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Enzymatic Peeling of Citrus Fruits: Factors Affecting Degradation of the Albedo

María Teresa Pretel[1*] • Paloma Sanchez-Bel[2] • Isabel Egea[2] • Félix Romojaro[2]

[1] EPSO, Departamento de Biología Aplicada. Universidad Miguel Hernández, Carretera de Beniel Km 3.2, 03312 Orihuela, Alicante, Spain
[2] CEBAS, Departamento de Biología del Estrés y Patología. Consejo Superior de Investigaciones Científicas, Campus de Espinardo, 30080 Murcia, Spain

Corresponding author: * **mteresa.pretel@umh.es**

ABSTRACT

The environmental problems caused by chemical peeling of citrus, together with the establishment of new applications for enzymatic preparations with degradative activities led several investigators to study enzymatic peeling as an alternative to traditional chemical peeling. The principle of enzymatic peeling is based on the digestion, through an enzymatic preparation, of the pectic substances existing in the cell wall of plants. The efficiency rate of enzymatic peeling is influenced by the morphological characteristics of citrus fruits, the correct vacuum application and the incubation time, as well as by the type and concentration of enzymatic solution, and temperature, among others. For the enzymatic peeling of citrus, it is important to take into account the ripening stage of the fruits, since variations this parameter modify the concentrations of enzymatic preparation necessary for the peeling process and, probably, the optimum conditions of vacuum. Optimum conditions for enzymatic peeling may not depend on the composition of the albedo in different citrus species. A temperature range between 35 and 40°C, besides being within the optimum range of peeling, would become economically profitable for the industry since a minimum addition of energy is required. For enzymatic peeling the pH range could be wider, between 3.5 and 4.5. The optimum concentration for obtaining a quality product with Peelzym® II is 1 ml L^{-1}, but depends on the composition of the enzymatic solution. The aim of this paper is to update information about the most important parameters implied in enzymatic peeling of citrus fruits, especially factors affecting degradation of the albedo.

Keywords: *Citrus*, enzyme concentration, fluidity, pH, ripening, temperature

CONTENTS

INTRODUCTION

The need for innovation in the agri-food sector has increased over the last years as a consequence, essentially, of the changes in demand and in a rise in the standard of living, higher requirements from the consumer concerning the nutrition and health quality and the preferential situation facing competitors. It is worth realizing that innovation in products can be achieved at different levels, ranging from making a new food to the improvement of a previously existing one, through the application of a new process, or even through changes made in the design of the packaging.

Although the capacity for innovation in the food manufacturing industry depends on many factors, the most outstanding factors concern the innovative capacity of business men and both the company and the public sector's suitable level of I+D. These two factors have not yet reached the required critical level in countries producing fruits and vegetables, becoming the main causes for stagnation in the production process and loss of significant market shares (Mermelstein 2002).

About 65% of total production of citrus fruits is destined to the fresh consumption whereas the 35% remaining is used for in industrial processesing. Peeling fruits is one of the necessary steps in the manufacture of some of these processed products. Traditional industrial processes for peeling citrus fruits consist of manual or mechanical skin removal and further chemical degradation of both the albedo remains and segments' membranes. These methods incur a high cost in labour force, require large amounts of water for the washing stages and cause serious damage to the environment due to the use of caustic agents in the peeling process. Moreover, in the manufactured product strange tastes appear due to the aggressive chemical treatment that the product undergoes (Ben-Shalom *et al.* 1986; Coll 1996). This sector is currently facing the need to develop technological innovations which improve the current manufacturing processes, thus allowing a higher range of manufactured products, the improvement of quality and the reduction of both large quantities of water used and generated wastes. In view of these circumstances it is quite interesting to improve an already existing process (the chemical peeling) and to adjust system improvements to the new products (McArdle and Culver 1994; Baker and Wicker 1996).

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Invited Review

On the other hand, the cell wall of plants have important functions in the different tissues that they are part of. This component of the cell affect in an essential way, the behaviour of the fruits and vegetables, either during manipulation, when they are still fresh, or during various processes of industrial manufacturing that the plant material undergoes (Suutarinen *et al.* 2003). The enzymatic modification of the cell wall offers wide possibilities for the development of innovative processes and products. During the last decades, the food industry has developed applications for the enzymatic preparations with degradative activities on the structural polysaccharides in cell wall to modify the smell, texture, look or to preserve the capacity of food. Throughout the enzymatic degradation of the cell wall of plants small structural changes in the polysaccharides that they are made of can alter considerably the properties of the wall (Ros *et al.* 1996). To carry out this process the combination of a large amount of enzymatic activities such as polygalacturonase (EC 3.2.1.15), pectinlyase (EC 4.2.2.10), pectinesterase (EC 3.1.1.11), cellulase (EC 3.2.1.4) is needed. The selection and combination of these activities depends on the kind of tissue that is intended to be degraded. The commercial pectolytic preparations used in the food industry for the processing of fruits and vegetables are heterogeneous mixtures of pectinases, hemicellulases (EC 3.2.1.8) and cellulases. Furthermore, the activity of these enzymes in the degradation of the cell wall is particularly influenced by temperature and pH (Ben-Shalon *et al.* 1986; Pretel *et al.* 1997; Prakash *et al.* 2001; Pagan *et al.* 2005).

The environmental problems caused by the chemical peeling of citrus, together with the establishment of new applications for enzymatic preparations with degradative activities have led several investigators to study enzymatic peeling as an alternative to traditional chemical peeling.

