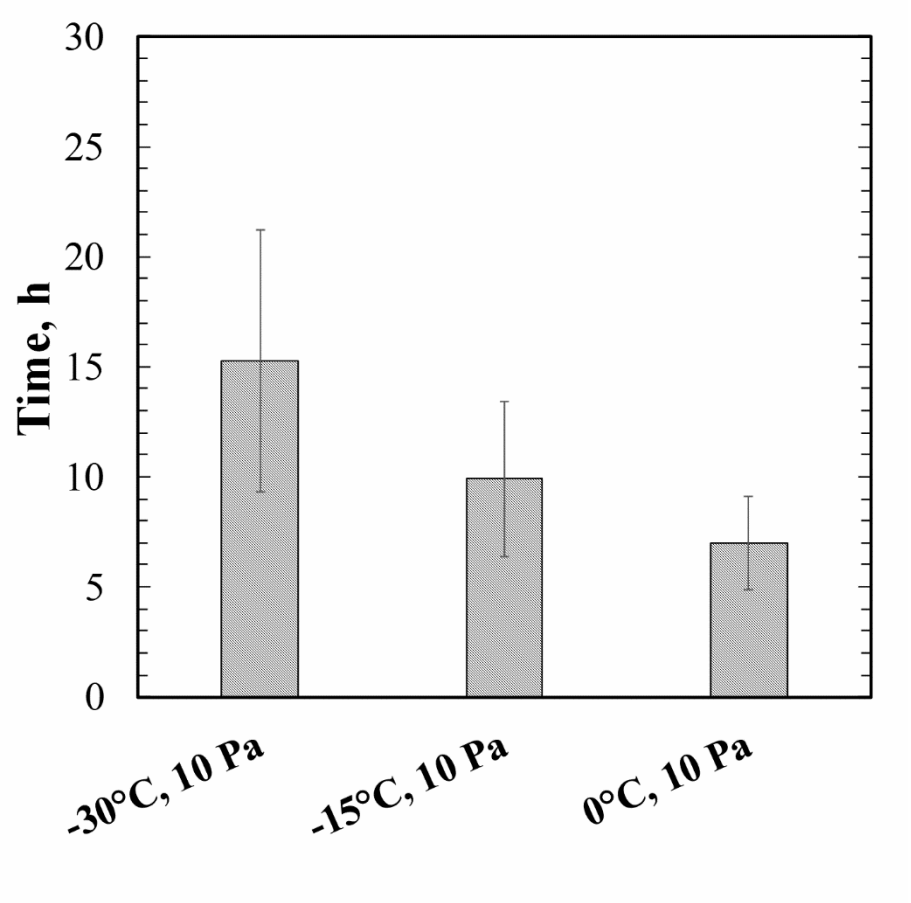


Figure 3

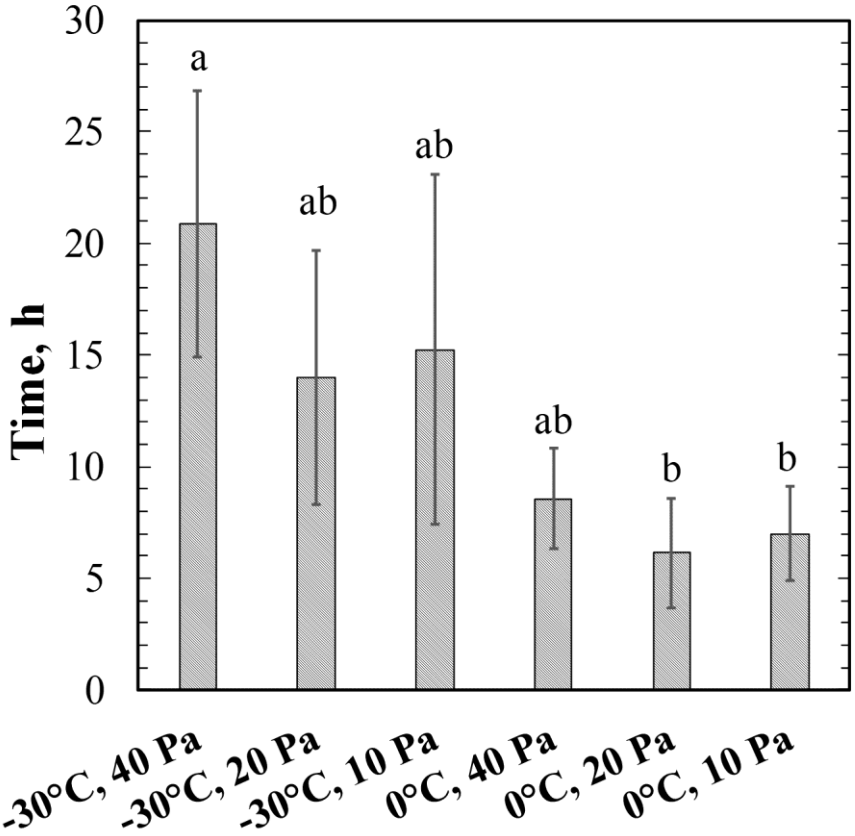

Time, h
30
25
20
15
10
5
0
a
ab
ab
ab
b
b
-30°C, 40 Pa
-30°C, 20 Pa
-30°C, 10 Pa
0°C, 40 Pa
0°C, 20 Pa
0°C, 10 Pa

