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Influence of Degradative Enzymatic Activities on the Shelf Life of Ready-to-Eat Prickly Pear Fruits

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***Abstract*—**Prickly pear fruit (*Opuntia ficus indica* L. Miller) belongs to the Cactaceae family. This species is very sensitive to low storage temperatures (< 5°C) which cause damages. The fruits can be peeled, suitably packaged and successfully commercialized as a ready-to-eat product. The main limit to the extension of the shelf life is the production of off-flavors due to different factors, the growth of microorganisms and the action of endogenous enzymes. Lipoxygenase (LOX) and Pectinesterase (PE) are involved in fruit degradation. In particular, LOX pathway is directly responsible for lipid oxidation, and the subsequent production of off-flavours, while PE causes the softening of fruit during maturation. They act on the texture and shelf-life of post-harvest, packaged fruits, as a function of the the grown of microorganisms and packaging technologies used. The aim of this work is to compare the effect of different packaging technologies on the shelf life extension of ready-to-eat prickly pear fruits with regards for the enzymes activities.

***Keywords*—**Enzymes, packaging, prickly pear, shelf life.

I. Introduction

APPEARANCE, flavor, texture and nutritional value, are the factors considered by consumers. Consumers are increasingly demanding convenient, ready-to-use and ready-to-eat fruits and vegetables with a fresh-like quality, and containing only natural ingredients. Processing of fruits and vegetables into ready-to-use products aims at maintaining freshness without reducing nutritional and sensory quality, hence obtaining a longer shelf life which allows a wider distribution and consumption.

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The problem of enzyme degradations in ready-to-use products, obtained following GMPs, is still of primary importance. In the entire vegetable tissues, cellular components such as enzymes, substrates, metabolites and reserve substances, are located in the subcellular organs and do not come in contact with each other. The eventual rupture of cell structures during processing and senescence can determine the out flow of substances and the subsequent onset of enzyme chain reactions, which speed up the decay of qualitative characteristics. Prickly pear is a fruit characterized by a high susceptibility to spoilage at ambient temperature and is sensitive to refrigerated storage temperatures (0 - 4°C) which cause chilling injuries (0 - 4°C) [1].

The main limits to storage of prickly pears are the loss of consistency and the development of off-flavors, due to different factors, such as microbial growth and oxidation and cellular degradation phenomenons by endogenous enzymes, whose natura has not been fully clarified. However, basing on studies performed on other fruits [2]-[3], Pectinesterase (PE, EC 3.1.1.11) and Lipoxygenase (LOX, EC 1.13.11) were addressed.

PE is an index of pulp firmness during postharvest of fruits, it was extensively studied in fruits and vegetables by several authors.

In prickly pear fruits changes in the cell wall constituents and enzymes were observed, especially pectins and pectinases [4]. There were no changes in the pectin content of the pulp during ripening, whereas total pectin content of the peel was notably higher and decreased with ripening. The percentage of soluble pectin, however, remained relatively constant during ripening [5].

LOX, found in plants, animals and fungi, catalyzes the dioxygenation of polyunsaturated fatty acids in lipids containing a cis, cis 1,4-pentadiene structure. LOX action changes the membrane composition, it has been implicated in membrane degradation during fruit ripening and senescence [6]-[7]. It is directly responsible of C6 aldehydes *n*-hexanal and *(E)*-2-hexenal production in ripe fruits associated to the production of ethylene during postharvest of fruit stored at 20°C [8]. The same was observed in ripe prickly pear fruits that are the object of the present work.

LOX activity has been found to increase in tomato (*Lycopersicon esculentum* Mill.) during fruit ripening and senescence [9] resulting in a decrease in product quality and commercial value. In pear fruits was observed that LOX and PE were higher during maturation and harvesting.

PE and LOX have never been investigated in prickly pear fruits. Aim of this work is to examine the effect of different treatments and packaging on PE and LOX activities.

II. Materials and Methods

A. Samples Preparation

Mature prickly pear fruits were harvested in Paternò (province of Catania, Italy), of these 150 were dethorned by removing the glochids and peeled. A part of fruits were used for physico-chemical determinations, as described below.

The fruits were packaged, four in each drip, into a barrier film, PET 30 Melinex 850, permeability to O_2, cc/m^2/24h: 56, permeability to H_2O vapour, g/m^2/24h: 13 under normal atmospheric conditions (Control), and under two modified atmosphere having different O_2 and CO_2 composition (MA1: 5% O_2, 2% CO_2, 93% N_2; MA2: 2% O_2, 5% CO_2, 93% N_2) [10]-[11], the same gas concentrations are generally used for modified atmosphere.

