FREEZING AND STORAGE OF ORANGE AND ORANGE/MELON JUICES: EFFECTS ON PECTINESTERASE ACTIVITY AND QUALITY

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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 pasteurization process. However, this processing causes also the degradation of quality parameters, such as flavor and color. Therefore, if freezing and frozen storage inactivates part of the PE, this unit operation could be used as a pre-treatment before pasteurization.
The objective of this work is to study the freezing and frozen storage effects on PE activity and quality of fresh pure orange and mixed orange/melon juices.
Samples of fresh orange 'Valencia' and orange/melon 'Cantaloupe' juices (ratio 1:1) were frozen and stored at -20°C during 120 days.
The parameters analyzed along storage were: PE activity, pH, °Brix, acidity, Brix/acid ratio, color, 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 juices. After 120 days of storage, it was verified a reduction on PE activity of approximately 28% and 9% for orange and orange/melon juices, respectively. 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 resistant fraction.
The freezing and frozen storage did not change significantly the pH, Brix, acidity and total color difference (TCD) of the two juices.
The freezing and frozen storage caused the lost of the cloud stability in the orange juice. However, this effect was not observed in the orange/melon mixture.
For the sensory evaluation the juices were thawed and pasteurized (85°C/2min for orange juice and 90°C/2min for orange/melon mixture). The fresh samples used in the sensory tests were also thermally treated during 2 min at 90°C and 95°C for orange and orange/melon juices, respectively. Using a Triangular test it was concluded that there is a significant difference between the samples (within 99% confidence). The Hedonic test showed that there is a greater preference for the fresh samples.

1. INTRODUCTION

The most consumed fruit juice in the world is pure orange juice. However, in the latest years, mixed juices, such as orange/carrot, orange/peach, peach/grape, have been commercialized with a very good success.
Just after squeezed the orange juice has a characteristic very appreciated by the consumer, that is the "cloudy" appearance. However, the pectinesterase (PE) enzyme causes the deesterification of the pectin and hence the cloud is destabilized. A drastic thermal treatment (e.g.: 90°C/2min for orange juice) is necessary to inactivate the heat resistant forms of PE (Amstalden and Montgomery, 1994; Marshall et al., 1985; Seymour et al., 1991a,b; Versteeg et al., 1980). These isoenzymes, thermostable (PE I) and high molecular weight (HMW), are responsible for the cloud loss in orange juice (Rombouts et al., 1982). Due to its low pH value (pH < 4.0) the microorganisms in the orange juice are less thermal resistant than PE enzyme. Thus, the microbiological quality of the juice is warranted (Argáiz and López-Malo, 1995; Eagerman and Rouse, 1976; Irwe and Olson, 1994; Nath and Ranganna, 1977). However, this excessive heat treatment develops "off-flavors" in the juice further causing nutrient losses (Baker and Bruemmer, 1972; Kiefer, 1961; Seymour et al., 1991b).
There is need to find alternative methods that combine maximum retention of natural properties of the fruits together with the inactivation of PE enzyme. Versteeg et al. (1980) recommended, for PE inactivation in orange juice concentrates the storage at -20°C. During frozen storage, Seymour et al. (1991a) also got some PE inactivation in grapefruit juice. Therefore, freezing and frozen storage followed by a less severe thermal treatment could be used to inactivate PE enzyme in orange juice. Hence, the juice quality could be improved because the freezing and frozen storage are less detrimental to the product than other methods (Del Rio and Miller, 1979). However, it is known that these operations do not prevent all changes. At low temperatures the reactions continue to occur at a slower rate and product deterioration can be observed (Ganthavorn and Powers, 1988; Jansen, 1969; Reid, 1990).

The main task of this research is to quantify the effects of freezing and frozen storage on the orange and orange/melon juices PE activity and quality. The final goal is to investigate the potential use of freezing and frozen storage as a pre-treatment before a less severe thermal processing.