ENZYMATIC PEELING OF CITRUS FRUITS

The principle of enzymatic peeeling is based on the digestion, through an enzymatic preparation, of the pectic substances existing in the cell wall of plants (Bruemmer *et al.* 1978; Berry *et al.* 1988). From a molecular point of view, pectin, cellulose and hemicellulose are the polysaccharides responsible for the adherence of the peel to the fruit (Whitaker 1984). Therefore, both pectinases and cellulases are necessary for enzymatic peeling. Cellulases are probably necessary for the release of pectins in the albedo and pectinases complete the process by contributing to the hydrolysis of polysaccharides making up the cell wall (Ben-Shalom *et al.* 1986; Rohuana and Mannheim 1994; Soffer and Mannheim 1994; Coll 1996). Later it was proved that the most important enzymatic activity leading to degradation of the albedo is the activity of polygalacturonase (Pretel *et al.* 2005). Bruemmer *et al.* in 1978 were the first to use this enzymatic method in the peeling process of grapefruit, by means of vacuum infusion of commercial pectolytic preparations. The reported that the sections of the fruit maintained their original taste and texture, with higher output and quality than those obtained through conventional peeling procedures (steam-peeled, lye-finished grapefruit, taken directly from the sectionizing table and sections were cut from the fruit segments). These same authors also observed that commercial pectinases differed greatly in their efficiency of peeling. Thus, Berry *et al.* (1988), using the method developed by Bruemmer *et al.* (1978), proved that less juice is lost in the segments of grapefruit and in whole-peeled grapefruits than in those obtained by conventional methods. To better understand the enzymatic degradation processes, Ben-Shalom *et al.* (1986) in studies of commercial pectolytic enzymes (C-80®, Pectinex® 3x and Ultrazyme® 100) with grapefruit membranes, clarified the importance of the evaluation of different commercial enzymes on the substrates that are intended for degradation (pectin, albedo, carpelar membrane, segments, etc.), and other authors confirmed this later with fourteen commercial preparations on of grapefruit (Rouhana and Mannheim 1994), five commercial preparations of orange and grapefruit (Soffer and Mannheim 1994) and four commercial preparations of citrus pectin, polygalacturonic acid, carboximethylcellulose, albedo and carpelar membrane of citrus fruit (Pretel *et al.* 2005).

In addition to the kind of enzymatic preparation used, there are numerous parameters that affect the quality of the finished product and successful peeling. Among them, the morphologic characteristics of fruits, such as skin adherence and its thickness and the unity rate between segments, are important factors which determine enzymatic peeling efficiency (McArdle and Culver 1994; Pretel *et al.* 1997, 2001). Citrus are very suitable fruits for enzymatic peeling because the mesocarp or albedo is an extremely porous tissue; thus when a vacuum is applied, the air in the intercellular spaces is replaced by the enzymatic solution. Nevertheless, the porosity of the citrus albedo varies with the species and the variety. Therefore, is an important parameter to take into account when a suitable vacuum is selected, since the vacuum should be more intense when the albedo is not very porous (McArdle and Culver 1994; Pretel *et al.* 2005). Likewise, the capacity of the fruit skin to absorb the enzyme solution depends on peel thickness (Toker and Bayindirli 2003; Pretel *et al.* 2007a, 2007b). However, not all citrus fruits are amendable to obtain segments through enzymatic peeling, since some morphological characteristics such as the compression of the albedo or the fragility of the segment peel could hinder this process. If only the entire peeled citrus is needed, the most important parameter to consider is the adherence rate of the albedo to the segments. Other critical parameters include the presence of the navel, the adherence between segments and the homogeneity of the membrane (Pretel *et al.* 1997, 2007a, 2007b).

The effect of scalding prior to enzymatic peeling has also been studied by some authors. Rouhana and Mannheim (1994) examined the effect in grapefruit and Soffer and Mannheim (1994) worked with 'Valencia' orange and grapefruit. Botth groups reported that scalding at 100°C from 2 to 4 min, depending on the thickness of the fruits' skin, is a necessary step prior to enzymatic peeling. Increasing the scalding time improved the efficiency of enzymatic peeling and decreased the time taken. The probable explanations for this result are that heat treatment decreased the viscosity of pectin, changed the crystalline structure of cellulose to an amorphic structure (Alberts *et al.* 1989), and improved the ability of the peel to absorb the enzyme solution. As a result, the peel components were readily digested by the enzymes. On the other hand, too long a scalding time resulted in decreased quality of the final product (Rouhana and Mannheim 1994). Javeri *et al.* (1991) also carried out scalding of 3 min at 100°C before infiltrating a pectinmethylesterase solution in peaches to study the increase in firmness in fruits. Some authors, however, consider sufficient a treatment of the fruits in a hot water bath, independent of the period of time, until the albedo reaches 40°C prior to peeling (Pretel *et al.* 1997, 1998a, 2007a, 2007b), while others start the peeling process directly, without heating the fruits (Pao and Petracek 1997; Liu *et al.* 2004; Pagán *et al.* 2005). The treatment of fruits by hot water dipping shows the best results in order to obtain a good enzymatic peeling. In most cases, fruits are placed into chlorinated water (300 ppm) prior to transfer to the hot water bath, to reduce possible microbial contamination of the finished product (Pretel *et al.* 1997, 1998b, 2007a, 2007b) or, as Pinnavaia *et al.* (2006), suggested fruits are pretreated with an acid solution (0.1 N HCl).

