Process Optimisation and Minimal Processing of Foods

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Foreword

This third series of booklets collects the presentations made at the third and final main project meeting, held at the Leuven Catholic University, under the auspices of the EU Copernicus Programme.

During its lifetime, the "Process Optimisation and Minimal Processing of Foods" project organised a series of initiatives to foster international scientific exchange in the area, with particular emphasis on EU-Eastern Europe co-operation, of which the main meetings, organised as workshops, were the source of dissemination material. Thus, the plenary lectures, short communications and posters presented were collected in booklets to provide a wide ranging dissemination.

Besides these booklets, a book is also being published by CRC press, entitled "Processing of Foods: Quality Optimisation and Process Assessement" with contributions specially written for this effect.

A large number of contributions has thus been collected and the deepest word of appreciation is given to the generous contribution of all the researchers involved. In particular, the role of the area leaders in promoting the project activities in their area is most greatly appreciated.

On the other hand the complex task of organising, collecting, computer-editing and publishing the large amount of contributions could only be done properly thanks to the commitment and efforts of Mrs. Isabel Lino, Mrs. Manuela Pascoal and Mr. Kai Sprecher, who are thus gratefully acknowledged.

Finally, everyone involved in the project would like to express their appreciation for the incentive given by the COPERNICUS Programme to the excellence of European research, adding in particular to the significant efforts made in Agro-Industrial Research.

Porto, June 30th, 1998
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Freezing and Storage of Orange Juice: Effects on Pectinesterase Activity and Quality

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Abstract

The pectinesterase (PE) enzyme is responsible for the citrus juices clarification. Usually, to prevent this undesirable effect, 90 to 100 % of the PE is thermally inactivated by a pasteurisation process. However, this processing causes also the degradation of quality parameters, such as flavour and colour. Therefore, if freezing and frozen storage inactivates part of the PE, this unit operation could be used as a pre-treatment before pasteurisation.

The objective of this work is to study the freezing and frozen storage effects on PE activity and quality of fresh orange juice. Samples of fresh orange "Valencia Late" juice were frozen and stored at –20 °C during 120 days. The parameters analysed during storage were: PE activity, pH, °Brix, acidity, °Brix/acid ratio, colour, cloud stability and sensory evaluation The PE activity was determined at two temperatures (30 °C and 55 °C) using the method described by Köner and adaptations suggested by Amstalden and Montgomery. The freezing process increased the initial PE activity of the juice. After 120 days of storage, a reduction on PE activity of approximately 28 % was verified. This enzyme did not reactivate after 24 hours at 5 °C. As observed by other authors, two main enzyme fractions, a thermolabile and another thermostable, were identified. The frozen storage inactivates the less thermal resistant fraction. The freezing and frozen storage did not change significantly the pH, °Brix, acidity and total colour difference (TCD) of the juice. However, it caused the loss of its cloud stability. For the sensory evaluation the juice was thawed and pasteurised (85 °C/2 min). The fresh samples used in the sensory tests were also thermally treated during 2 min at 90 °C. Using a Triangular test it was concluded that there was a significant difference between the samples (within 99 % confidence). The Hedonic test showed that there was a greater preference for the fresh samples.

1. Introduction

Fresh orange juice contains particulates in suspension that give it a "cloudy" appearance (Amstalden and Montgomery, 1994, 1995; Baker and Bruemmer, 1972; Köner *et al.*, 1980). This characteristic gives the consumer an idea of a natural fresh product. The commercial value is lost

if the juice clarifies (Amstalden and Montgomery, 1994, 1995; Atkins *et al*., 1952). Cloud loss is due to the pectinesterase (PE) enzyme activity (Amstalden and Montgomery, 1994, 1995; Atkins and Rouse, 1953; Marshal *et al*., 1985; Seymour *et al*., 1991a,b; Versteeg *et al*., 1980).

