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FROZEN STRAWBERRYQUALITY ENHANCEMENT USING HIGH HYDROSTATIC PRESSURE AND VACUUM INFUSION WITH PECTIN METHYLESTERASE AND CALCIUM CHLORIDE SOLUTION

Konyole SO[1]*, Oiye SO[1] and GP Okiror[2]

Silvenus O Konyole

*Corresponding author email:konyole2000@yahoo.com

[1]Department of Nutritional Sciences, Masinde Muliro University of Science and Technology P.O. Box 190 - 50100, Kakamega, Kenya

[2]Department of Biosystems Engineering, Faculty of Agriculture & Environment, Gulu University, P.O. Box 166, Gulu, Uganda

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ABSTRACT

Fresh strawberry (*Fragaria x ananassa*) is a delicious fruit that is an excellent source of micronutrients and an array of beneficial phytochemicals. Emerging technologies like high pressure freezing and thawing have gained interest recently due to their ability to preserve the structure of the food during storage, albeit the possible shortcomings of degradation of texture and sensory quality. In this study, the effects of high hydrostatic pressure freezing and thawing on the physical quality attributes (texture, drip loss and Degree of Methylation (DM)) of strawberry were investigated. Strawberry samples were infused in a vacuum using pectin methyl esterase (PME) and $CaCl_2$ solution at 170 hPa pressure for 5 min at room temperature to improve textural integrity; infused with distilled water; and another group of samples further incubated at 40°C for 20 min. Fresh samples and non-pretreated samples were also studied. All the samples were subjected to high pressure freezing and thawing processes at 200 MPa and the effect of the processing conditions on texture were evaluated. The high pressure processed samples were compared with conventionally frozen and thawed samples. Pectin methyl esterase, $CaCl_2$ infusion and incubation gave a relative hardness of 1.5 pretreatment compared to water only infused at 0.5. The $CaCl_2$and PME-infused samples had a higher relative hardness after one day of storage at -18°C compared to non-pretreated and water-infused samples at 0.3 compared to 0.1, respectively. Pretreated, water-infused, and PME with $CaCl_2$-infused samples showed no significant difference in hardness when measured immediately after conventional thawing and high pressure induced thawing HPIT but are the former are a third less hard compared to the latter upon storage after 3 hours and three months. Galacturonic acid per mg of Alcohol insoluble residues AIR sample for the fresh strawberry was 0.002±2.699E-05 and 0.002±1.070E-05moles for the one-day vs. three months storage, respectively, for samples conventionally frozen and thawed in duplicate experiments. Therefore, a combination of high-pressure shift freezing (HPSF) with PME and $CaCl_2$infusion improved the texture of the strawberry compared to those that were not pretreated, and gained weight by 15%. The PME and $CaCl_2$-infused strawberry showed less degradation than the non-pretreated and water-infused during both long- and short-time frozen storage. Conventional freezing processes caused more degradation compared to HPSF. Degradation of 3 months conventional freezing was comparable to 1-day storage. For frozen storage, enzyme pretreatment combined with high pressure freezing can be used to enhance the quality of strawberry.

Key words: High pressure freezing, drip loss, strawberry, pectin methyl esterase, vacuum infusion

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INTRODUCTION

Strawberries are delicious fruits, with good aroma and taste, nice appearance and mostly used fresh. They are an excellent source of vitamin C and manganese, as well as a very good source of dietary fiber and iodine and a good source of potassium, vitamin B_2, vitamin B_5, vitamin B_6, omega-3 fatty acids, vitamin K, magnesium, and copper [1]. Strawberry also contain an array of beneficial phytochemicals, including flavonoids, anthocyanidins and ellagic acid [1, 2, 3]. According to FAOSTAT 2020, China was the key world strawberry producing country, with an output of 3.8M tonnes in 2016, which accounted for 42% of the global output with other major producers being the U.S. (16%), Mexico and Egypt (5%, each), Turkey and Spain (4%, each) [4].

The conventional methods of food processing such as freezing and thawing are increasingly being used despite their shortcomings. One of the main shortcomings of the conventional freezing and thawing processes is the degradation of texture of the product. At the same time, texture is increasingly becoming a major sensory quality parameter as the consumers are becoming more aware of the initially neglected food quality attributes, especially for fruits and vegetables [5]. It is in this context that emerging technologies like high pressure freezing and thawing have gained interest in the past two decades due to their ability to preserve the structure of the food during freezing and thawing [5, 6, 7]. Moreover, high pressure freezing and thawing have been demonstrated to minimize texture degradation and drip losses that are associated with conventional freezing and thawing processes [8, 9, 10, 11].

In addition, pretreating the products prior to freezing has been reported to improve the texture of frozen products. Some of such pretreatments include the addition of calcium salts and exogenous enzymes like pectin methyl esterase (PME) [12]. The texture improvements resulting from these pretreatments are related to the changes in the structure and composition of the pectin present in the plant material. Similarly, vacuum infusion can be used to transfer the added substances to the tissue cells of the food material [13, 14]. Since texture is such an important quality parameter for frozen and thawed products, the pre-freezing treatments of incorporating $CaCl_2$ and PME in strawberry under vacuum are particularly of interest in improving texture of frozen strawberry. These processes have been successfully used to improve the texture of some frozen products such as sweet cherries and strawberry [13, 15, 16, 17].

The firming effects of calcium have been known and used for long to prevent excess softening in processed fruits and vegetables [18, 19, 20, 21]. The firming effects of calcium ions are associated with pectin as evidenced by the fact that no firming occurs in oyster mushrooms whose cell walls do not contain pectin [22, 23]. The use of calcium salts to treat strawberry in a vacuum before freezing and thawing to avoid texture degradation has been reported and were observed to increase calcium content of the fruit, reduce drip loss and vitamin C loss upon thawing, and to enhance fruit firmness without significantly affecting the fruit sensory quality [15, 24, 25, 26].