For the enzymatic solution to penetrate the interior of the albedo and among the segments, it is necessary to make cuts in the flavedo of the fruits before creating a vacuum (Bruemmer *et al.* 1978). Soffer and Mannheim (1994) scored orange peels with at least four radial lines, while Bruemmer *et al.* (1978) hand-scored the peel of grapefruits in quadrants. Pretel *et al.* (1997) found that cuts recommended by these authors (Bruemmer *et al.* 1978; Soffer and Mannheim 1994) did not result in a good peeling process since areas of undegraded albedo remained and it was dif-

ficult to separate the segments. Presumably the furthest albedo from the cuts had not been saturated with the enzymatic solution. To obtain whole oranges, the best results were obtained with three transversal cuts made in the calycinal, peduncular and equatorial zones of the fruit, and two longitudinal cuts. Using this cut pattern, there was minimal difficulty in separating the remnants of the rind, as the enzyme solution spread easily through the albedo without penetrating the segments. However, when orange segments were needed, the best results were obtained when the transversal cuts near the calycinal and peduncular zones were substituted by removal of the peel in these zones. Under these conditions, the peel remnants were easily removed and the solution penetrated between several segments of the orange (Pretel *et al.* 1997). In subsequent studies, Pretel *et al.* (1998a) examined different cut patterns in the flavedo for the enzymatic peeling of mandarin by analysing characteristics of enzymatic saturation at different pressures and times of vacuum. The most suitable pattern that allowed for mandarin segments was a cut in the equatorial zone, the removal of part of the peel of the calycinal and peduncular zones and two longitudinal cuts. This cut design, with the application of vacuum conditions permitted between 85 and 95% of enzymatic saturation, and allowed for a homogeneous distribution of the enzymatic solution in the interior of the albedo, favouring easy separation of remains of the residual peel. In addition, removing a portion of the skin from the peduncular and calycinal zones allowed the entrance of the solution into the fruit, assisting the passage of enzymatic solution between the various segments, with a subsequent increase in efficiency of segment separation (Pretel *et al.* 1998a). In following studies (Pretel *et al.* 2007a, 2007b), the entire fruit surface was homogeneously perforated by rolling the oranges over a 1 m^2 wood plate to which cylindrical metal projections 5 mm long and 1 mm in diameter, were attached. The distance between each cylindrical metal projection was 10 mm. With this system, 8 ± 3 perforations per cm^2 in the albedo fruit were produced. This method, which favoured the penetration of the solution into the interior of the albedo, could be easily be adapted for industrial peeling than those used in earlier studies (Pretel *et al.* 1997, 1998a, 2007a, 2007b).

The suitable application of a vacuum after perforation of the flavedo is basic in the process of enzymatic peeling (Baker and Wicker 1996; Pretel *et al.* 1997; Prakash *et al.* 2001; Pagán *et al*. 2006; Pretel *et al.* 2007a, 2007b). This allows the penetration of the enzymatic solution under the fruit skin when entire peeled fruits are required and also the penetration of the enzymes within the segments to obtain peeled segments. However, to subject some porous tissues, such as citrus albedo, to sudden excessive pressure can cause irreversible tissue collapse, preventing the entry of the enzyme solution (McArdle and Culver 1994). In addition, low vacuum pressures may not be enough to obtain good peeling efficacy (Pretel *et al.* 1997). In spite of the importance of vacuum pressure for enzymatic peeling of citrus fruits at the industrial level, only few studies have been carried out. Most authors do not measure the real vacuum pressure applied (Rohuana and Mannheim 1994; Soffer and Mannheim 1994), while others (Prakash *et al.* 2001) apply 93 kPa for 0.5-4 min. Using grapefruit, Bruemmer *et al.* (1978), considered the absorption of 100 ml of solution in the albedo as a parameter to indicate peeling efficacy.

Furthermore, the effectiveness of a determined vacuum pressure in allowing the entrance of the enzymatic solution towards the internal fruit tissues depends on the morphological characteristics of each variety (McArdle and Culver 1994). In addition, the type of final product required needs to be taken into consideration. Thus, if the aim is to obtain peeled segments the vacuum conditions should allow for the saturation of the albedo by the enzymatic solution, and moreover, its penetration between the segments (Baker and Bruemmer 1989; Baker and Wicker 1996). However, if the aim is to obtain a whole peeled fruit for fresh consumption or for canning, the enzymatic solution should not penetrate between the segments, because the residual enzyme could result in alterations during preservation, such as undesired flavour and the destruction of juice vesicles (Pretel *et al.* 1997).

Another important parameter affecting quality of the final product is the incubation time with the enzymatic solution at atmospheric pressure after vacuum application (Soffer and Mannheim 1994; Pretel *et al.* 1998a; Prakash *et al.* 2001). An incubation time above the optimum could cause the degradation of the juice vesicles from the surface and the softening of the segments (Pretel *et al.* 1997). Given their importance, there have been several studies dealing with the incubation time of fruits with an enzymatic solution (Soffer and Mannheim 1994; Prakash *et al*. 2001; Liu *et al*. 2004). For instance, Pretel *et al.* (2007) ascertained that 40 min was optimum to obtain entire fruits the 'Thomson' variety enzymatically peeled with Peelzym® II (1 ml L^{-1}) a 40°C, while 30 min was optimal to obtain peeled segments form 'Mollar' and 'Sangrina' varieties with Peelzym® II under the same conditions (Pretel *et al.* 2007a, 2007b). However, Prakash *et al.* (2001) found that incubation time beyond 30 min. damaged the texture of Indian grapefruit when different enzymatic solutions were tested. On the other hand, Soffer and Manheim (1994) found that the optimum incubation time varies according to the species, the enzymatic solution used and its concentration.

Thus, the degree of effectiveness of enzymatic peeling is influenced by the morphological characteristics of citrus fruits, the correct vacuum application and the incubation time, as well as by the type and concentration of enzymatic solution, the temperature (Bruemmer *et al.* 1978; Berry *et al.* 1988; Baker and Bruemmer 1989; McArdle and Culver 1994; Soffer and Manheim 1994; Coll 1996; Pretel *et al.* 1997).

In addition to all the factors directly concerning enzymatic peeling, other indirect factors such as the ripening stage can influence the ease of enzymatic peeling of citrus. Throughout the ripening process a series of irreversible physical, biochemical and physiological changes are brought about in the fruits, which included breathing intensity, colour changes, increase in sugar content and decrease in firmness (Serrano *et al.* 2005). Among these changes, the enzymatic degradation of the glucidic polymers increases, in particular the pectin and hemicellulose substances, weakening the cell wall and their disposition (Brady 1987; John and Dey 1986). More importantly, the behaviour of the enzymes during the degradation of the cell walls is influenced by the physical and chemical properties of the plants, which can vary from one species to another, or even within the same variety, during the ripening process (Coll 1996; Ismail *et al.* 2005). In spite of its importance, there are not enough studies to date that analyze the relationship between the ripening condition of fruits, particularly citrus species with enzymatic peeling and more specifically with the degradation of the albedo.