2. MATERIALS AND METHODS

2.1. Materials

'Valencia Late' oranges (*Citrus sinensis* L.) and 'Cantaloupe' melon (*Cucumis melo*) grown in Portugal was used to carry out this study. The orange juice was squeezed manually. To obtain the melon juice, the pulp was extracted and smashed with a mixer. To prepare the orange/melon juice 50% of each one were mixed. The juices were filled into polyethylene bags, frozen in a blast freezer (Armfield FT36) at -25°C and stored at -20°C±1°C. At pre-determined time intervals, samples were taken out of the cold store and thawed in a water bath at 25°C. PE activity, pH, Brix, acidity, Brix/acid ratio, color, cloud stability and sensory evaluation, were analyzed.

2.2. Methods

2.2.1. Cloud determination

Just after thawing and after three days stored at 5°C the cloud loss of the juices was measured. Ten ml of sample were centrifugate for 10 min at 360 x g. By measuring the absorbance at 660 nm in a spectrophotometer (Shimadzu UV-1601, Japan) the turbidity was monitored.

2.2.2. Pectinesterase assay

PE activity was determined by the method described by Köner *et al.* (1980). 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 55C. Some modifications, suggested by Amstalden and Montgomery (1994) were introduced.

2.2.3. Color, acidity, Brix and pH measurements

To characterize the color a portable tristimulus colorimeter (Minolta Chroma Meter CR-300) was used. For each time interval the measurements were taken in triplicate. To determine the Total Color Difference (TCD) a Hunter L, a, b tristimulus scale was used.

By titration with NaOH the acidity was determined and expressed as acid citric. To measure the Brix degree, a hand refractometer (Atago) was used. A digital pH meter (Crison model 2002) previously calibrated was used to characterized the juices pH.

2.2.4. Sensory Evaluation

The juices were thawed and pasteurized (85°C/2min for orange juice and 90°C/2min for orange/melon mixture). The fresh samples were also thermally treated during 2 min at 90°C and 95°C for orange and orange/melon juices, respectively. Panelists were trained to participate in the study. The work was carried out in a portable booths, for 8 periods of frozen storage. Judges were asked to taste and indicate which sample was different. Overall liking was evaluated using a 9-point hedonic scale. A Triangular Test was performed to verify whether there was difference between the samples (Askar and Treptow, 1993). Frequency distributions of the difference test were analyzed by Chi-square analysis. Hedonic test results were analyzed by ANOVA. A cut-off value of =0.05 was used for all tests. Data were analyzed with the software Excel (Version 4.0).

3. RESULTS AND DISCUSSION

Figure 1 presents the residual PE activity determined at 30°C and 55°C for orange and orange/melon juices. During frozen storage, the total enzyme activity decreased 28% and 9% in orange and orange/melon juices, respectively. Probably, the reduction in the enzyme activity of the orange juice was greater due its lower pH value (Table 1). This enzyme is more easily inactivated in media with lower pH values (Marshall et al., 1985).

Initially, just after freezing, an increase in PE activity can be verified (Figure 1). This can be explained by the freezing process used in this study, that was not too fast. Hence, the ice crystals formed were bigger. Therefore, this phenomenon may cause cells disruption and liberation of enzymes.

During frozen storage the solute concentration of the unfrozen medium increases. Changes in pH, ionic strength, viscosity, can occur (deMan, 1990; Reid, 1990 and 1993). Thus, several factors can

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be responsible for the PE inactivation, such as low pH that causes protein denaturation (Belitz and Grosch, 1987). Probably, the low pH of the unfrozen part of the juices could have caused the enzyme inactivation.
Two enzyme fractions, PE I and PE II (thermolabile) can be observed. About 90% of the total PE activity comes from the thermolabile PE isoenzyme. The most heat resistant isoenzymes are responsible for the cloud destabilization (Seymour et al., 1991b; Rombouts et al., 1982;Versteeg et al., 1980).