Pectin methyl esterase is a pectic enzyme that catalyzes the splitting of glycosidic bonds in pectic substances. It is mainly electrostatically bound to the plant material cell walls

where it can be easily removed by use of high ionic strength saline solutions, although microbial sources also exist [27]. Pectin methyl esterasehydrolyzes (demethylates) pectin giving mainly methanol and pectin with a lower degree of esterification (pectic acid). The relative quantities of the products of pectin hydrolysis depend on variety, pectin content and level of active PME on the substrate [28, 29]. Catalyzing the cleavage of the ester bonds between the methyl groups and the carboxyl groups leads to a decrease in degree of esterification (DE) and the formation of anionic COO^- groups with which calcium ions can form salt bridge cross-links. Previously, it has been reported that the activation of PME in the cell walls of fruits and vegetables is increased by the presence of cations such as calcium [27, 30]. The objective of the present study was therefore to investigate the effect of high hydrostatic pressure freezing and thawing processes in combination with vacuum infusion of samples with PME and $CaCl_2$ along with incubation pretreatments on the quality of frozen strawberry in comparison with conventional freezing and thawing processes. The quality attributes studied were texture, drip loss and the degree of methylation (DM) after 24 hours (short-term) and 3 months (long-term) of frozen storage.

MATERIALS AND METHODS

Sample preparation and pretreatments

Fresh strawberries (*Fragaria x ananassa var. Elsanta*) in prime condition were used for these experiments. No discrimination was made with regard to shape or size but apparent ripeness was considered. The strawberries were bought every week and stored in a refrigerator at 2^0C for a maximum of three days. The study was carried at the laboratory of Food Technology, Katholieke Universiteit of Leuven in Belgium.

Before use, the refrigerated strawberries were equilibrated to room temperature for 30 min after which hulled manually, cut and mixed into homogenous lots during use. The fresh strawberries were cut into quarters and randomly distributed among the experiments. The samples were weighed and put into infusion solution before putting in the infusion chamber at 125 g berries per 150 ml solution as shown in Table 1. Non-pretreated samples were used as reference, while those infused with pure water were used as controls. For the incubated samples, the incubation was 20 min at 40°C because this is the optimum temperature for the activity of fungal PME, after which the samples were weighed again to determine weight changes due to incubation [27]. The results for infusing $CaCl_2$ only or PME only are not reported in the present study since the combination proved optimal, and is in accordance with previous studies [17, 26].

Vacuum infusion

Vacuum infusion was done in specially fabricated equipment with a chamber to control pressure at room temperature. The samples to be infused were initially weighed, placed in a plastic beaker and covered with a lid to ensure complete immersion in the infusion solution. At the beginning, the airtight chamber was opened and equilibrated at atmospheric pressure. The infusion proceeded in three steps: immersing the sample in infusion solution, application of the vacuum pressure at 170 hPa for 5 min and restoring the atmospheric pressure before removing the samples. Samples were then drained and blot dried on a paper towel for 5 min and weights taken. The infusion solution was added

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at 150 ml of infusion liquid per 125 g of strawberry as in previous studies [17, 26, 31]. Weight gain upon infusion was determined by difference at the end of the vacuum infusion process and the percent weight gain was computed as in Equation 1:

(Equation. 1)

$$\% \text{ Weight Gain} = \frac{\text{Weight after infusion (g) - Weight before infusion (g)}}{\text{Weight before infusion (g)}} \text{ x } 100\%$$

Two infusion solutions were used:(a) calcium chloride ($CaCl_2.2H_2O$) at 0.5% w/w and 0.12% by volume of PME enzyme in distilled water, and(b) pure distilled water (as control). PME (E.C.3.1.1.11) used in the experiments was a commercial product of *Aspergillus oryzae*PME (NOVOSHAPE, Novo Nordisk Ferment Limited, Dittingen Switzerland). The product is free of polygalacturonase activity [28]. The calcium chloride dihydrate used in the experiments was pure form (FlukaChemika 21100 from FlukaChemie GmbH, Germany).

High pressure treatment

A pilot scale high pressure unit by Engineered Pressure Systems International (EPS International, Belgium) was used for the experiments. Its operating characteristics are: a maximum pressure of 6000 bars, operating temperature range of -30°C to 100°C and an internal volume of 590 ml (50 mm internal diameter and a height of 300 mm). A mixture of 60% DowcalN (in distilled water) is used as the pressure transferring liquid (Dow Chemical Company, Switzerland) and 56% (in water) ethylene glycol is used as the heat exchange media. Closing the vessel is done by screwing the upper lid into the vessel. The temperatures and pressure were monitored at regular time intervals of approximately 4 seconds by a computerized data acquisition system (SCXI controlled by LabVIEW, National Instruments, USA).

Texture measurement

Texture was measured objectively as hardness using a texture analyzer (model TA-XT2, Stable Microsystems, Surrey, UK) by recording maximum force to give a depth of 5 mm at plunger test speed of 2 mm/s. A 5 mm diameter aluminium cylinder was used with a load cell of 5 kg on a non-lubricated flat platform. The samples were placed with cut surfaces facing the platform and the side with achenes facing the cylindrical probe. The recorded values of texture for each sample were averages of 16 measurements for all except for the high-pressure processed samples that were averages of 12 measurements due to the limitation of the high-pressure unit. Using the fresh strawberry as a reference, the texture was expressed as relative hardness defined as shown in Equation 2. The use of relative hardness thus minimized the batch variability effect on the texture values obtained and allowed for comparison of different batches.

(Equation 2)

$$\text{Relative Hardness} = \frac{\text{Hardness of pretreated samples in gram-force}}{\text{Hardness of fresh samples of the same batch in gram-force}}$$

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Freezing

Conventional freezing and high-pressure freezing methods were used. The conventional freezing was done at -18°C followed by storage for 24 hours and 3 months in a conventional domestic freezer. The strawberry samples were packed in polyethylene bags following standard practice [13, 17]. At the end of the storage time, the samples were removed, thawed and their quality analyzed. The high-pressure freezing was done in a single vessel unit with the strawberry quarters packed in polyethylene bags of smaller size. A food model, tylose, was used to determine the best process pathway in the pressure-temperature (PT) diagram to apply for strawberry. This was done by following the temperature profile in the tylose gel at different temperature and pressure combinations. Two high hydrostatic pressure (HHP) freezing pathways: high-pressure assisted freezing (HPAF) and high-pressure shift freezing (HPSF) were investigated. Before the start of the high-pressure freezing of the samples, the whole system was equilibrated at 0°C after inserting the samples in the unit to reduce the cooling time.