In recent years, changes in people's socioeconomic habits have favoured the appearance in markets of minimally processed or ready-to-eat vegetable products. Under the term ready-to-eat are gathered fresh, washed and cut fruits and vegetables ready to be consumed. Usually these products are prepared in plastic film bags and preserved at temperatures lower than 10°C, and have a useful life ranging from 7 to 10 days. The minimally processed products of the salad type have been very intensely studied; however, the ready-to-eat products manufactured from fruits are currently little developed, despite the wide range of possibilities (Suutarinen *et al.* 2003). Citrus show suitable conditions for manufacture as ready-to-eat products, because besides being non-climacteric fruits with a long preservation period, their segments with radial disposition allow for fruit to be cut, without changing the integrity of its vesicle structure. Loss of liquid as a consequence of cellular desorganisation is practically non-existent. However, the removal of the natural protection given by both the flavedo and the albedo implies increased the risk of physiological changes, such as,

excessive dessication, accelerated aging and anaerobic metabolism; not to forget that peeled citrics are potentially sensitive products to microbial attacks (Terry and Overcast 1976; Pretel *et al.* 1998b).

Obtaining enzymatically peeled citrus could be a main option in the development of new food and in its processing in the agri-food industry. It is considered that enzymatic peeling methods can be applied to a wide range of fruits and vegetables (Roe and Bruemmer 1976; Bruemmer *et al.* 1978; Berry *et al.* 1988; Coll 1996; Pretel *et al.* 1997). With enzymatic peeling methods, the marketing possibilities of these products could be remarkably expanded in order to face the demand for innovation in the world-wide market, either as minimally processed products or to replace traditional processes. The improvement of these biotechnological processes that allow the possibility of achieving both higher quality manufactured products and brand new ones, depends mainly on a more thorough knowledge of the materials to degrade, together with the development of technological applications that optimize its use. Thus, we are going to expose the results of some recent studies carried out by our work group, which can add valuable information for the improvement of enzymatic peeling process of citrus. Among them, the influence of the ripening stage and those of species, temperature, pH and enzymes' concentration on enzymatic degradation of the albedo.

ENZYMATIC DEGRADATION OF ALBEDO DEPENDS ON THE RIPENING STAGE OF THE FRUITS

To determine the influence of the ripening stage on enzymatic degradation of the albedo, six of the most representative species of citrus were selected: *Citrus maxima* (Burm.) Merrill etnovar 'Cimboa' (**Fig. 1**), *Citrus limon* (L.) Burm etnovar. 'Fino' (**Fig. 2**), *Citrus sinensis* (L.) Osbeck etnovar. 'Blanca' (**Fig. 3**), *Citrus limettoides* Tanaka 'Lima dulce' (**Fig. 4**), *Citrus reticulata* 'Blanco' subsp. *Deliciosa* (Ten.) D. Riveraand cols. etnovar. 'Mandarina del terreno' (**Fig. 5**) and Citrus *x paradisi* Macfad. in Hook. etnovar. 'Pomelo' (**Fig. 6**) (Rivera *et al.* 1998), in different stages of ripeness, although always within commercial period (between 8.5 and 15.6 °Brix). Fluidity was assessed on lyophilised albedo according to Levinson and Reese (1960) and Pretel *et al*. (2005) when the enzymatic solution was added (Peelzym® II). Soluble solids were determined on the endocarp.

Soluble solids are a good indicative parameter of the ripening stage in fruits (Artés 2000; Pretel *et al*. 1998b). Therefore, using this parameter, we attempted to establish a correlation between the ripening stage of fruits from different species and degradation facility in the albedo, since optimum conditions for enzymatic peeling could probably vary depending on the ripening stage of the fruits. On the other hand, the increase in fluidity of a solution was directly related to the decrease in its viscosity, caused by the degradation of huge polymers in smaller molecules when an enzymatic mixture is added. Thus, the lower the viscosity, the higher the fluidity.

When the levels of soluble solids were increased, within the range of ripeness studied, there was an increase in fluidity. This principle was fulfilled for every species of citrus examined (**Fig. 7**). However, differences among the species can were apparent. *Citrus x paradisi*, *Citrus sinensis* and *Citrus reticulata*, showed greater modifications of fluidity, as the °Brix increased, whereas in the remaining species (*Citrus limon*, *Citrus limettoides* and *Citrus maxima*) the increase in degradation ease of the albedo that depended on their ripening stage was less obvious. In support of these results, when the content of soluble solids from different species increased, the time taken for the solution to reach its maximum fluidity diminished (**Fig. 8**), with a good correlation between them (r^2=0.74). That is to say, in general terms, with the progression of the ripening of the fruits, the ease of enzymatic degradation in the albedo is favoured. These results allow us to conclude that, during the enzymatic peeling of citrus, it is important to take into account the ripening stage of the fruits, since variations of this parameter will

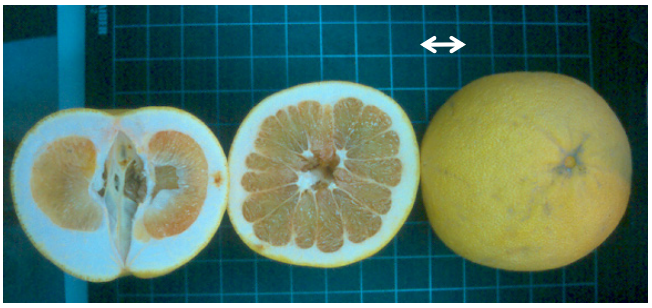

Fig. 1 *Citrus maxima* 'Cimboa'. (↔: 3 cm)

