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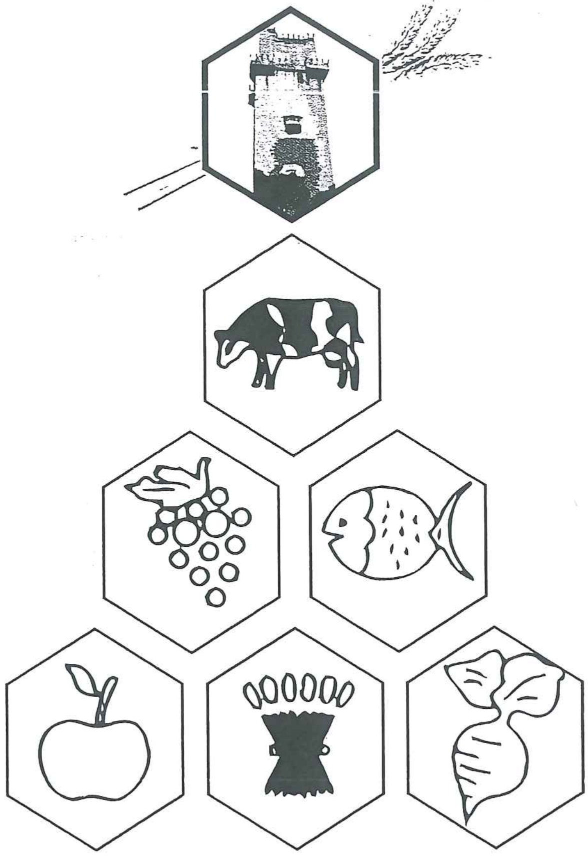

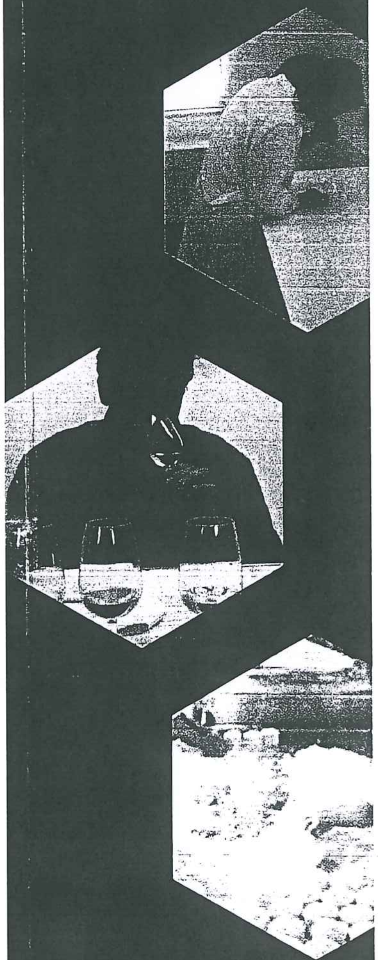

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EFFECT OF THE THERMAL AND HIGH PRESSURE PRE-TREATMENTS AND OF HIGH PRESSURE SHIFT FREEZING ON GREEN PEPPERS FIRMNESS

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Abstract: Industrially, bell peppers are usually frozen, after being thermally blanched, to be consumed in salads after thawing. However, texture tend to decrease after this process. Therefore, several thermal (55ºC) and thermal/high-pressure (200MPa, 25ºC and 55ºC) pre-treatments, with and without calcium (0.50%, w/v) addition, were studied for their possible effect on texture improvement of green bell peppers. The temperature-pressure-calcium conditions revelead a significant ($P<0.05$) enhancement on the firmness of peppers. Peppers pre-treated at the conditions that caused the best firmness improvements were used to further study the effect on firmness of freezing at atmospheric pressure and of high-pressure shift freezing (HPSF). Only the pre-treated samples that were frozen by HPSF (200MPa, -18ºC) showed no detrimental effect on firmness ($P>0.05$), while those frozen at atmospheric pressure (-18ºC, -40ºC) presented a significant decrease on firmness ($P<0.05$). The pre-treated samples showed no changes on firmness ($P>0.05$) after overnight storage, but after 2.5 months storage, only the high pressure pre-treated peppers showed no decrease in firmness ($P>0.05$), clearly indicating the beneficial effect of the high pressure pre-treatments to retain firmness. These results illustrate the potential of thermal, pressure, combined thermal/high-pressure pre-treatments on firmness improvement of green bell pepper and of HPSF to retain firmness during freezing and frozen storage.

