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Effects of thermal treatments on pectinesterase activity determined in blood oranges juices

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Abstract

The citrus processing industry aims to maintain turbidity and attractive colour of the juice obtained from Sicilian blood oranges. Nevertheless, the presence of pectinesterase (PE, E.C. 3.1.1.11) causes the loss of these peculiar characteristics, due to precipitation of colloids and very fine pieces of pulp in suspension, with negative effects on colour and clarification of the juices. This study compares total PE activity of Sicilian blood oranges (*Sanguinello*, *Moro*, *Tarocco*) with the blonde cultivar *Navel*, checking enzyme stability with various pasteurisation times and temperatures conditions. Decimal reduction time and temperature (*D* and *z*) as well as the kinetic constant (*k*) were established to optimise and increase the shelf-life of the pasteurised juice. Finally, a heat treatment (85 °C × 3 min) of both microbiological and enzymatic efficacy has been developed that does not compromise anthocyanin stability; this treatment could be used by the citrus fruit processing industry as a valid alternative in the production of blood orange juices.

Keywords: Pectinesterase; Sicilian blood oranges; Pasteurisation

1. Introduction

The sweet oranges (*Citrus sinensis* L.) are classified in accordance with the colour of the pulp as either blonde or blood (or pigmented) oranges. The latter are commonly grown in the Mediterranean Basin, but for the area's special pedoclimatic characteristics, it is Sicilian ones that express their particular qualities to the best effect [1–3]. They are oranges with high aroma profile and antioxidant activity, but the most striking feature is the red colour, of varying intensity and prevalence, caused by the anthocyanins, found in the flavedo or juice vesicles, mainly consisting of cyanidin-3-glucoside [2,4]. The intensity of the colouring depends on the cultivars, but also on the ripeness and climatic conditions, in particular a wide daily temperature range in the months October–November is essential for producing the distinctive red colour. Sicilian blood oranges account for around 60% of the Italian orange harvest. The most common in descending order are *Tarocco*, *Moro* and *Sanguinello* [3].

In citrus fruits, pectic substances are amongst the most abundant, being found above all in the albedo and flavedo, but also in the pulp tissues and so-called "cloudy" juice [1].

In the production of the juices the presence of pectinolytic enzymes plays a remarkable role in their commercial quality. The pectinases catalyze the degradation of pectic polymers in plant cell walls; depolymerization of pectins is generally associated with the process of fruit ripening [5]. Particularly, pectin esterase (PE, E.C. 3.1.1.11) is the most important enzyme involved with citrus fruit containing around 12 isoenzymes can be distinguished by their expression patterns and by their physical and biochemical properties [6,7].

PE is a deesterification enzyme [5] produced by higher plants, fungi and some bacteria and yeasts. It must start from the reducing end of the molecule [6] with release of methanol and mono and polygalacturonic acids that react with calcium ions by precipitating under the form of pectinates, thus

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causing spontaneous clarification [8–13]. In the case of turbid juices such as those of blood oranges, in order to maintain turbidity for as long as possible, it is necessary to keep the juice frozen or inactivate PE with heat treatment [2].

Deesterification is never fully completed due to competitive inhibition of the product formed, the pectate, and lowering of the degree of esterification that must in fact exceed 10% before a fall in enzyme activity will be observed [5]. PE is inhibited by high sugar concentrations; therefore in concentrated juices activity will be restored after reconstituting with water. The juice of ripe fruits shows slightly higher PE activity in blood than blonde cultivars, whilst grapefruit juice shows greater activity than orange juice [6].

In this study, following a comparison of PE activity between blood and *Navel* oranges (the most common blonde cultivar) using the previously method of determination [11], a check was made on enzyme stability under various pasteurisation conditions in order to optimise the heat treatment and increase the shelf-life of the pasteurised juice through the determination the decimal reductions times and temperatures (*D* and *z*) as well as the kinetic constant (*k*) of degradation of PE.