There are different forms of PE in citrus juices. The thermostable (PE I), thermolabile (PE II) and high molecular weight PE (HMW) are present in all citrus species already studied. These isoenzymes have different temperature stability. PE I and PE II account for almost 90% of the total PE activity and the HMW activity is variable in the different species of citrus. Furthermore, the main agents causative of cloud loss are HMW and PE I (Versteeg *et al*., 1980; Seymour *et al*., 1991b; Rombouts *et al*., 1982). Due to the thermostable isoenzymes a thermal treatment (pasteurisation) at relatively high temperature is used commercially for PE inactivation (Amstalden and Montgomery, 1995; Atkins and Rouse, 1953; Eagerman and Rouse, 1976; Marshall *et al*., 1985; Seymour *et al*., 1991b, Versteeg *et al*.,1980). Thus, the adequacy of a pasteurisation process depends on the extent of the PE inactivation. Because of the low orange pH (generally pH < 4.0) the microorganisms occurring in the juice are less thermal resistant than the PE enzyme (Eagerman and Rouse, 1976; Nath and Ranganna, 1977; Irwe and Olsson, 1994; Argáiz and López-Malo, 1995; Tajchakavit and Ramaswamy, 1997). However, the orange juice acquires off-flavours from excessive heat treatment (Baker and Bruemmer, 1972; Kiefer, 1961; Seymour *et al*., 1991b). There is a need to develop alternative methods that allow maximum retention of natural fruit properties. Versteeg *et al*. (1980) recommend pasteurisation at rather high temperatures or storage at –20 °C for PE inactivation in orange juice concentrates. Seymour *et al*. (1991a) obtained some PE inactivation in grapefruit during frozen storage. Therefore, freezing and frozen storage may be used as pre-treatment for PE inactivation followed by a less severe thermal treatment. The freezing and frozen storage processes cause less damages to the product than other preservation methods (Del Rio and Miller, 1979). However, this technology does not prevent all changes. During frozen storage product deterioration can occur (Ganthavorn and Powers, 1988; Jansen, 1969; Reid, 1990). Enzymatic and non-enzymatic changes occur at a slower rate at low temperatures. However, they can limit the storage life of frozen foods (Kramer, 1979). These reactions can develop off-flavours or losses of flavours in fruits and vegetables (Kermasha *et al*., 1988).

The juice extraction can be responsible for development of undesirable taste in orange juice. For example, a drastic juice extraction can liberate greater quantity of limonin in juice. This substance causes a bitter taste. It is present in the albedo, in the rags and in the seeds (Lanzarini and Pifferi, 1989; Versteeg, *et al*. 1977). Oxidative processes are also responsible for the development of off-flavours. The lipoxygenase enzyme is a potential deteriorative factor even at low temperature and very low residual activity (Baardseth, 1978, Kermasha and Metch, 1986; Munoz-Delgado, 1977).

With the final goal of investigating the potential use of freezing and frozen storage as a pre-treatment before a less severe thermal treatment, the objective of this work was the quantification of the freezing and frozen storage effects on the orange juice PE activity and quality.

2. Materials and Methods

2.1. Materials

Portuguese "Valencia Late" oranges (*Citrus sinensis L.*) were bought and squeezed to conduct this study. A polyethylene bag was filled with 0.5 l of orange juice. Table 1 presents the characteristics of the fresh orange juice. The juice was frozen in a blast freezer (Armfield) at –25 °C and stored at –20 ± 1 °C. At pre-specified time intervals (0, 1, 3, 7, 13, 20, 27, 34, 48, 69, 83, 96 and 120 days) samples were taken out from the cold store and thawed in a water bath at 25 °C. The following parameters were determined: PE activity, pH, °Brix, acidity, °Brix/acid ratio, colour, cloud stability and sensory evaluation.

Table 1
Characteristics of fresh and frozen (during 120 days) "Valencia Late" orange juice.

Parameter	Fresh juice	Frozen juice
pH	3.54	3.50
Brix	14	12
Brix/acid[a]	11.02	10.00
Acidity (%)	1.27	1.20
L, a, b	31.61, -4.75,12.27	32.62, -4.65, 12.97
PE activity	23.9[b]	17.2[b]
(PEUx10^4/ml)	26.2[c]	19.7[c]

[a] Expressed as citric acid, [b] Test temperature: 30 °C, [c] Test temperature: 55 °C