The other fruits were blanched at 80 °C for 10 minutes in water (Blanching Treated -BT), and in a solution of 2% citric acid (Blanching + Citric Acid Treated -BCT), and packaged, four in each drip, in the film described above.

All samples were stored at 4°C and all analysis were carried out until 13 days from packaging.

B. Composition and Nutritional Characteristics of Ripe Fruits

The following determinations were carried out on fruits: weight, yields of pulp, peel, seeds and juice. Pulps were homogenized by Ultraturrax (Janke & Kunkel) and the following determinations were performed: pH, acidity (expressed as mg citric acid/10 mL of pulp). The determination of pH was conducted by potenziometric method at 20°C, pHmeter (Inolab) was calibrated with buffer solutions.

All determinations were carried out in triplicate and all not specified reagents were provided by Sigma.

C. Microbiological Analyses

The microbiological analyses of each trial were performed in triplicate at 0, 3, 5, 7, 9, days of storage at 4°C. Each sample was homogenised and serially diluted in sterile physiological solution (0.9 % NaCl) up to 10^{-9}. Mesophilic Viable Counts were performed by inclusion in Plate Count Agar (PCA). Yeast and mould counts were carried out in Sabouraud Dextrose Agar (SAB) media. All media were provided by Oxoid.

D. Enzymatic Determinations

1. Pectinesterase Assay

Pectinesterase (PE) extraction and quantitative determination was carried out on 20 g of homogenised pulp by a suitably modified method [12]-[13], natural pectin from apple was used as substrate.

2. Lipoxygenase Assay

Lipoxygenase (LOX) extraction and quantitative determination was carried out on 20 g of homogenised pulp [14] – [15], and linoleic acid (free acid) 25 nM (Sigma) was used as substrate.

III. Results and Discussion

The chemical-physical characterization of fruits showed that some of the measured parameters are in agreement with data reported in literature [16]. The yield in edible fruit ranges from 61±8% and can be differentiated into two fractions: pulp and juice, corresponding to 62 and 38%, while peels amounted to 38±5%. The major components of the fruit pulp are water, about 85%, carbohydrates, 10-12%, expressed as Brix. Soluble solids and total sugar content, generally, increase during ripening. pH and acidity of fruit was 6.0-6.5, as reported for ripe fruits, the acidity was 1.92 (mg citric acid/10 mL of pulp), also in accordance with literature [17].

The microbiological results show the microbiological degradation of packaged fruits during refrigerated storage. The modified atmospheres (***MA1*** and ***MA2***) determine only a slight decrease of the total bacterial counts on PCA, with a significative decrease of yeasts and molds on SAB (data not shown).

The most interesting results are related to enzymatic determinations, in the different packaged or treated samples during storage. Both enzyme activities, Pectinesterase (PE) and Lipoxygenase (LOX), are responsible for nutritional and sensorial degradation in ripe fruits. From a sensorial point of view, a correlation has been found among PE activity and loss of texture. Fig. 1 shows that PE activity at zero is lower for Control, MA2 and BT samples, compared with MA1 and BCT samples, but their activity is similar during all the period of storage.

For MA1, the activity increases slightly until 8 days, then a sharp decrease of such activity was observed. This could be correlated with the higher initial CO_2 content and with the decrease of O_2 down to values proximate to 0 after 3 days of refrigerated storage. In Fig. 1 it is also possible to note that heated samples show higher PE activity respect to the control, this due, probably, to the breakdown of cell wall during heating. This result is confirmed in Fig. 2, in which BCT shows a low total activity, but higher compared with the control. Generally, PE activity does not affect the pectin content of the pulp during ripening. Studies reported few changes in pectin content during ripening until the prickly pear became over-ripe, when total pectin content increased.