Figure 4

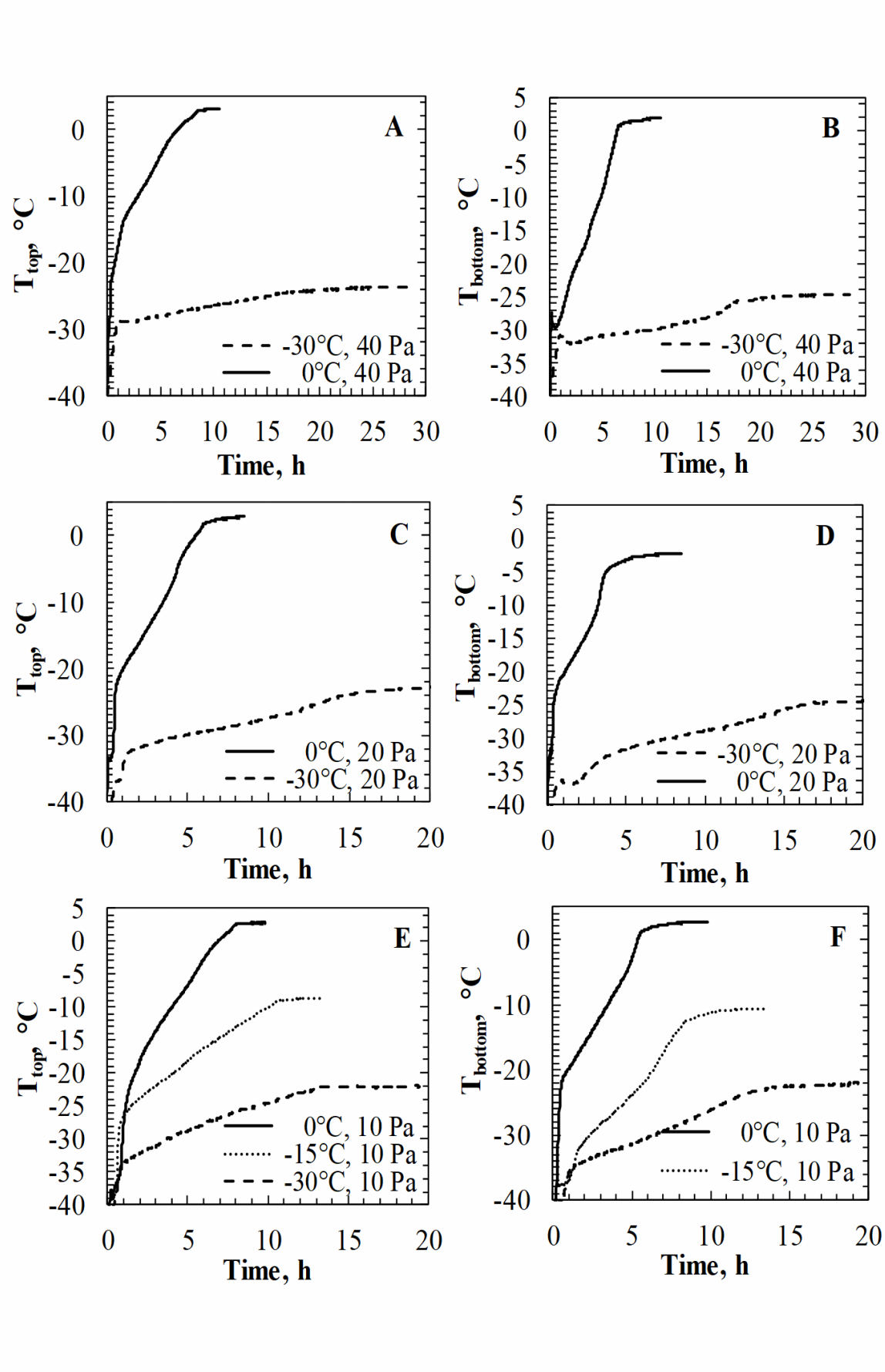

A
B
C
D
E
F
T_{top}, °C
T_{bottom}, °C
Time, h
-30°C, 40 Pa
0°C, 40 Pa
0°C, 20 Pa
-30°C, 20 Pa
-15°C, 10 Pa
-30°C, 10 Pa
0°C, 10 Pa