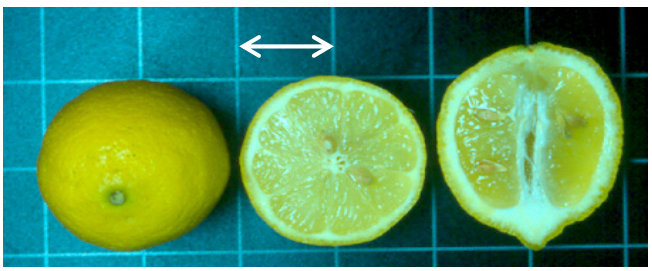

Fig. 2 *Citrus limon* 'Fino'. (↔: 3 cm)

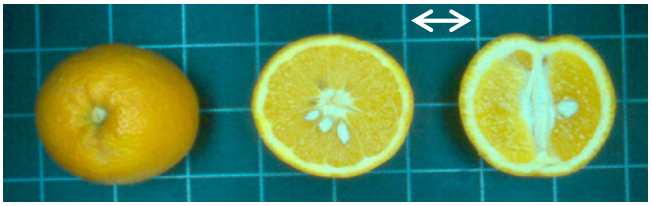

Fig. 3 *Citrus sinensis* 'Blanca'. (↔: 3 cm)

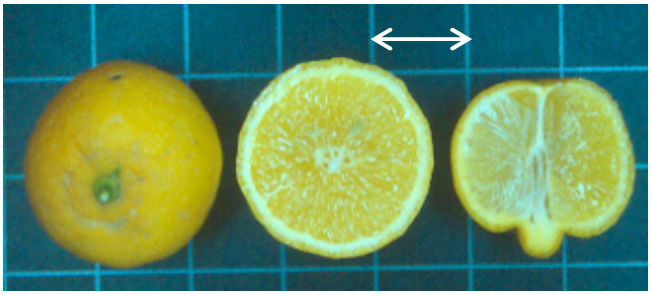

Fig. 4 *Citrus limettoides* 'Lima dulce'. (↔: 3 cm)

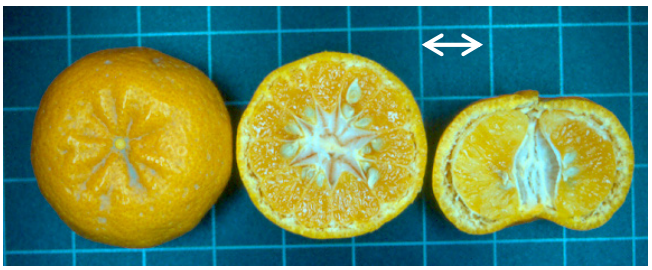

Fig. 5 *Citrus reticulata* 'Mandarina del terreno'. (↔: 3 cm)

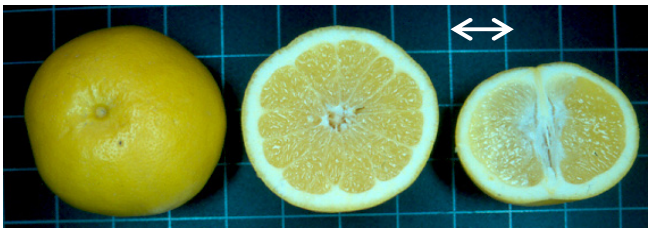

Fig. 6 Citrus *x paradisi* 'Pomelo'. (↔: 3 cm)

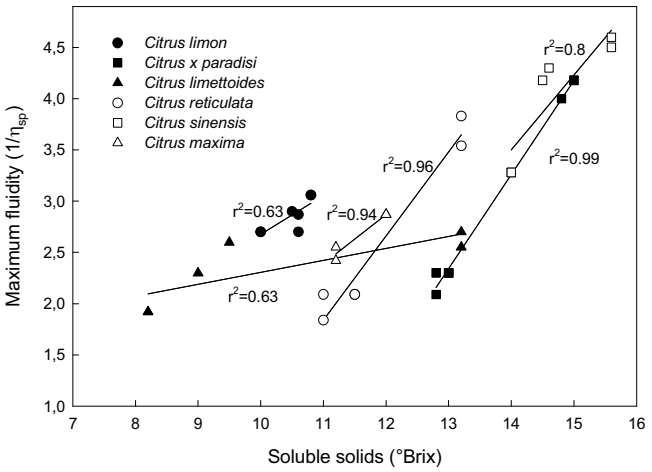

Fig. 7 Relationship between soluble solids (°Brix) measured in endocarp and maximum fluidity (1/ηsp) measured in a 20 mg/mL solution of albedo and 4 µL of Peelzym® II (1 ml L^{-1}) prepared in 0.1 M sodium acetate buffer at pH 4.0.

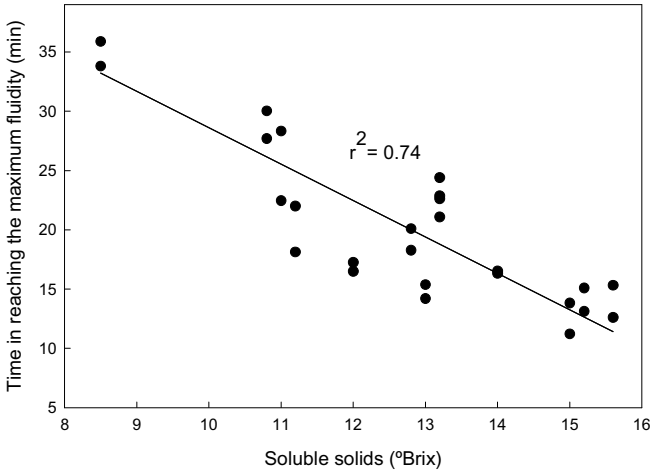

Fig. 8 Relationship between the time taken by an albedo solution (20 mg mL^{-1}) from different species of citrus and 4 µL of Peelzym® II (1 ml L^{-1}) prepared in 0.1 M sodium acetate buffer at pH 4.0 to reach maximum fluidity (min) and its content in soluble solids (°Brix).

probably modify both the concentrations of enzymatic preparation necessary for the peeling process and the optimum vacuum conditions. In relation to these results, Ismail *et al.* (2005) found a great variability in the efficiency of peeling in 'Valencia' orange, depending on the season in which the fruits were harvested. This efficiency became higher as the date of harvesting advanced (lower efficency of peeling in February than in June). These same authors (Ismail *et al.* 2005) found a slight increase in soluble solids as the date of harvesting advanced.