2.2. Methods

2.2.1. Pectinesterase assay

For PE analysis, the method described by Köner *et al*. (1980) was used. Basically, the method consisted of a titrimetric measurement of the rate of carboxyl group liberation from a 1 % pectin (Unipectin Up Slow Set 150), 0.15 M NaCl solution at pH 7.0 and controlled constant temperature. The activity was expressed in PEU, which corresponds to the miliequivalents of acid liberated per min per ml at pH 7.0 and specified temperature. The test temperatures used to determine residual activity were 30°C and 55°C. Some modifications, suggested by Amstalden and Montgomery (1994), were introduced. A 20 ml sample of juice was added to 40 ml of pectin solution (previously heated up) with constant stirring and quickly adjusted to pH 7.0 with 0.1 NaOH. When the pH 7.0 was reached the stopwatch was put in action simultaneously with the addition of 1 ml of 0.05 N NaOH. The stopwatch was stopped when the pH was back to 7.0. A constant temperature was maintained during the titration. PE activity was calculated by the equation:

$$PEU = \frac{(1ml\ NaOH\ *\ N\ of\ NaOH\ *\ 10^4)}{(time\ (min))\ *\ (ml\ of\ sample)} \quad (1)$$

where PEU is unit of pectinesterase/ml of sample.

In order to take into account the effect of pectin degradation by the alkali, which causes a pH decrease, an analysis was made with the pectin solution heated with distilled water instead of juice. The value determined was subtracted from the PE activity calculated by equation (1). The accuracy of the method was ± 0.7 PEUx104/ml.

2.2.2. Cloud determination

Ten ml of sample were placed in a conical centrifuge tube for 10 min at 360 x g. The turbidity was monitored by measuring the absorbance at 660 nm in a spectrophotometer (Shimadzu UV-1601, Japan). It was calibrated at 660 nm using distilled water as a blank. Cloud loss of the supernatant was measured just after thawing and after three days of storage at 5°C.

2.2.3. Sensory Evaluation

Thirty panellists were trained to participate in the study. The judges were pre-selected among the graduate students of the University, using a Triangle Test. The study was carried out in portable panel booths, for 8 periods of frozen storage (13, 20, 27, 34, 48, 69, 83 and 96 days).

Each panellist received three 20 ml samples of orange juice presented in 30 ml plastic souffle cups at 18 ºC. For each panellist two samples were equal and one sample was previously frozen and pasteurised at 85 ºC during 2 min. The fresh sample was pasteurised at 90 ºC during 2 min. Panellists were asked to taste and indicate which sample was different. Overall liking was evaluated using a 9-point fully anchored hedonic scale with the lowest category labelled "dislike extremely" and the highest category labelled "like extremely". The neutral point was labelled "neither like nor dislike". To determine whether there was a perceived difference between the samples a Triangle Test (Askar and Treptow, 1993) was used.

Frequency distributions of the difference test were analysed by Chi-square analysis. Overall liking scores were analysed by ANOVA. A cut-off value of a=0.05 was used for all tests. Data were analysed with the software Excel (Microsoft, Version 4.0).

2.2.4. Acidity, ºBrix, pH and colour measurements

Acidity was determined by titration with NaOH and expressed as citric acid. Brix degrees were measured with a hand refractometer (Atago) at 25 ºC. The pH of orange juice was measured using a digital pH meter (Crison model 2002), previously calibrated.

The colour parameters were measured using a portable tristimulus colourimeter (Minolta Chroma Meter CR-300). The sample was placed in a glass Petri dish and the measurements were taken in triplicate. The Hunter L, a, b tristimulus scale was used to characterise the Total Colour Difference (TCD), determined using the equation:

$$TCD = [(L_O-L)^2+(a_O-a)^2+(b_O-b)^2]\Omega \quad (2)$$

where the subscript "o" refers to the value of the original unfrozen orange juice.

3. Results and Discussion

Figure 1 presents the residual PE activity determined at 30 °C and 55 °C, as a function of frozen storage. It can be concluded that there are at least two enzyme fractions, one more heat resistant (PE I) and another more heat sensitive (PE II). In average the thermolabile fraction represented 90 % of the total PE activity in the original samples. There was an increase in PE activity after immediately freezing which probably resulted from cellular disruption. The structure of the cells probably changed or was damaged by ice crystals, and thus enzymes may have been more easily extracted. A reduction in PE activity was observed at test temperatures of 30 °C and 55 °C (table 2). Cloud destabilisation occurred after 3 days at 5 °C. This confirms that only the thermostable isoenzyme was not inactivated. During the formation of ice the solute concentration in the unfrozen medium increases. Changes in pH, ionic strength, viscosity and other properties can occur (deMan, 1990 and Reid, 1993). Several factors can be responsible for the enzyme inactivation: pH effect can cause protein denaturation (Belitz and Grosch, 1987). Therefore, the PE enzyme was possibly inactivated because of the low pH value in the unfrozen juice.