Figure 5

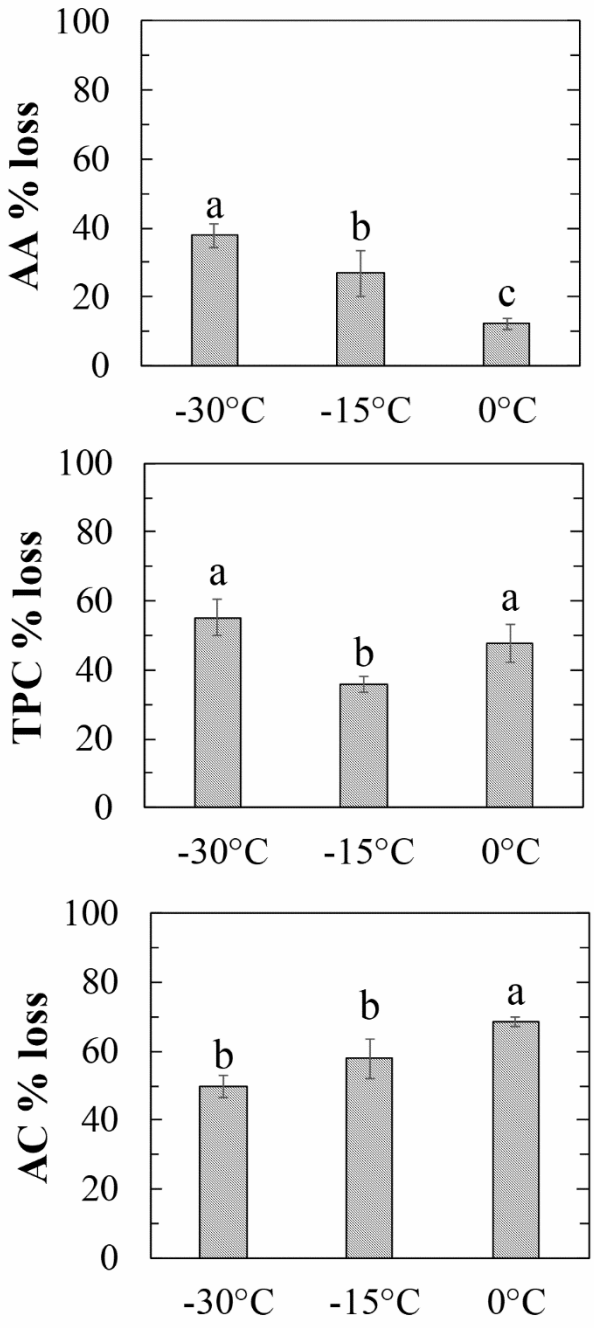
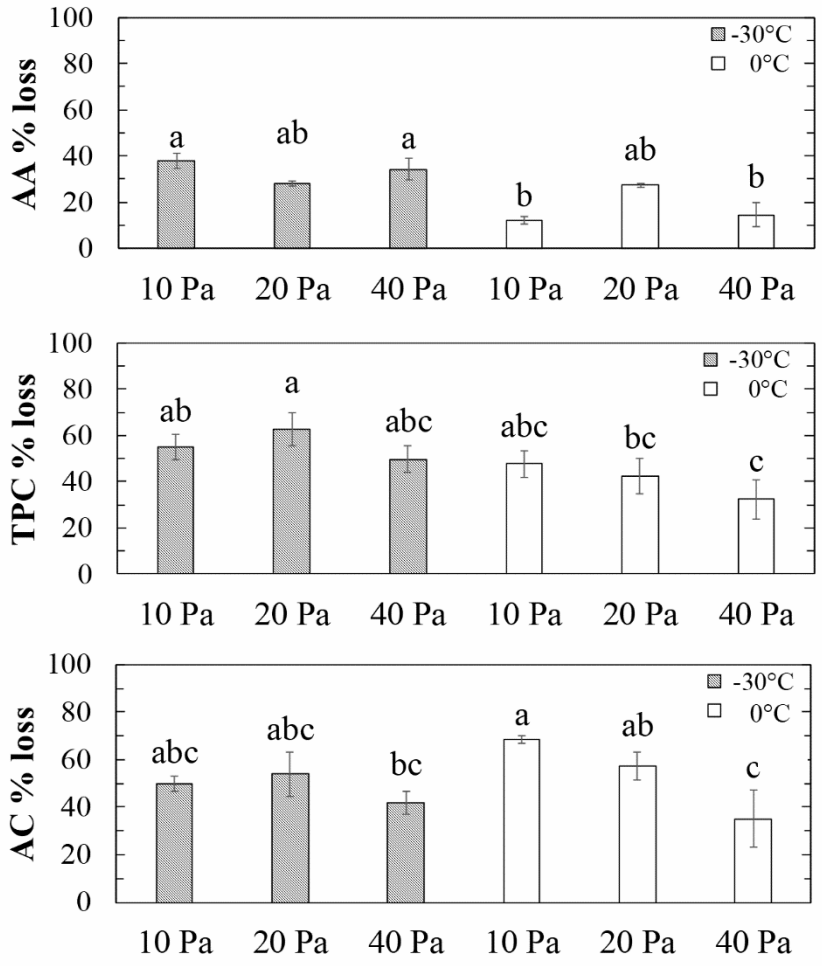

AA % loss
100
80
60
40
20
0
a
b
c
-30°C
-15°C
0°C
TPC % loss
100
80
60
40
20
0
a
b
a
-30°C
-15°C
0°C
AC % loss
100
80
60
40
20
0
b
b
a
-30°C
-15°C
0°C

Figure 6


AA % loss
TPC % loss
AC % loss
-30°C
0°C
100
80
60
40
20
0
a
ab
a
b
ab
b
ab
a
abc
abc
bc
c
abc
abc
bc
a
ab
c
10 Pa
20 Pa
40 Pa
10 Pa
20 Pa
40 Pa