Similar studies were carried out for the carpelar membrane and no correlation was found between fluidity and endocarp content in soluble solids (data not shown), which suggest that, within the levels of commercial ripeness used for this experiment, the disposition of the membrane undergoes no modification that can affect its ease of degradation with pectolytic preparations.

ENZYMATIC DEGRADATION OF THE ALBEDO ON DIFFERENT SPECIES USED

To determine the influence of different species on albedo degradation, five fruits in commercial ripening stage were selected. Fluidity was based on the lyophilised albedo from different species when an enzymatic solution (Peelzym® II) was added according to the method described by Levinson and Reese (1960) and Pretel *et al*. (2005).

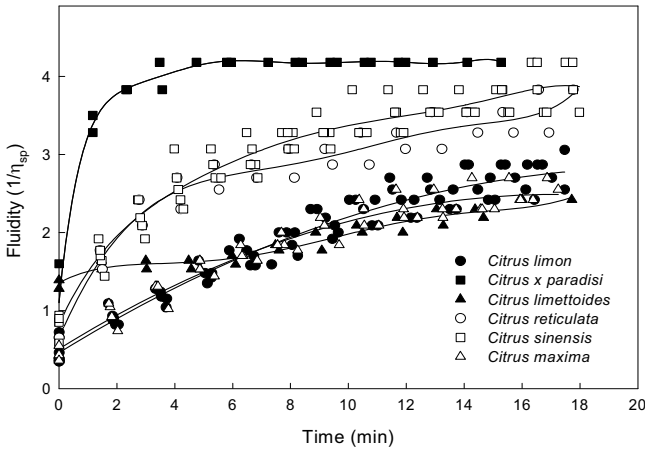

Fig. 9 Evolution of fluidity (1/ηsp) in several albedo solutions from different citrus species throughout reaction time. Reaction medium was 10 mL of albedo solution (20 mg mL^{-1}) and 4 µL of Peelzym® II (1 ml L^{-1}) prepared in 0.1 M sodium acetate buffer at pH 4.0.

Citrus species can affect the efficiency of the enzymes used in the process of enzymatic peeling (Ismail *et al.* 2005). To prove this fact, fruits were selected with the highest °Brix level within the limits of commercial ripeness and belonged to six different species of citrus: *C. maxima* (Burm.) Merrill etnovar 'Cimboa', *C. limon* (L.) Burm etnovar. 'Fino', *C. sinensis* (L.) Osbeck etnovar. 'Blanca', *C. limettoides* Tanaka 'Lima dulce', *C. reticulata* 'Blanco' subsp. *Deliciosa* (Ten.) D. Rivera and cols. etnovar. 'Mandarina del terreno' and *C. x paradisi* Macfad. in Hook. etnovar. 'Pomelo' (Rivera *et al.* 1998). **Fig. 9** shows that the fluidity of different solutions after the addition of the enzymatic mixture increased with the reaction time. Maximum fluidity was reached at around 2.5 (1/ηsp) for *C. limon*, *C. maxima* and *C. limettoides* and slightly higher, between 3 and 4 (1/ηsp), for the other species, *C. sinensis*, *C. x paradisi* and *C. reticulata.* Those variations are probably due to the ripening stage more than to differences among the species, since the three species showing a higher fluidity are those which showed a larger amount of soluble solids (*C. sinensis*, *C. x paradisi* and *C. reticulata*). Therefore, optimum conditions for the enzymatic peeling may not depend only on the composition of the albedo in different species, but other factors, such as changes occurring during the ripening, or morphological characteristics belonging either to species or varieties, as has been demonstrated by other authors (McArdle and Culver 1994; Pretel *et al.* 1997, 2001).

Species and varieties are important factors in the enzymatic peeling process, but parameters, such as albedo thickness could affect the amount of enzymatic solution necessary to carry out the process, since that quantity will be higher if the fruit to be peeled shows a larger amount of albedo (Pretel *et al.* 2007a). Thus, to establish the optimum conditions for the enzymatic peeling process for different citrus species, one of the determining parameters was the degradation ease in the albedo that, as shown before in this work, is directly connected with the ripening stage of the fruits. Likewise, other factors, such as morphological differences among species, can influence the quality of citrus segments obtained, as has been found in other studies.

EFFECT OF TEMPERATURE AND PH ON THE ALBEDO ENZYMATIC DEGRADATION

For this study, *C. maxima* (Burm.) Merrill etnovar 'Cimboa' (Rivera *et al.* 1998) fruits (**Fig. 1**) in commercial ripening stage were selected, and the albedo was immediately removed and lyophilised. Lyophilised and sifted albedo, were used as substrates for the reactions. Fluidity was assessed on lyophilised albedo after adding the enzymatic solution (Peelzym® II), according to the method described by Levin-