1. INTRODUCTION

Nowadays, the consumer demand for natural, fresh-like, more convenient and, at the same time, safe food products, has driven the food industry to apply minimal (mild) efficient techniques to process plant products. Besides, frozen fruits and vegetables, nutritionally similar to fresh ones, are becoming an important part of a healthy, balanced and nutrient rich diet. Unfortunately, the ice crystals formation, an integral part of the freezing process, can damage the fragile membranes causing release of enclosed contents and changes in the microenvironment of food tissues and, as a consequence, affecting the food product texture. This situation points to the need for optimization of conventional technologies as well as their combination with new emerging technologies, such as high-pressure (e.g., combined thermal/high-pressure treatments and high-pressure shift freezing), in order to produce high-quality (including firmness) processed fruit and vegetable products.

The possibility of using milder pre-processes for preservation of tissue integrity and the improvement of quality has been explored in the last years. Pre-heating of some vegetable tissues at temperatures between 50-60ºC before canning can reduce canning-induced softening in several plant tissues [1]. It has been suggested that the firming effect obtained

from mild heat treatments alone or even combined with $CaCl_2$ treatments [3-5] may be attributed to the action of heat-activated pectin methylesterase (PME, EC 3.1.1.11), a well-known cell-wall bound enzyme, and/or to increased Ca^{2+} diffusion into the tissue. Also, high-pressure (HP) pre-treatments alone or even combined with temperature seem to have a similar positive effect when compared to thermal treatments [6]. Recently, the positive contribution of HP pre-treatments to improve the firmness as well as to preserve food structure during freezing [7,8] has been demonstrated. High-pressure freezing (HPF) promotes uniform and rapid ice nucleation and growth through the whole sample due to the high and uniform degree of supercooling [9], and has been applied with success to several food products. Softening caused by freezing/thawing can sometimes be minimized by pre-treatments of the tissue with $CaCl_2$ [10] in combination with mild heat treatments [11,12].
In this work, several high pressure pre-treatments were studied for their possible effect on texture improvement of green bell peppers (*Capsicum annuum*). Considering that freezing is generally preceded by blanching, the effect of several thermal pre-treatments were also investigated, as well as, combined T/HP pre-treatments. For all cases the effect of calcium addition was studied. The pre-treatments showing the best firmness improvement were further studied for the effect of freezing and frozen storage on firmness of peppers.

2. MATERIALS AND METHODS

2.1 Thermal and thermal/high-pressure pre-treatments, with/without calcium soaking

Green bell peppers (*Capsicum annuum*) obtained from a local auction (Mechelen, Belgium), were washed, the stems were cut off, and the core removed by hand. Individual samples were prepared by cutting small circles of 20mm diameter, and placed in a double plastic bag and vacuum sealed. The thermal treatments were performed in a pre-set thermostated water bath at 55°C. After each treatment, the samples were immediately cooled in an ice-water bath (5min) and equilibrated at room temperature before texture measurements. The combined thermal/high-pressure treatments (200MPa, at 25°C and 55°C, 15min) were carried out in a single vessel HP equipment (590mL volume, i.d. 50mm, height 300mm), with a maximum operating pressure of 600MPa and a working temperature ranging from -30°C to 100°C (SO. 5-7422-0, Engineered Pressure Systems International EPSInt, Belgium). The pressure medium used was a glycol water mixture (60% Dowcal N, Switzerland). After each treatment, the samples were left at room temperature for 45min, to allow PME catalysed de-esterification of the methylated pectin-substrate, not only during the treatments [13], but also after the pressure release [9,14]. The combination of calcium soaking with thermal and thermal/high-pressure treatments was also evaluated. For calcium soaking, pepper circles were removed from the sealed plastic bags and immersed in a beaker with $CaCl_2$-solution (0.50%, w/v) for 60min at room temperature and 55°C. The samples, treated simultaneously with temperature and calcium, were soaked in a $CaCl_2$-solution, previously equilibrated at 55°C.

2.2 Freezing treatments

The effect of the freezing process on texture of peppers was evaluated for non- and pre-treated samples. Pepper samples were frozen by Cryogenic Freezing (CF) and High-Pressure Shift Freezing (HPSF). A programmable cryogenic freezer (Nicool PC Plus, Air. Liquide, Paris, France) was used to freeze the pepper samples. Liquid nitrogen was supplied by a self-pressurized (0.04MPa) vessel (TP60, Air Liquide, Paris, France). The CF conditions were established at pre-set temperatures (-18°C, -40°C). The frozen samples were kept overnight in a conventional freezer at the respective temperature and thawed at room temperature before texture measurements. The HPSF experiments were performed in the same high-pressure equipment used for combined thermal/high-pressure treatments. The pepper samples at room temperature were placed inside the vessel, with the pressure medium already at -25ºC (by external cooling) and the pressure was built up to 200MPa. When the temperature at the centre of the pepper circles reached -18°C, the pressure was released to atmospheric pressure to freeze the pepper samples. Due to the pressure release, the sample temperature increases until the initial freezing point at atmospheric pressure. The samples were kept in the system until they reached -18ºC. The frozen samples were transferred to a conventional freezer at -18°C, stored for the appropriate time (overnight or 2.5months), thawed at room temperature and used for texture measurements.