For the HPAF, once equilibrated, the freezing cycle was started by building the pressure to 2000 bars and setting the cryostat temperature to -25°C to achieve HPAF to ice III / I in the phase diagram of pure water [32, 33, 34]. The temperature profiles of the samples were then monitored using the thermocouples connected to a data logger until the desired temperature was attained, at which stage the system was depressurized. The frozen samples were removed, thawed for 2 hours at room temperature and the texture was measured. The same was done at a pressure of 900 bars and 1500 bars at a temperature of -25°C with an aim to attain the phase of ice I [32, 33, 34]. For the HPSF, upon system equilibration, the freezing cycle started by building the pressure to 2000 bars at a temperature of -18°C with a target to attain the phase of ice I [32, 33, 34].

Storage and thawing

The samples were stored at -18° C for 24 hours and3 months. This was to investigate the differences between short- and long-time storage. Thawing time was defined as the time taken by the fully frozen sample to achieve 10 °C at the thermal centre during thawing. The time was determined using a timer and a thermocouple at room temperature and it was found that 2 hours at room temperature conditions was enough to adequately thaw the samples. Conventional thawing was done for two hours at room conditions after which texture measurements were done. The high pressure induced thawing (HPIT) pathway in the water phase diagram was also determined using the food model, tylose. The samples previously frozen in a conventional freezer at -18°C were placed in the high-pressure unit. The temperature was equilibrated at -15°C and the pressure built to 2000 bars. The system was heated to 10 °C and depressurized. The temperature of the sample was then allowed to increase to 10 °C at atmospheric pressure when the samples were removed and texture measured.

Alcohol Insoluble Residue extraction

To determine the degree of methylation, Alcohol insoluble residues (AIR) are extracted. Ten grams of strawberry were frozen using liquid nitrogen to make the blending easier and homogenized using 50 g of 95% ethanol. The obtained suspension was filtered with SS595 filter paper (FlukaChemie GmbH, Germany) and the remaining insoluble fraction re-suspended in 25g of 95% ethanol, homogenized and filtered again. The obtained

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insoluble fraction was then suspended in 25 g acetone, homogenized and filtered. The obtained AIR was dried in a vacuum oven overnight at 40°C and thereafter kept in a desiccator until analysis over phosphate (P_2O_5) [35].

Analysis of galacturonic acid in the cell wall extracts

This analysis involved two steps. The first was AIR hydrolysis for galacturonic acid [36]. Briefly, to 20 mg AIR in a beaker containing a magnetic stirring bar, 8ml chilled, concentrated (96%) sulphuric acid was added. The beaker was chilled in a stirred ice-water bath. 2 ml water was then added to the beaker drop-wise while gently stirring. The preparation was allowed to stir for 5 min as the cell walls began to dissolve. Another 2 ml water was added drop-wise and the mix was stirred until complete dissolution. The sample was transferred to a 200 ml volumetric flask. The beaker was rinsed several times with distilled water and the rinse transferred to the volumetric flask. The flask was then filled to designated volume with distilled water.

The second step was the actual galacturonic acid analysis [37]. To 0.6 ml of the above solution, 3.6 ml chilled tetraborate reagent (0.0125M solution of sodium tetraborate in 96% H_2SO_4) was added in an ice-water bath. The samples were mixed and heated in an oil bath (ThermoHaake W13, Haake GmbH, Germany) at 100°C for 5 minutes. After cooling in running water until room temperature, 60 µl of the meta-hydroxydiphenyl reagent (0.15% solution of meta-hydroxydiphenyl in 0.5% NaOH solution) was added and mixed well for 1 minute. Absorbance at 520nm at 25°C was determined in a spectrophotometer. To correct the slight pink color produced when materials containing neutral sugars are heated in H_2SO_4/ tetraborate, a blank was used in which 60 µl of 0.5% NaOH solution was added (instead of meta-hydroxydiphenyl). The absorbance of the blank was subtracted from the absorbance of each sample. To avoid the sensitivity of the method to water content of the surrounding (RH %), the tetraborate-sulfuric acid solution was fresh every week for use [38].

Finally, different D-galacturonic acid standard solutions were prepared, with a concentration between 0 to 100 µg/ml. Absorbance was measured according to the earlier defined procedure for galacturonic acid analysis. Readings at time intervals of 30 seconds were taken and after 1 minute, the absorbance value recorded was highest. One minute was the used as the standard time to read the absorbance values of samples.

Analysis of methanol in cell wall extracts

The analysis of methanol in the cell wall extracts proceeded in two steps as follows. The first step was AIR hydrolysis for methanol [39]. A 20 mg sample of AIR was suspended in 8 ml water, shaken and sonicated for 10 min. The sample shaken again after sonication then de-esterified by adding 3.2 ml 2M NaOH and incubating for 1 hour at 20°C with occasional shaking. The sample was neutralized by the addition of 3.2 ml 2M HCl and allowed to equilibrate at 25°C for 15 min. The sample was transferred to a 200 ml volumetric flask. The beaker was rinsed several times with phosphate buffer 0.0975 M (pH 7.5) and the washing was also transferred to the volumetric flask. The flask was filled to its designated volume with phosphate buffer.

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The second step was the actual methanol analysis [40]. To 1 ml of the above solution, 1 ml of alcohol oxidase solution (1unit/1ml) water was added. The sample was shaken to mix and incubated at 25°C for 15 min in a water bath (Haake W13, Haake GmbH, Germany). During this time, pentandione solution consisting of 7.8653g ammonium acetate, 143µl acetic acid solution and 103µl 2, 4 pentandione solution in 50 ml volume was prepared. 2 ml of the freshly prepared pentanedione solution (0.02 M 2, 4 pentadione in 2 M ammonium acetate and 0.005 M acetic acid) was added. To the blank, 2 ml of distilled water was added instead of pentanedione solution. The sample was mixed and incubated for 15 min at 58°C in a water bath (Haake W19, Haake GmbH, Germany). After cooling till room temperature, the sample was mixed again, and the absorbance was read at 412 nm and at 25°C. The absorbance of the blank was subtracted from the absorbance of the sample. A standard curve for methanol was then developed using different methanol standard solutions, with concentrations varying from 0 to 20 µg ml^{-1}.

Determination of the degree of methylation (DM)

Degree of methylation (DM) is the ratio of methanol to that of galacturonic acid in a sample expressed as a percentage (Equation 3).

(Equation 3)

$$DM\% = \frac{\text{N methanol/g AIR}}{\text{N galacturonic acid of fresh sample/g AIR of fresh sample}} \times 100\%$$

Where: N methanol is the moles of methanol; N galacturonic acid is the moles of galacturonic acid; and AIR is the alcohol insoluble residue.