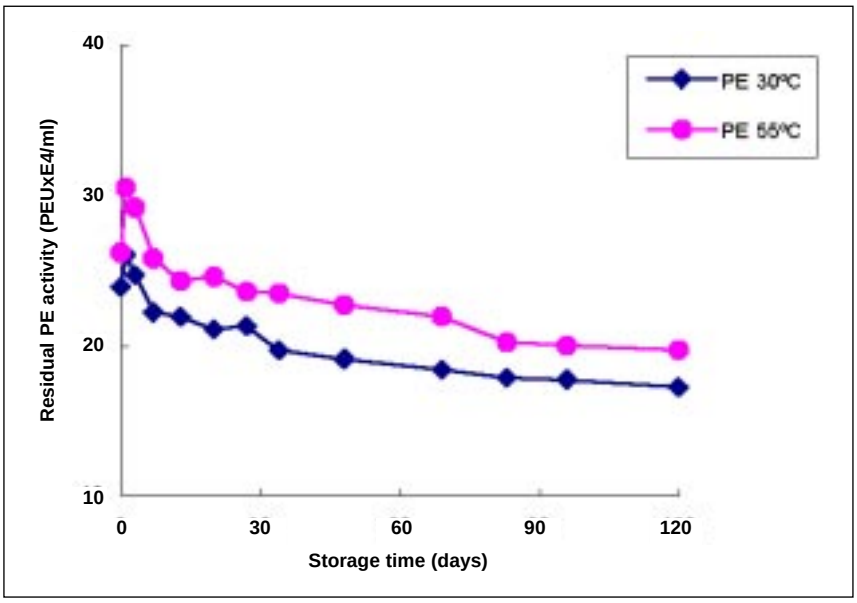

Figure 1 – Effect of frozen storage time on PE activity of orange juice at two different test temperatures (30 °C and 55 °C)

Table 2
PE activity for fresh and frozen stored orange juice (during 120 days) and percent inactivation.

PE activity ($PEUx10^4$/ml)	Fresh	Frozen	% inactivation
at 30 °C	23.9	17.2	28
at 55 °C	26.2	19.7	25

The pH value did not change after freezing and during the frozen storage (table 1). In figure 2, a decrease in Brix degrees of the samples until 15 days of storage can be observed. After this period and until the end of storage time an almost constant value was observed. The °Brix/acid ratio presented a similar behaviour to the °Brix values (figure 2). However, there was not a very significant variation of the juice acidity after freezing and during the frozen storage.

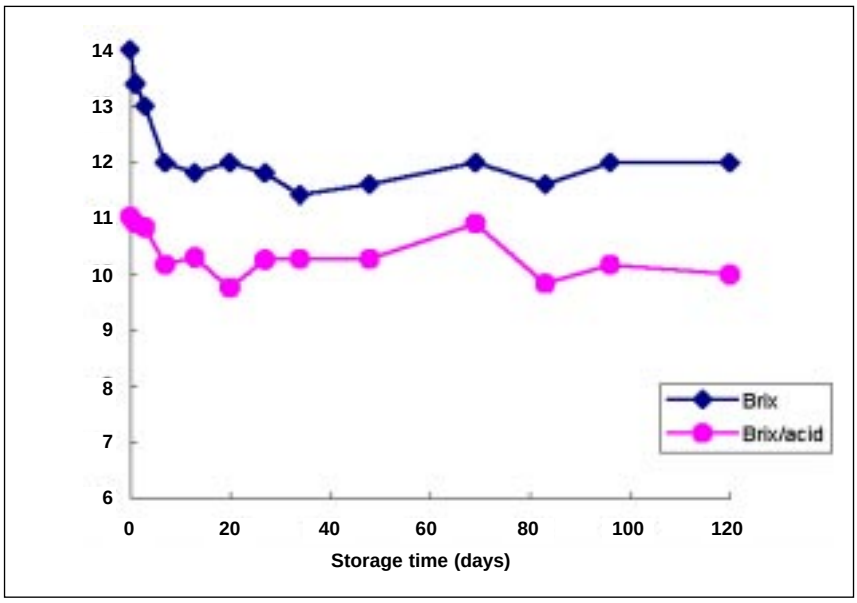

Figure 2 - Effect of frozen storage time on °Brix and °Brix/acid ratio of orange juice