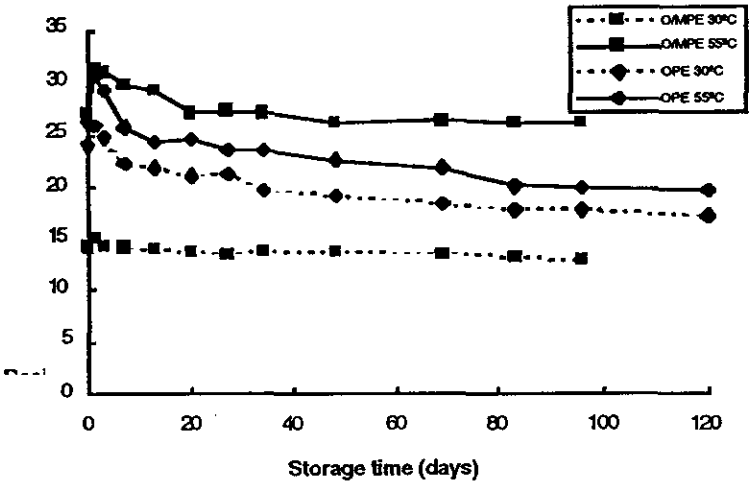

Figure 1: Effect of frozen storage time on PE activity of orange (O) and orange/melon (O/M) juices at two different test temperature (30°C and 55°C).

Immediately after and three days after thawing it was observed the cloud destabilization of the samples. Thus, only the thermolabile isoenzyme was inactivated.
After freezing and during frozen storage, no significant changes in acidity and pH value of both juices were observed (Table 1).

Table 1: Characteristics of fresh and frozen orange (during 120 days) and orange/melon (during 96 days) juice.

Parameter	Orange Juice		Orange/Melon Juice	
	Fresh juice	Frozen juice	Fresh juice	Frozen juice
pH	3.54	3.50	4.00	4.00
Brix	14	12	9	8.8
Brix/acid[a]	11.02	10.00	10.3	11.3
Acidity (%)	1.27	1.20	0.87	0.78
PE activity (PEUx10^4/ml)	23.9[b]	17.2[b]	14.1[b]	12.9[b]
	26.2[c]	19.7[c]	26.8[c]	26.1[c]

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

In Figure 2, it can be observed that a decrease in Brix values of the orange juice samples occurred until 15 days of storage. On the other hand, the orange/melon juice Brix values did not have a significant change. An almost constant Brix after this period and until the end of the storage was observed. A similar behavior between Brix values and brix/acid ratio also can be observed (Figure 2).

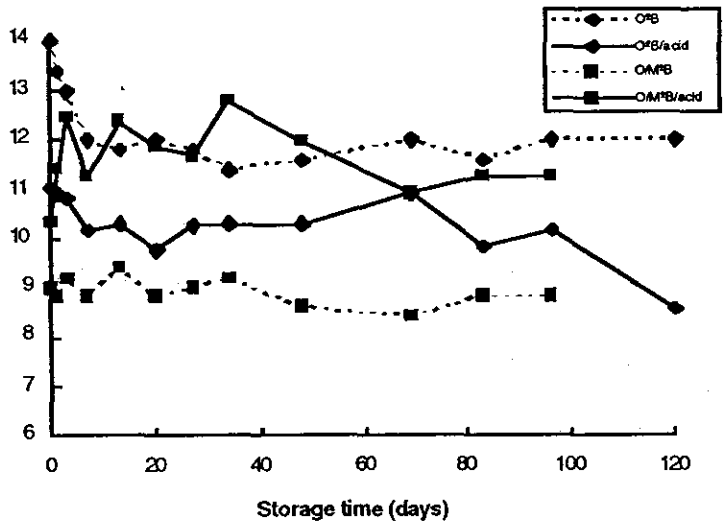

Figure 2: Effect of frozen storage time on Brix and Brix/acid ratio of orange (O) and orange/melon (O/M) juices

TCD value for both juices did not present a distinct difference between fresh and frozen samples. Using a Chi-square analysis a significant difference within 99% confidence, between the sensorial quality of the fresh and frozen samples for both juices was observed. After two weeks of frozen storage, there was a development of an "off-flavor" in the samples, mainly in the orange juice. This undesirable taste intensified with one month of storage. However, the frozen samples still had a good acceptability. Although the juice had been obtained by a hand process, this "off-flavor" probably occurred due to the limonin or to an oxidative process. Three to six ppm of limonin can be perceptible (Lanzarini and Pifferi, 1989). On the other hand, lipoxygenase is a potential deteriorative factor even at low temperature and very low residual activity (Baardseth, 1978, Kermasha and Metch, 1986; Munoz-Delgado, 1977).
Due to the development of this bitter taste the potential use of freezing and frozen storage as pre-treatment before pasteurization of orange and orange/melon juices can be compromised.

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