Drip loss determination

The drip loss was determined upon thawing using a gravimetric method where a balance was used to weigh the samples before and after thawing. Drip loss was then calculated as a ratio of weight loss to that of initial weight of the samples before thawing as shown in Equation 4.

(Equation 4)

$$\text{Drip loss} = \frac{\text{weight before thawing (g)- weight after thawing (g)}}{\text{weight before thawing (g)}} \times 100\%$$

RESULTS AND DISCUSSION

Determination of Hydrostatic High Pressure (HHP) pathways using tylose

To determine the optimal HPAF and HPSF pathways in the high-pressure processing domain, tylose was used as a model food. In addition, for the high-pressure thawing process, only HPIT was studied. For all strawberry experiments, only HPSF and HPIT were employed. The HPSF process pathway for tylose is shown in Figure 1. From the various combinations possible for pressure and temperature, a pressure of 2000 bars was chosen.

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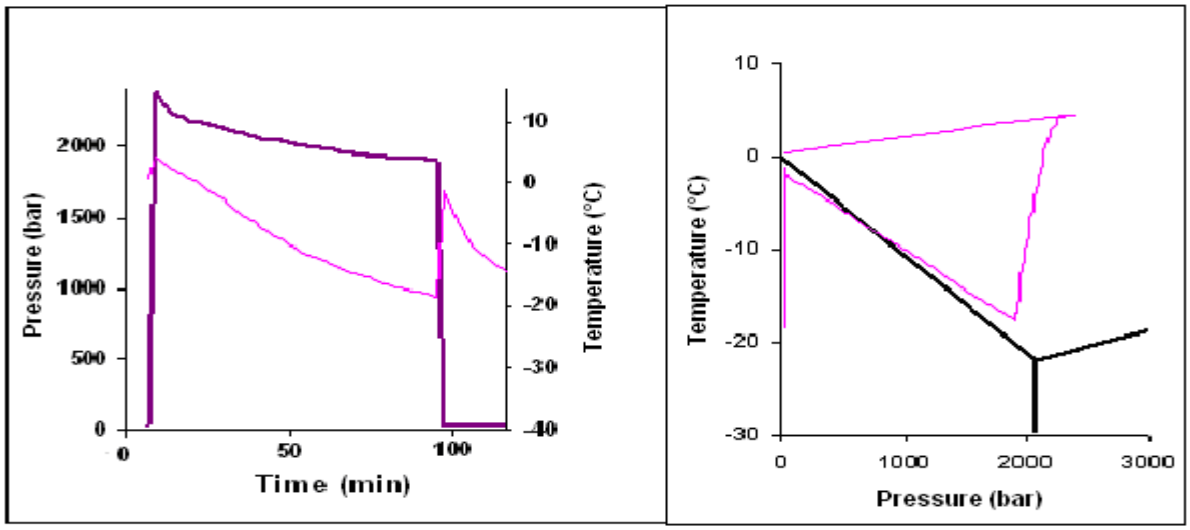

(A) (B)

Figure 1: HPSF Pressure-time (A) and temperature-pressure (B) profiles for tylose. The thick lines in each diagram represent the phase transition lines of pure water. The thin lines represent the thermal centre temperature of the sample

During HPSF, the system was stabilized at 0°C and then pressurized to 2000 bars. An initial temperature increase is observed due to adiabatic heating on pressure build up from the atmospheric pressure to 2000 bars followed by a temperature reduction to about -16°C as the system cools. On depressurization, a phase change occurs and this causes a very fast increase in sample temperature to near the freezing point at atmospheric pressure. The sample temperature increase is due to the release of latent heat as nucleation occurs and the inability to remove the latent heat immediately from the system. As the heat is removed, the system restores to equilibrium. This shows that the phase change is initiated at high pressure and then continued at atmospheric pressure.

As soon as the sample is placed in the vessel, the temperature starts changing. In the opposite way of HPSF, a sudden temperature decrease is seen when the pressure is increased to 2000 bars. This is due to latent heat absorption. Since the system cannot simultaneously supply the needed amount of latent heat, there is a sudden increase in the temperature in the high pressure transferring medium caused by compression but the sample temperature decreases during this pressure increase. The temperature starts to decrease before the phase transition line of pure liquid water is reached since the latent heat needed to thaw tylose is absorbed over a range of temperatures [41].

Thawing continues at high pressure in the second phase resulting in a reduction in sample volume as well as pressure that cause the freezing/thawing point to increase. The sample is then completely thawed and the temperature increases to the pre-set desired value. The system is depressurized and the sample temperature drops. Therefore, care has to be taken to ensure that the vessel temperature is maintained low enough to avoid partial freezing of the sample due to the pressure release. The increase in the thawing rate associated with HPIT is due to the larger temperature gradient resulting after

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compression between the temperature of the melting product and that of the ambient pressure transfer medium. It is concluded that during the HPSF and HPIT, the removal or absorbance of the latent heat is the major factor determining the economic potential of the process.

Effect of pretreatment on texture

The effect of the pretreatments alone on the hardness of the strawberry was investigated using the same batch of the strawberry as illustrated in Figure 2.

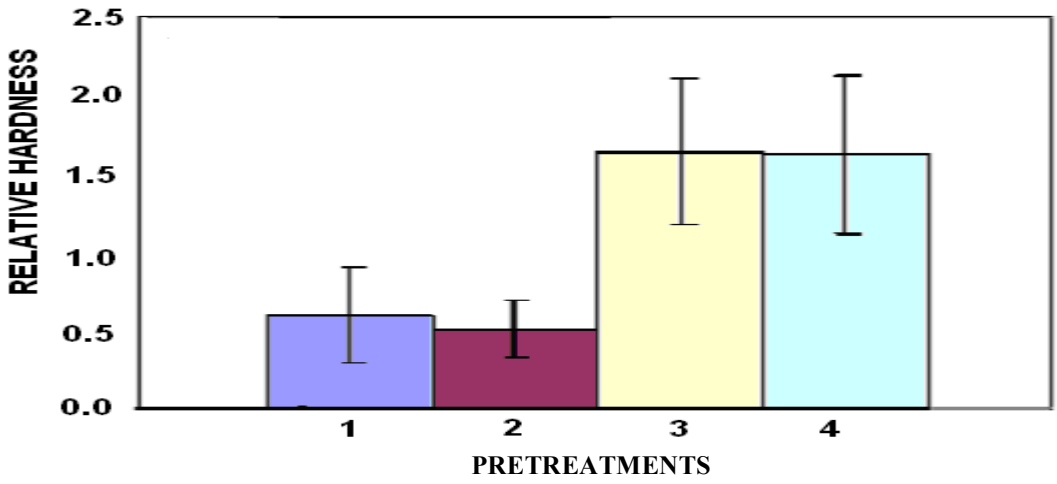

Figure 2: Relative hardness of the strawberry after different pretreatments Pretreatment 1: water infusion; Pretreatment 2: water infusion and incubation at 40°C for 20 min; Pretreatment 3: PME and $CaCl_2$ infusion; Pretreatment 4: PME and $CaCl_2$ infusion and incubation at 40°C for 20 min