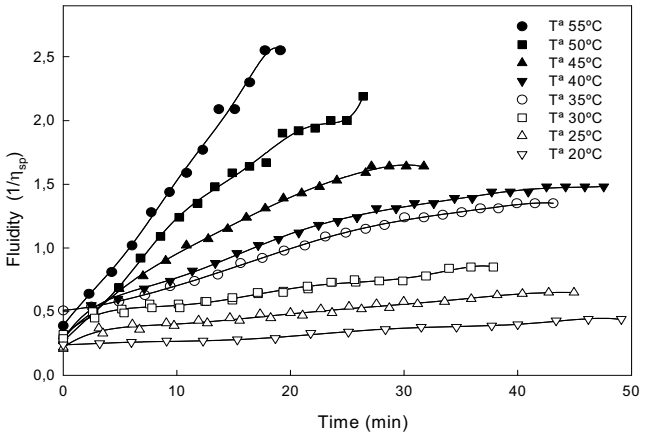

Fig. 10 Evolution of fluidity (1/ηsp) in a *Citrus maxima* albedo solution over time. Reaction medium was 10 ml of a *Citrus maxima* solution of albedo (20 mg mL^{-1}) and 4 µL of Peelzym® II (1 ml L^{-1}) prepared in 0.1 M sodium acetate buffer at pH 4.0. Reaction temperature: 20, 25, 30, 35, 40, 45, 50, 55, 60°C.

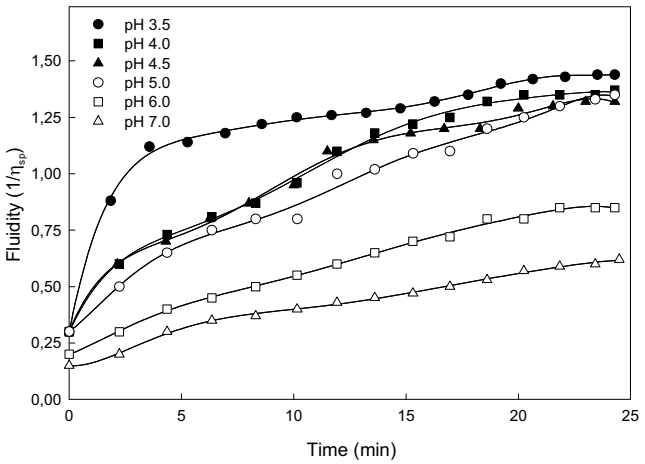

Fig. 11 Evolution of fluidity in a *Citrus maxima* albedo solution over time. Reaction medium was 10 mL solution of albedo (20 mg mL^{-1}) and 2 µL of the enzymatic compound Peelzym® II (1 ml L^{-1}) prepared in 0.1 M sodium acetate buffer, under different values of pH (3.5, 4.0, 4.5, 5.0, 6.0, 7.0). Reaction temperature: 40°C.

son and Reese (1960) and Pretel *et al*. (2005).

The increase of fluidity produced in the degradation of the albedo solution belonging to *C. maxima* at different temperatures versus reaction time is represented in **Fig. 10**. At temperatures of 50 and 55°C, maximum fluidity is achieved, reaching after 15 minutes of reaction 2.17 (1/ηsp) and 1.57 (1/ηsp), respectively. However, the speed with which fluidity in the albedo solution increases at 50 and 55°C could be due to enzyme denaturation as described previously by Pagán *et al.* (2005) in their study of the effect of temperature on the enzymatic peeling of oranges. Ben-Shalom *et al.* (1986) found out that the optimum temperature for degradation of segment's membranes by pectinase C-80® was 55°C, whereas optimal temperature for degradation of commercial pectin was 50°C, thus concluding that the optimum conditions for the action of the enzymatic preparations depends on the degraded substrate. On the contrary, with temperatures ranging from 20 to 30°C, very low maximum fluidity values are reached only around 0.5 (1/ηsp), showing that the albedo is not suitably degraded, as described by Pagan *et al.* (2005), who could not find an increase in the efficiency of orange peeling with temperatures lower than 30°C. With temperatures ranging from 35 and 40°C, similar maximum fluidity values are obtained, 1.48 (1/ηsp) and 1.35 (1/ηsp) respectively. Those are the more suitable temperatures leading to enzymatic degradation of the albedo, since acceptable fluidity values are achieved with a constant rise against time. In connection with these results, Rohuana and Manheim (1994) described that 40°C was the best temperature for enzymatic peeling of grapefruit, since lowering the temperature extended the process time for peeling, and working at higher temperatures caused a decrease in the integrity of the fruits. The epicuticular wax, which holds the juice sacs of the segments, melts at temperatures above 45°C, causing the segments to soften and disintegrate (Soffer and Mannheim 1994; Rouhana and Mannheim 1994). However, the best temperature leading to enzymatic peeling of Salustiana orange was 35°C (Pretel *et al.* 1997). This range of temperature between 35 and 40°C, in addition to being within the optimum range of peeling, would become economically profitable for the industry since minimum addition of energy is required, being in this way our experiments the closest to industrial peeling requirements.

One of the most important properties of enzymes is its high dependence of pH (Ben-Shalom *et al.* 1986). As can be seen in **Fig. 11**, in the pH range from 3.5 to 5.0, maximum fluidity is produced reaching values between 1.31 and 1.44 (1/ηsp), whilst with pH 6.0 and 7.0, maximum fluidity of 0.85 and 0.62 (1/ηsp) is respectively reached. With pH 3.5, the highest increase of fluidity is achieved in the first minutes of reaction, reaching the maximum 1.44 (1/ηsp) after 5 minutes. Maximum fluidity reached with pH ranging between 4 and 5 is similar to that reached with a pH 3.5, though it is reached later. Similar results were obtained by Soffer and Mannheim (1994), who found that the optimum pH for pectinase and cellulase action on citrus albedo and membranes was established between 3.5 and 3.8. Ben-Shalom *et al.* (1986), when studying the pH dependence of pectinase C-80® proved that, to achieve the maximal degradation in the membranes of segments, the pH ranged between 4 and 5. Also Rouhana and Mannheim (1994) established that optimal pH for the enzymatic digestion lies between 4 and 5, since when citrus fruits are placed into an enzymatic solution, the pH gets reduced to 3.5–3.8, probably due to acids dissolution. Usually the enzymatic peeling solution is stabilized with a buffer solution of sodium citrate/citric acid; thus, the incubation time to achieve a quality product considerably diminishes (Rohuana and Mannheim 1994; Pretel *et al.* 1997). However, the optimum pH for enzimatic peeling is established according to both the level of activity shown by the enzyme preparation and its stability under operational conditions. Because of this reason, the stability of the preparation was ascertained by studying the evolution regarding time of its cellulase and pectinase activities at different pH, using specific substrates (citrus pectin and CM-cellulose, respectively) as standard substrate, thus finding that in both overall enzymatic activities, the half-life decreased with increased acidity of the medium. Minimum deactivation was obtained for both at pH 4-4.5. Cellulase activity showed greater pH stability, while pectinase activity was reduced to 36% under the same conditions. Since peeling efficiency is established at pH 4, this value was chosen as the best for peeling process (Pretel *et al*. 1997). In this way, it could be concluded that, although the most suitable pH to achieve enzymatic degradation of the albedo is 3.5, the pH range leading to enzymatic peeling could be wider, between 3.5 and 4.5.