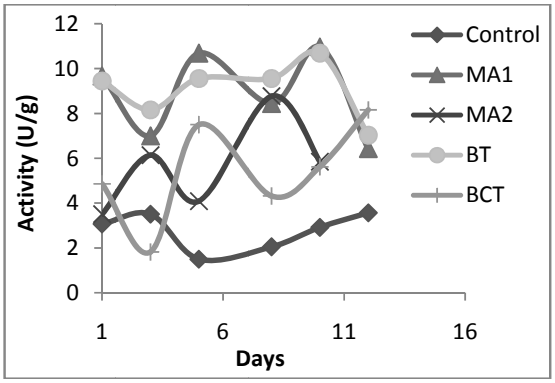

Fig. 1 PE activity (U/g) in different samples

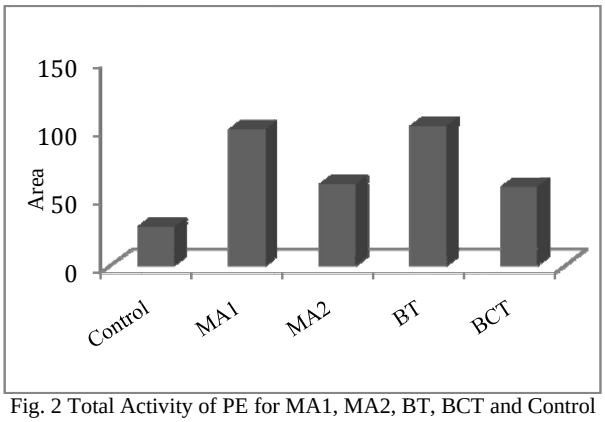

Fig. 2 Total Activity of PE for MA1, MA2, BT, BCT and Control

Figs. 3 and 4 show the course of LOX in the three atmospheres and in the thermally-treated and control samples, respectively. Fig. 3 shows that LOX activity increases in both the samples in MA respect control, after nine days of storage the activity is lower. This is probably due to the inhibitory effect of CO_2 in correspondence to a decrease of O_2. In Fig. 4 LOX activity is lower in heated samples with respect to the control. The effect of blanching is more evident in the samples treated with citric acid. Fig. 5 shows the total activity of LOX expressed as area and also this data confirms the above mentioned results.

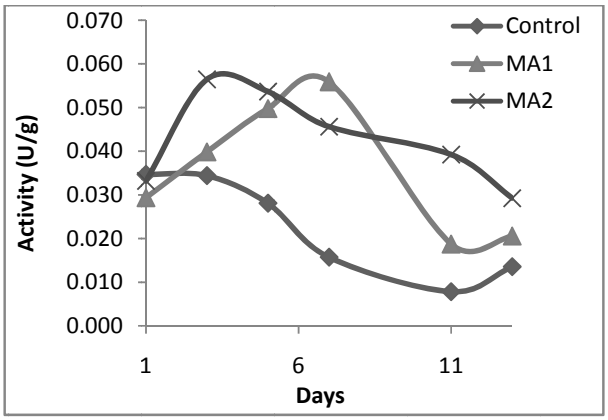

Fig. 3 LOX activity (U/g) in MA1, MA2 and Control

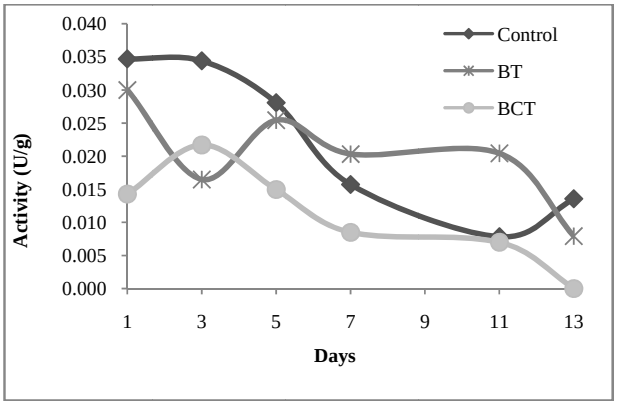

Fig. 4 LOX activity (U/g) in BT, BCT and Control

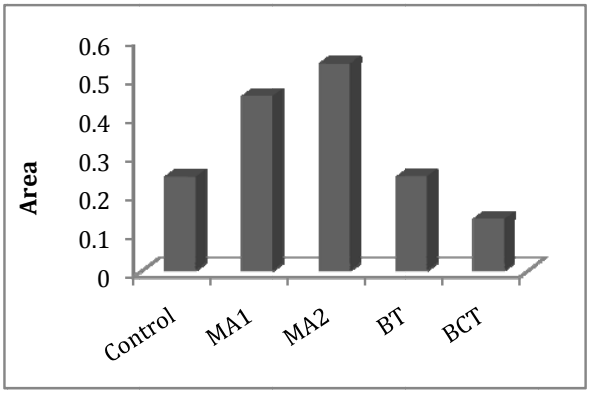

Fig. 5 Total Activity of LOX for MA1, MA2, BT, BCT and Control

In conclusion, PE and LOX activities are involved in prickly pear shelf-life. Heating treatment and citric acid affect both enzymes, in particular the LOX activity. Results suggest that a chemical-physical characterization of LOX could indicate the best parameters for the control of LOX activity in association with a sensorial evaluation to increase the shelf-life of minimally processed fruits.

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