The samples infused with PME and $CaCl_2$solution are significantly harder than those infused with water. This is true for both incubated and non-incubated samples and confirms results obtained by previous studies [20, 25, 26]. It is also worth noting that the strawberry infused with PME and $CaCl_2$ solution are harder than the fresh strawberry. The improved hardness may be attributed to the calcium-pectate complex formation that is believed to enhance the texture. The formation of the calcium-pectate complex could be taking place after the infused exogenous PME de-methylates the endogenous strawberry pectin and yields the free carboxyl groups (COO-) that complex with the calcium ions in the infused solution as reported by other workers [16].

Effect of HHP processes on strawberry texture

Texture degradation is used as an indicator to evaluate the freezing and thawing processes. To study the potentials of HPSF and HPIT concerning texture retention, the texture degradation during each of the processes was investigated and compared on relative terms. To investigate the effect of frozen storage time on the texture degradation, the samples were kept at -18°C for different times (1 day and 3 months).

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Effect of conventional freezing and pretreatments on texture

After 1 day of frozen storage at -18°C, the hardness of the 16 strawberry samples was measured (Figure 3).

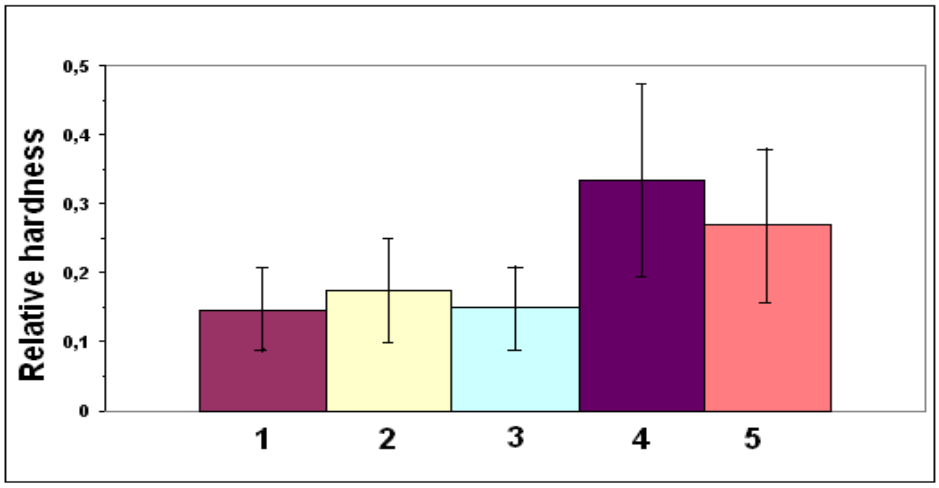

Figure 3: Relative hardness of strawberry after one day storage at -18°C Pretreatment 1: No pretreatment; Pretreatment 2: Water infusion; Pretreatment 3: Water infusion and incubation at 40 °C for 20 min; Pretreatment 4: PME and $CaCl_2$ infusion; Pretreatment 5: PME and $CaCl_2$ infusion and incubation at 40 °C for 20 min

The results in Figure 3 are averages of 2 replications. The trend of each set of results is similar to the reported average values. The $CaCl_2$and PME infused samples show a higher relative hardness after one day of storage at -18°C compared to non-pretreated and water-infused samples. This is in line with the results reported by other workers about the ability of $CaCl_2$and PME to enhance the texture of fruits and vegetables [20, 26]. The hardness of the samples infused with water is significantly higher than those of the non-pretreated samples. The high variations could be due to the variability in the strawberry hardness, which is a characteristic of biological materials. It also appears that the incubation process only slightly affects the texture of the samples and so all subsequent analyses for HPIT do not include the pretreatments with incubation.

Effect of high-pressure shift freezing on texture

The strawberry samples were high-pressure shift frozen after pre-treatment. Figure 4 shows the results of hardness obtained from the average of 12 samples. The experiment was replicated twice and the mean is presented. The trend of each set of the results resembles the averages recorded.

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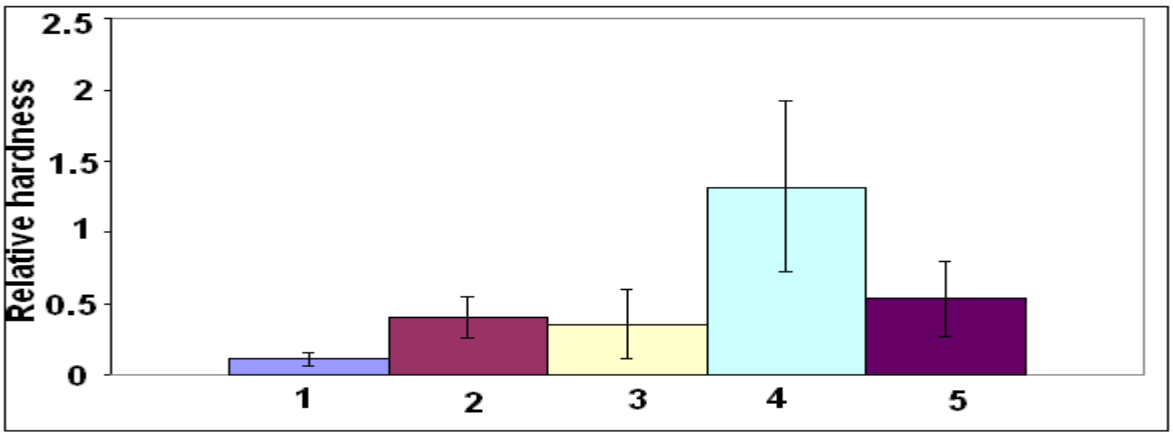