INFLUENCE OF ENZYME CONCENTRATION ON ALBEDO ENZYMATIC DEGRADATION

Citrus sinensis L. variety 'Sangrina' fruits in a commercial ripening stage have been selected to carry out this study, being the albedo removed and lyophilised inmediately. The lyophilised and sifted albedo was used as a substrate for reactions. Fluidity was assessed on lyophilised albedo when enzymatic solution was added (Peelzym® II), according to the method described by Levinson and Reese (1960) and Pretel *et al*. (2005).

Enzymatic solution should be infiltrated under vacuum conditions through the perforations made in the flavedo to

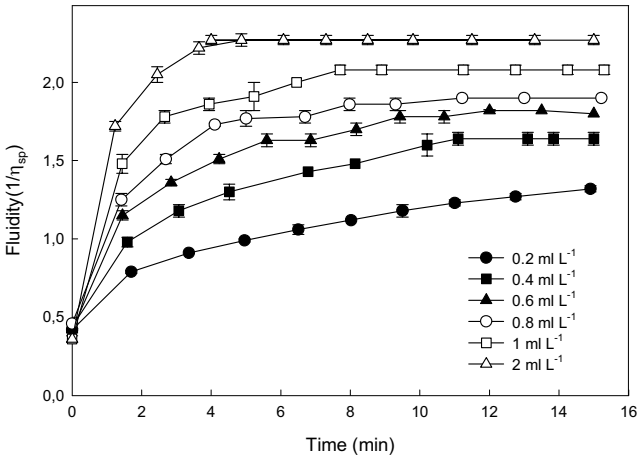

Fig. 12 Evolution of albedo solution fluidity against time with different Peelzym® II concentrations. Reaction medium: 10 ml of albedo solution (20 mg ml^{-1}) in 0.1 M sodium acetate buffer, pH 4 and 2 µl of Peelzym® II at different concentrations (0.2, 0.4, 0.6, 0.8, 1, 2 ml L^{-1}) (Pretel *et al*. 2007b).

achieve both an effective degradation of the albedo and segment separation (Baker and Wicker 1996). However, some authors, by evaluating the final product quality, showed that the effectiveness of the process varied a great deal depending on the enzyme concentration used (Bruemmer *et al*. 1978; Pretel *et al*. 1997; Prakash *et al*. 2001; Toker and Bayindirli 2003). In the present work the degradative capacity of different Peelzym® II concentrations (from 0.2 to 2 ml L^{-1}) using a saturate solution of lyophilised albedo is studied. **Fig. 12** (Pretel *et al*. 2007b) shows the increase in fluidity produced by the degradation of albedo solution from Sangrina variety against reaction time. As Peelzym® concentration increased, final fluidity also increased and the time required to reach the maximum fluidity decreased. When the lowest concentration of Peelzym® was used (0.2 ml L^{-1}), fluidity increased slowly from 0.42 ± 0.001 units till 1.32 ± 0.02 units after 15 minutes, showing a deficient degradation of the albedo. In the concentration range of Peelzym® from 0.4 to 1.0 ml L^{-1}, when enzyme concentration is increased in 0.2 ml L^{-1}, final fluidity increased proportionally, which indicates a gradual increase in the ease of degradation of the albedo, as has been shown to occur in grapefruit peeling (Prakash *et al.* 2001).

However, our results from this study showed that with concentrations higher than 1 ml L^{-1}, fluidity changed from 2.08 ± 0.03 to 2.27 ± 0.03 units when the concentration of Peelzym® II was doubled from 1 to 2 ml L^{-1}, showing a different dose response factor. This indicates that even doubling the concentration of enzymatic solution, degradation of the albedo was only slightly modified. Pretel *et al.* (1997) ascertained that, to obtain a good peeling efficacy of Salustiana orange, it was necessary to use 10 g L^{-1} of the glycolhydrolase enzyme preparation Rohament® C. Likewise, those results indicated that optimum concentration for obtaining a quality product depends on the composition of the enzymatic solution (Soffer and Mannheim 1994). On the other hand, and due to the high price of enzymatic solutions, it is possible to reduce the amount of enzymatic solution by increasing incubation time (Prakash *et al*. 2001), thus reducing production costs.

CONCLUSION

Enzymatic peeling is a good alternative to traditional chemical peeling. There are many parameters to take into account to obtain good peeling effectiveness. To achieve enzymatic peeling of citrus is important to consider the ripening stage of the fruits. Optimum conditions leading to enzymatic peeling may not depend on the composition of the albedo in the differents citrus species. The range of temperature between 35 and 40°C and pH range between 3.5 and 4.5, are the most suitable values for enzymatic peeling of citrus. As a final conclusion, it is important to point out that the optimum concentration of enzymatic solution depends on the ratio of the different enzymes presents in the mixture (mainly pectinase and cellulase).

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