The Total Colour Difference (TCD) is a parameter that quantifies the overall colour difference (Minolta, 1994). The TCD value found between the fresh and frozen samples (during 120 days) was in average 1.6 (table 1). Using the difference classification scale for TCD (table 3) the colour of the fresh and frozen orange juice presents a distinct difference. However, it can be concluded that there is not a significant difference in colour due to the frozen storage.

Table 3
Classification of colour differences (Drlange, 1994).

TCD	Colour Difference Classification
0.0 - 0.2	not perceptible
0.2 - 0.5	very small
0.5 - 1.5	small
1.5 - 3.0	distinct
3.0 - 6.0	very distinct
6.0 - 12.0	great
> 12.0	very great

Using a Chi-square analysis a significant difference was observed, within 99 % confidence, between the sensorial quality of the fresh pasteurised and frozen pasteurised samples for all time intervals analysed. The panel was unanimous in its judgement, the difference between the samples was marked. After two weeks of frozen storage, there was a development of an off-flavour in the samples. The majority of judges identified a bitter taste in the juices. This off-flavour intensified with one month of storage. However, the frozen samples still had a good acceptability. The mean sensory score (figure 3) between the frozen pasteurised and fresh pasteurised juices presented a significant difference, within 95 % confidence interval, at all time intervals.

Although the juice was obtained by a manual process, this off-flavour probably occurred due to the limonin or to an oxidative process. The limonin can be perceptible at 3-6 ppm (Lanzarini and Pifferi, 1989). On the other hand, a small activity of lipoxygenase and the presence of low levels of fatty acid and oxygen are enough to develop off-flavour.

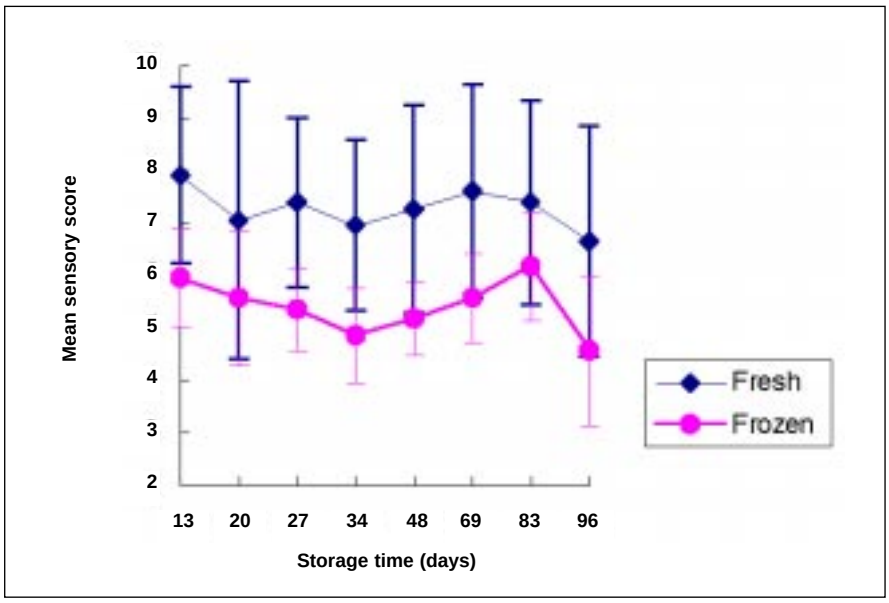

Figure 3 - Mean sensory scores obtained for fresh pasteurised and frozen pasteurised orange juice

Due to the development of this bitter taste the potential use of freezing and frozen storage as pre-treatment before pasteurisation can be compromised.

4. Conclusions

PE inactivation in single strength orange juice obtained by frozen storage was remarkably smaller than obtained from conventional thermal inactivation. The frozen storage inactivated only the thermolabile isoenzyme and it was not reversible after 24 h at 5 °C.

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