Figure 4: Relative hardness of high-pressure treated frozen strawberry Pretreatment 1: No pretreatment; Pretreatment 2: Water infusion; Pretreatment 3: Water infusion and incubation at 40 °C for 20 min; Pretreatment 4: PME and $CaCl_2$ infusion; Pretreatment 5: PME and $CaCl_2$ infusion and incubation at 40 °C for 20 min

The results show a similar trend in hardness as those of conventionally frozen strawberry. The $CaCl_2$ and PME-infused samples are harder than the water-infused and non-pretreated samples. The hardness of HPSF processed samples is higher than that of conventionally frozen strawberry for both the water-infused and PME and $CaCl_2$-infused. Although the variability is high, the PME and $CaCl_2$-infused samples without incubation are harder than the rest of the samples while for the non-pretreated samples, there is no significant difference in hardness between HPSF samples and conventionally frozen samples without pretreatment.

Effect of high-pressure induced thawing on texture

Figures 5 and 6 show hardness values recorded after HPIT and conventional thawing. Hardness measurements were carried out in three stages: immediately after thawing, after 3 hours of storage at room temperature and after storage overnight at 4°C. The samples used were initially stored at -18°C for 3 months. All the measurements are for batches thawed at the same time and of same pretreatment. Non-pre-treated, water-infused, and PME with$CaCl_2$-infused samples show no significant difference in hardness when measured immediately after conventional thawing and HPIT. After storage for 3 hours at room temperature, the non-pre-treated and water-infused samples show no significant hardness changes. Following overnight storage at 4°C, both samples became mushy and thus not measurable. For PME and $CaCl_2$-infused samples, there is a significant hardness difference between conventional thawing and HPIT samples after 3 hours storage at room temperature. The difference is not so significant after an overnight storage at 4°C (Figure 5).

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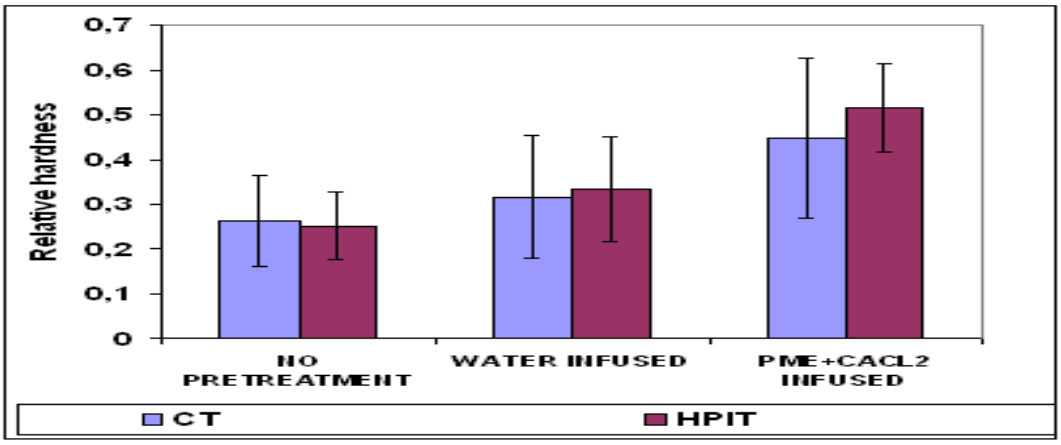

Figure 5: Hardness comparisons for different pretreatments immediately after thawing for HPIT and conventional thawing. CT: Conventional thawing; and HPIT: High-pressure induced thawing

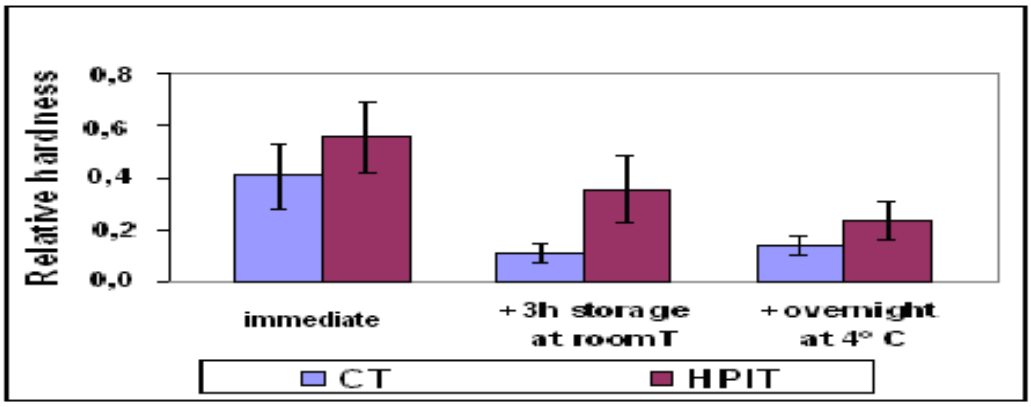

Figure 6: Hardness comparisons for PME and $CaCl_2$-infused strawberry 3 hours at room temperature and overnight at 4°C for HPIT and conventional thawing. CT: Conventional thawing. HPIT: High-pressure induced thawing

The relative hardness of the thawed strawberry shows significant differences between the two processes after 3 hours of storage at room temperature, where HPIT process is better. However, for both processes the hardness drops off sharply within 3 hours of storage at room temperature after thawing. Even extended chilled storage no longer preserves the texture of the thawed product. This indicates that frozen strawberry should be consumed immediately after thawing in order to benefit from the preserved texture.

Effect of long-term frozen storage on strawberry texture

The conventionally frozen samples were stored for 3 months at -18 °C after which they were thawed at room temperature for 2 hours and hardness determined. The values obtained are averages of 16 samples. The aim of this investigation is to determine the effect of the storage conditions and pretreatments on the hardness of the strawberry.