2. Materials ad methods

2.1. Pulp and juice extraction

Twenty kilograms of the orange cultivars *Tarocco*, *Moro*, *Sanguinello* and *Navel* (Azienda Vasile & Co., Lentini, Italy) were immersed for 10 min in chlorate solution at a concentration of 200 ppm and squeezed under sterile conditions using a household juicer (Moulinex, Milan, Italy) and the cloudy juice obtained was then centrifuged at $2000 \times g \times 10$ min at 6.0 °C in order to separate the pulp from the juice. The products obtained were stored at −50 °C [11].

2.2. Pectin esterification

Seventy-five grams of apple pectin (degree of esterification 72–75%) were washed, in order, with 60% acid ethanol (following the addition of concentrated HCl), 60% ethanol, 96% ethanol and lastly pure methanol in order to remove the polyhydric phenols and simple sugars. The pectin was added to 1 L of anhydrous methanol in 1 M H_2SO_4 at 3.0 °C × 4 weeks, stirring at regular intervals. The product was then filtered and washed, in order, with pure methanol, 60% ethanol and 96% ethanol. Finally it was dried at 55 °C until constant weight was achieved [11].

2.3. Total PE extraction and assay

Seventy-five grams of juice of the four cultivars were taken to pH 7.0 using 1N NaOH. The solution was placed at 4.0 °C for 2 h, and centrifuged at $4000 \times g$ for 10 min, then ultrafiltered using a membrane with 10 kD cut-off (Millipore, Bedford, MA, USA). Twenty-five grams of pulp of the four cultivars were added to 100 mL of a solution of 0.2 M citric-phosphate (C-P) buffer at pH 7.0, 1 M NaCl, 1 mM DTT. Each of these mixtures was homogenised for 2 h at 4.0 °C, centrifuged at $4000 \times g \times 10$ min, filtered (589^2, Schleicher & Schuell, Dassel, Germany) and ultrafiltered. The samples obtained were stored in test tubes at a temperature of −20 °C [11].

The assay was conducted according to the method previously devised [11]. Two millilitres of 0.6% apple pectin in 0.05 M C-P buffer, pH 3.6 and 0.5 mL of the previously extracted solution were introduced into 10 mL test tubes, stirred continuously in a bath at 20 ± 1 °C; the reaction was stopped using 0.5 mL of 1N H_2SO_4. The same procedure as already described was followed with an aliquot of 0.2 mL. The blank consisted of a mixture of the same reagents, but added in reverse order. The enzyme activity, expressed in enzyme units per gram of edible part, was calculated in accordance with the formula, U/g edible fraction = $(\text{Abs} \times V_1 \times V_3)/(\gamma \times \text{PM} \times t \times V_2 \times w)$ where Abs is the absorbance; γ the gradient ($M^{-1}\,cm^{-1}$); PM the methanol molecular weight; t the reaction time (min), V_1 the assay final volume (mL); V_2 the enzyme volume (mL); V_3 the extracted solution final volume (mL); w the initial weight of edible part (g). The solvents and reagents not expressly specified had a high degree of purity (RPE) and were supplied by Carlo Erba (Rodano, Italy). Each analysis was carried out in triplicate. The variability was ±0.5% (with a 95% confidence interval).

2.4. Effect of pasteurisation on the extracts

Eppendorf tubes with 100 μL of pulp or juice from the extracted samples were incubated for varying periods of time at 70–85 ± 0.5 °C. After the heat treatment the sample were immediately frozen in liquid nitrogen. The activity was determined in accordance with the method previously described [11].

2.5. Stability determination

Eppendorf tubes with 100 μL of pulp or juice from the extracted samples were incubated, after the heat treatment and immediately frozen in liquid nitrogen, for varying temperatures of 4, 15 or 25 ± 0.5 °C for periods ranging from 10 min to 50 days. The PE activity was determined in accordance with the method previously described [11]. Anthocyanins in blood oranges were determined according to Rapisarda et al. [4].

3. Results and discussion

The cultivars used, *Navel*, *Sanguinello*, *Moro* and *Tarocco*, showed different yields of juice and pulp (Table 1); in particular, compared to the other, *Sanguinello* and *Navel* had the