2.3 Texture measurements

The texture was measured at room temperature by a texture analyser (TA-XT2i, Stable Micro System), using a holed plate, with the following parameters: 5kg force load cell, 2mm diameter aluminium cylinder probe, and 2.0mm.s^{-1} test speed. The property "firmness", the maximum force (*TP*) applied to puncture the pepper tissue, was measured as an indicator of texture. An average value of firmness from 9 puncture measurements (skin and flesh sides) was calculated for each experimental condition. It should be mentioned that the data shown was for flesh side measurements, even though tests were preformed for both sides (skin and flesh). This procedure was adopted, since the measurements performed from the flesh side gave more information (the maximum force registered varied more with the treatments applied) associated to the different conditions under study, probably due to a higher cell heterogeneity and heat sensibility [15].

2.4 Data analysis

The firmness decay as a function of heating time (TP_t), at a constant temperature, can be described by a fractional conversion model to explain vegetable tissue texture degradation kinetic data.

$$TP_t = TP_\infty + (TP_0 - TP_\infty)\cdot \exp(-k \cdot t) \quad (1)$$

The TP_0 is the initial firmness and TP_∞ is the non-zero equilibrium firmness after prolonged treatment time. For most irreversible first-order reactions TP_∞ approaches to zero. Both texture degradation rate constant (k) and non-zero equilibrium firmness after prolonged treatment time (TP_∞) were estimated using non-linear regression analysis on equation 1 [16].

3. RESULTS AND DISCUSSION

The effect of different temperature-pressure-calcium conditions can be visualized in **Table 1**. The relative firmness was calculated as the ratio between the firmness of the sample after a certain pre-treatment and the firmness of the non pre-treated pepper samples. While immersion in $CaCl_2$-solution alone did not improve firmness ($P>0.05$), all the treatments involving heating at 55ºC caused an increase ($P<0.05$) in firmness, compared to samples not pre-treated or only immersed in $CaCl_2$-solution. For several vegetables it has already been shown that relative firmness increments up to a maximum value, when heated at 50-55ºC for 30min [1]. At the same time, PME-activity from different plant sources also reveals a maximum around 50-60°C [17,18]. Previous process stability studies related to pepper PME indicated that after heat treatments at 50-55ºC, the relative pepper PME activity of intact tissue and puree samples increased, followed by a fast decay above 60°C [19].

Combined thermal/high-pressure treatments also increased pepper firmness ($P<0.05$). Comparing the firming effect of thermal and thermal/high-pressure treatments, it can be concluded that pre-treatments at 200MPa, 25°C for 15min (134±11%) are similar ($P>0.05$) to a thermal treatment alone at 55°C for 60min (132±4%), creating the possibility to use high-pressure as an alternative to thermal pre-processing in the improvement of texture of green bell peppers. Other studies have already revealed that firmness could be enhanced by low levels of pressure [14], and that these increments might be related to the effect of HP on the cell structure, which leads to an increase in the extractability of PME from the pepper tissue [19], and further contact of the enzyme with the pectin substrate. At low pressure levels, pepper PME seems to be protected from thermal inactivation [20] and when in presence of pectin substrate, the PME catalyzed reaction exhibited an optimum for enzyme activity [13] at 200MPa, which could be the reason for the improvement of the relative firmness observed. The free carboxyl groups formed during the action of PME could then interact with Ca^{2+} ions to form bridges between the pectin chains, resulting in higher firmness and resistance of the tissue to further cooking [1-2].

Table 1 - Relative firmness (%, ± standard deviation) of pepper samples with different thermal and thermal-high-pressure pre-treatment, with/without calcium soaking.

Pre-treatment	Relative firmness (%)
Not pre-treated	100.0±15.3
0.50% Ca^{2+} (60')	111.4±14.2
55°C (60')	132.0±4.2
0.50% Ca^{2+} (60') → 55°C (60')	136.7±20.6
55°C + 0.50% Ca^{2+} (60')	118.2±15.5
55°C (60') → 0.50%Ca^{2+} (60')	150.4±13.1
200MPa, 25°C (15')	134.2±11.0
200MPa, 55°C (15')	107.9±6.6
200MPa, 25°C (15')→ 0.50%Ca^{2+} (45')	158.7±11.5
200MPa, 55°C (15')→ 0.50%Ca^{2+} (45')	149.4±15.5

The pre-treatments yielding the best results in terms of firmness were evaluated for their effect on pepper thermal texture degradation kinetics at 90°C, a temperature typically used to blanch peppers prior to freezing. The kinetic parameter estimates for thermal texture degradation at 90°C for pre-treated pepper tissue are presented in **Table 2**.