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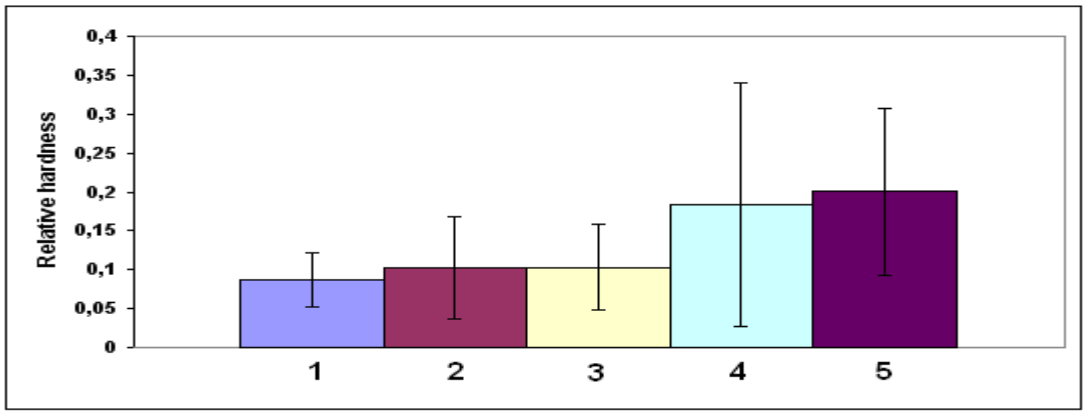

Figure 7: Relative hardness of conventional frozen strawberry after 3 months storage at -18 °C
Pretreatment 1: No pretreatment; Pretreatment 2: Water infusion; Pretreatment 3: Water infusion and incubation at 40 °C for 20 min; Pretreatment 4: PME and $CaCl_2$-infusion; Pretreatment 5: PME and $CaCl_2$ infusion and incubation at 40 °C for 20 min

The results in Figure 7 show that the $CaCl_2$and PME-infused samples are harder than the water-infused samples. The results also show that the samples stored for 3 months are not significantly different in hardness from those stored for 1 day at the same temperature (Figure 8). The non-significant differences in hardness despite the different frozen storage times can be attributed to re-crystallization that may occur at these conditions because storage was done above the glass transition temperature of strawberry, leading to reduced texture degradation [17, 26].

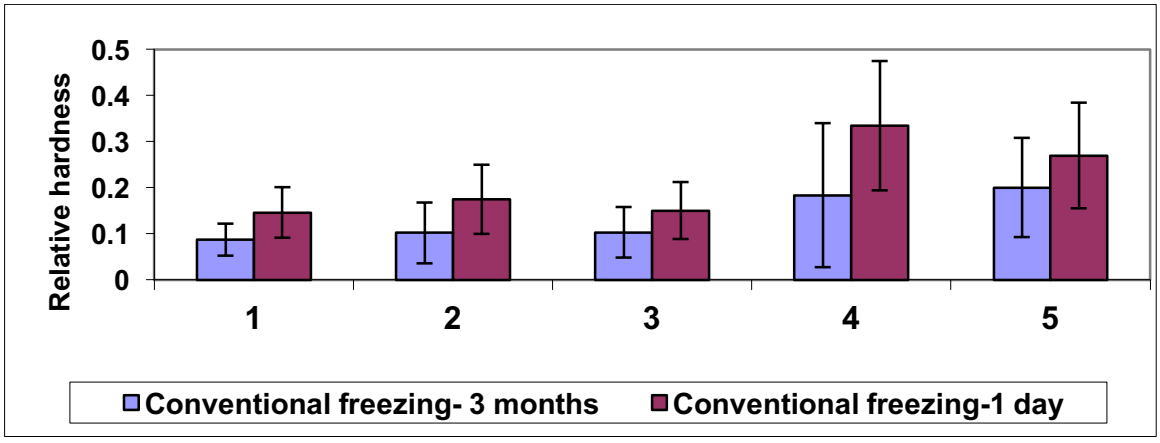

Figure 8: Comparison of strawberry hardness after 1 day and 3 months storage at -18°C
Pretreatment 1: No pretreatment; Pretreatment 2: Water infusion; Pretreatment 3: Water infusion and incubation at 40°C for 20 min; Pretreatment 4: PME and $CaCl_2$-infusion; Pretreatment 5: PME and $CaCl_2$-infusion and incubation at 40°C for 20 min

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Effect of HHP processes on drip loss

Drip loss was determined gravimetrically according to Equation 4. The aim was to compare the effect of pretreatments, HHP freezing and thawing processes and the effect of storage on the drip loss of the samples. These comparisons were intended to find out the pretreatment and process combination that minimizes drip loss (%) as compared to conventionally frozen samples (Table 2).

It is observed that drip loss is not minimized by any of the pretreatments after conventional freezing. Similarly, HPSF alone does not minimize drip loss of strawberry. It is combination of HPSF with the infusion PME and calcium chloride that significantly minimized drip loss. Incubation appears to improve the results. A non-significant difference in drip loss, however, exists between storage for one day and 3 months for non-infused samples. The non-significant difference can be due to re-crystallization (which may be affecting drip loss more than it affects texture) and other texture degrading processes taking place at the storage temperature of -18°C. This is possible because of the fact that the temperature of -18°C used to store the strawberry is above the glass transition temperature of strawberry, which is reported to be -38°C [17].

The effect of HPIT was also investigated and compared with conventional freezing both for short-and long-term storage periods and the results are shown in Table 3. The same batch of strawberry was used for each analysis. The measurement time is immediately after thawing (0 hr.), storage for three hours at room temperature after thawing (3 hrs), and chilled storage for twenty-four hours after thawing (24 hrs).

The drip losses are higher in conventional thawed samples than in HPIT samples for all pretreatments. Minimum drip loss is observed with PME and $CaCl_2$-infusion pretreatment for HPIT. This is agreement with previous studies done in beef and fish [9, 42]. It was also found that there is a reduction in thawing time when HPIT is used to one hour as compared to conventional thawing that takes two hours for similar sample dimensions. The reduction in thawing time may also account for the reduced drip loss observed. Water infusion, however, does not reduce drip loss compared to non-pretreated samples. Similarly, PME and$CaCl_2$-infusion alone does not minimize drip loss in conventionally thawed samples after 3 months storage.

Drip loss increases as the time after thawing increases both for conventional thawing and HPIT and for all the pretreatments. This may be because the longer the thawing time, the more water and solutes are released from the tissues. However, PME and $CaCl_2$-infused samples experience lower drip loss compared to the non-pretreated and water-infused samples. This is true both after three hours and overnight storage for HPIT. Drip loss is thus minimized by HPIT after storage at room temperature and overnight at 4 °C for PME and $CaCl_2$-infused strawberry.

Effect of vacuum infusion on weight gain

The percentage weight gain during infusion of water and PME and $CaCl_2$ solution was computed according to Equation 1. The results are average values of 10 analyses. The observed weight gain with both infusion solutions is due to the hydrodynamic mass

transfer taking place as the solutions move into the strawberry tissue when the atmospheric pressure is restored. The observed results are in agreement with published reports on vacuum infusion [43, 44]. The average weight gain on vacuum infusion is 15 ± 1.33 %. However, the weight gain during PME and $CaCl_2$-infusion (16%) is significantly higher than the case of water infusion (14%) and the difference can be due to the ability of the calcium-pectate network formed in the presence of PME and calcium ions that trap the infused solution more than in the water-infused samples that may occur without calcium-pectate formation.

Effect of freezing on the degree of pectin methylation

As detailed in Table 4, to correlate the DM% and the texture values obtained, the moles of galacturonic acid per mg of AIR sample for the fresh strawberry were 0.002±2.699E-05 for the one-day storage and 0.002±1.070E-05 for the three-month storage for samples conventionally frozen and thawed with a storage time of 1 day and 3 months in duplicate experiments. A higher value of DM% for the fresh than for the processed strawberry means freezing seems to lower the DM% possibly due to effect of extraction. The pretreatment influences the extent of the reduction of DM%. The PME and $CaCl_2$-infused samples show the lowest DM% after one-day storage. The infused PME possibly de-methylates the pectin in the strawberry that results in a lower degree of methylation. These results agree with those obtained with carrots where the DM% reduced during precooking and cooking of carrots due to increase in activity of PME during preheating [22, 39]. During the extraction, it is observed that extracting the water-infused samples is not easy and the samples have to be mixed for longer time to obtain a fine powder of AIR. The results are similar with long-term frozen storage. Generally, however, it is expected that as the DM% decreases, the texture will increase due to the formed calcium-pectate complexes as the pectin is de-methylated in the presence of PME and calcium ions [17].

CONCLUSION

The effect of PME and $CaCl_2$-infusion and incubation along with high-pressure processes on the texture, drip loss and DM% were investigated and compared to conventional processes. Vacuum infusion of PME and $CaCl_2$solution into the strawberry resulted in texture improvement. A 15% weight gain during vacuum infusion is desirable in keeping the strawberry hydrated. High pressure shift freezing enhances the texture of frozen strawberry when combined with PME and $CaCl_2$-infusion. The incubation process done at 40 °C for 20 minutes appears not to have extra-hardness enhancing effect and HPSF combined with the infusion of PME and $CaCl_2$also minimizes drip loss of the strawberry.

Long-term frozen storage (3 months) of conventionally frozen strawberry does not cause a significant difference in hardness compared to short-term (1 day) storage. The PME and calcium chloride infused strawberry; however, experience minimum textural degradation compared to the non-pretreated and water-infused both during long- and short-term frozen storage. After 3 months frozen storage, the strawberry infused with PME and $CaCl_2$were harder than the non-pretreated or water-infused strawberry. The DM% appears to decrease with conventional freezing but further analysis is necessary to establish the relation between DM% and texture in frozen strawberry.

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High pressure induced thawing does not minimize texture degradation of the strawberry although it minimizes drip loss. High pressure induced thawing (HPIT) also reduced thawing time compared to conventional thawing process and this is important in saving process times and energy. Generally, enzyme pretreatment combined with high pressure freezing improves the texture of frozen strawberry thawed for use. However, further research is still necessary to correlate other texture indicators to changes that occur during freezing and thawing and to also find out the acceptability of the frozen strawberry to the human consumers.

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Table 1: Pretreatments of strawberry samples prior to freezing

Pre-treatment Code	Pre-treatment Process
O	Fresh samples
I	No pretreatment
II	Water infusion only
III	Water infused and incubation at 40° C for 20 min.
IV	PME and $CaCl_2$-infused only
V	PME and $CaCl_2$-infused plus incubation at 40° C for 20 min

Table 2: Combined effect of pre-treatment and freezing processes on drip loss

Pretreatment	Conventional (1 day)	Conventional (3 months)	HPSF (1 day)
None	19.76 ± 7.08	35.03 ±14.81	47.93 ± 4.62
Water	37.41±9.86	35.53 ± 8.53	39.96 ± 6.52
Water and incubation	34.68 ± 14.45	31.99 ± 10.12	36.64 ± 3.73
PME/$CaCl_2$	28.29 ± 5.85	26.95 ±12.69	15.01 ± 5.92
PME/$CaCl_2$/incubation	27.28 ± 4.35	30.05 ± 8.13	8.94 ± 5.96

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Table 3: Combined effect of pretreatment, thawing process and measurement time on drip loss (%)

Pretreatment	Conventional Thawing			HPIT		
	0 Hr.	3 Hrs.	24 Hrs.	0 Hr.	3 Hrs.	24 Hrs.
None	25.96	66.62	82.15	23.65	55.66	66.75
Water-infused	59.58	60.84	75.18	49.14	56.51	71.75
PME/$CaCl_2$-infused	30.27	60.24	71.14	22.20	38.97	55.03

Table 4: Effect of frozen storage on the pectin degree of methylation

Pretreatment	1 Day Frozen Storage		3 Months Frozen Storage	
	N CH_3OH/mg AIR	(DM %)	N CH_3OH/mg AIR	(DM %)
Fresh	0.002 ± 9.6E-05	82.2 ± 0.10	0.002 ±3.8E-05	90.1 ± 0.09
None	0.001 ± 3.9E-05	64.1 ± 0.09	0.001 ±1.6E-05	71.7 ±0.09
Water	0.002 ± 4.1E-05	65.4 ± 0.98	0.002 ±7.1E-05	89.1 ±0.12
Water/incubation	0.002 ± 5.7E-05	68.4 ± 0.10	0.002 ± 4.1E-05	94.6±0.12
PME/$CaCl_2$	0.001± 1.9E-05	60.3 ± 0.09	0.001 ±2.4E-05	75.5 ± 0.09
PME/$CaCl_2$/incubation	0.001± 1.3E-05	45.9 ± 0.07	0.002 ±7.4E-05	86.3± 0.12

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