Table 2 - Texture degradation rate constant (k ± standard error of regression) and relative final texture value (TP_∞/TP_0), for thermal (at 90 °C) texture degradation of pre-treated pepper.

Pre-treatment	$k \times 10^2$ (min^{-1})	Relative firmness at t_∞ (TP_∞/TP_0, %)
Not pre-treated	7.16±0.72	10.1±3.9
0.50% Ca^{2+} (60')	4.71±1.27	23.5±8.9
55°C (60')	5.37±0.88	14.9±6.0
55°C (60') → 0.50%Ca^{2+} (60')	3.04±0.41	17.0±4.6
200MPa, 25°C (15')	3.90±0.14	16.0±1.1
200MPa, 55°C (15')	2.92±0.18	34.3±1.6
200MPa, 25°C (15') → 0.50%Ca^{2+} (45')	1.60±0.27	17.4±6.9
200MPa, 55°C (15') → 0.50%Ca^{2+} (45')	1.95±0.16	41.5±2.2

It can be seen that k-value decreases by 1.3- to 4.5-fold, which means that texture degradation at 90°C of pre-treated pepper is significantly slowed down by all the pre-treatment conditions

investigated: As already observed for other vegetables [6, 21], softness induced by processing at 90°C was clearly slowed down by mild thermal and mild thermal/high pressure pre-treatments. From the different selected conditions, pre-pressurized pepper samples, followed by calcium soaking yield the lowest *k*-values. However, higher temperature-pressure conditions (400MPa, 60°C) were necessary to reduce thermal texture degradation of carrots between 90-110°C [6]. Since TP_∞ only becomes important after a very long heating time, unrealistic for industrial applications, the optimisation of texture degradation should be in terms of texture degradation rate constants. Nevertheless, it should be emphasized that for TP_∞, improvements of 1.5- to 4.1-fold were obtained for all pre-treatments studied. This evidence, together with the observed decrease in the *k*-value, clearly illustrates that under the studied conditions, the texture degradation of blanched pre-treated peppers was slowed down.
The effect of freezing and frozen storage, on texture of peppers pre-treated by three of the pre-treatments (those showing the most significant beneficial effect on pepper firmness), was further evaluated (**Figure 1**).

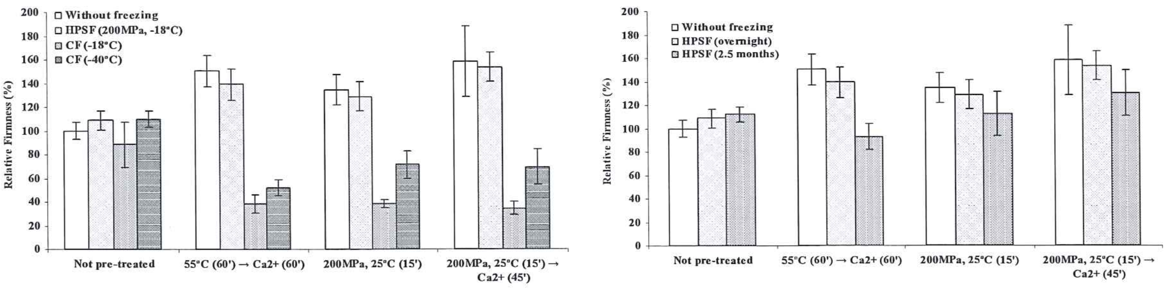

Figure 1 – Effect of freezing (left) and frozen storage (right) on relative firmness (%) of non pre-treated and pre-treated pepper samples. The bars represent the standard deviation of each sample.

Statistical analysis revealed that only the pre-treated samples frozen by HPSF (200MPa, -18°C) showed no detrimental effect on firmness (P>0.05), while those frozen by CF (-18°C, -40°C) presented a significant decrease on firmness (P<0.05), of about 40-60%, an effect that was more pronounced for CF at -18°C. This may be due to a lower freezing rate at -18°C and, as a consequence large ice crystals causing more detrimental effects on pepper tissue. For the non pre-treated pepper samples no significant differences between the three freezing processes were observed, although these samples showed a lower firmness before the freezing process. Our results seem to be in agreement with other studies where faster freezing improved the texture of frozen carrots [12]. While for overnight storage, all pre-treated samples showed no changes of firmness (P>0.05), after 2.5months storage, only the pre-pressurized samples showed no decrease on firmness (P>0.05), clearly indicating the beneficial effect of the pressure pre-treatments to retain texture (**Figure 1**).
The results reported in this work, clearly demonstrate the potential of thermal, pressure, and combined thermal/high-pressure pre-treatments, to improve green bell peppers firmness and of high pressure shift freezing to retain firmness during freezing and frozen storage.

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