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Phenolic extracts of the pod of *Ceratonia siliqua* new alternative in fruit maturation

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Abstract
Carob or *Ceratonia siliqua* is a typical mediterranean forest and fruit tree that has considerable ecological and socio-economic importance. It is frequently used in our culinary and medical traditions to combat cholesterol, acute diarrhea and digestive disorders. Our work was based on the study of the polyphenolic extract of *Ceratonia siliqua* pods on fruit oxidation and maturation. Our results show that the total polyphenols (PPT) carob tree pod play a role in apple, banana and lemon maturation. It is then suggested that carob PPT has a major role in accelerating fruit oxidation that play a role in fruit maturation. We suggest that our product can be used to replace chemicals that are used in the acceleration of fruit maturation.

Keywords: product development, ash gourd juice, value added products

1. Introduction

In the face of all the environmental problems affecting the planet, we must become more and more aware and we must protect nature. Feeding is a daily act and essential for our survival and health. If eating should remain a pleasure, it is not useless to ask ourselves some questions about our methods of production and food consumption, and to wonder about the consequences that these can cause for our environment.

Currently, food is at the heart of environmental challenges because it is one of the areas with the highest environmental impacts.

Indeed, the production of our food on an industrial scale requires land and a large consumption of water, raw materials (for the production of fertilizers and pesticides in particular) and energy (for the heating of greenhouses, the work of Earth …)

Food production requires the use of chemicals, including plant treatment products by farmers. These products can be in different forms: solid (powder, granules, fibers such as asbestos), liquid, gaseous. The use of chemicals is never trivial, and should be handled by observing basic precautions related to the characteristics of these products [1].

In fact, conventional farming frequently uses it for growing and maturing and preserving fruits and vegetables. Some plants have pesticide residue levels well above the standards, and unfortunately, we find them on our plates!

These modes of production have impacts, positive or negative, not only on our "Nourishing Earth" but also on our health. Dangerous for the health, they could be at the origin of pathologies.

Some chemicals are likely to be a source of ignition, or explosion, with serious consequences for humans and the environment. Among the effects of chemicals on the body: acute intoxication, chronic intoxications, allergies, some products are likely to cause serious or irreversible diseases such as cancer and also affect reproductive functions (reduced fertility, undermining the health of the child through breastfeeding).

We conducted research focused on the maturation of fruits and vegetables trying to find alternatives or natural substitutes of chemicals used during this period.

Following further research that shows that when the peak respiratory of fruits is reached, the maturation process begins and leads to an overproduction of free radicals. These free radicals in turn cause an increase in ethylene production, which boosts maturation, creating a chain reaction linking oxidation and maturation. We then tested the induction of maturation by testing the oxidation. Our choice was made on fruits with fast oxidation "the apple and the bananas" which are climacteric fruits whose maturation depends on the ethylene [2].

In a synthetic way, the oxidation of a food is caused by the oxidation of the phenols of the fruit by enzymes in the presence of the dioxygen on the air. The oxidation of these phenols is catalyzed by the enzymes contained in the fruit and the oxygen. This favors the production of free radicals, which are unstable molecules that seek to exchange one or more electrons on the outer layer of another atom or molecule. There is then a chain reaction that results in the production of melanin. We speak of browning for fruits and rancidity for lipids, these two phenomena giving rise to a color change of the food [3, 4]. In general, browning is caused by enzymatic oxidation of natural phenolic compounds. Polyphenol oxidase (PPO) is the key enzyme for this degradation [3, 5].

We have tried to test the effect of the total polyphenols resulting from the carob tree on oxidation fruits. The carob tree contains a high proportion of sugar (glucose, sucrose, fructose), which is also rich in tannins and has low levels of proteins and lipids, It is a good source of potassium and magnesium [6, 7]. The polyphenols of the carob tree have had a growing interest from nutritionists, agribusinesses and consumers over the past decade, and are therefore very

powerful antioxidants [8].

Polyphenols are associated with many physiological processes in food quality. The ability of a plant species to resist attack by insects and microorganisms is often correlated with phenol content [9]. These compounds show antioxidant [8, 10], anticarcinogenic, anti-inflammatory, antibacterial, antiviral [8], anti-allergenic and vasodilator activities [8]. In addition, this product is known to exert a regulating effect on intestinal function such as diarrhea, burning of the stomach and inability of the intestine to absorb certain foods [8].

We treated the fruit with a natural product "total polyphenols (PPT) carob tree", known by these biological and taste interests, to find out if there is stimulation and induction of oxidation causing the maturation of the fruit by improving the quality organoleptic of fruit.

2. Materials and methods

Preparation of carob extract

The carob used in this study was harvested in the region of Ait Abbas Azilal, July 2016. To prepare the carob extract, the pulp is washed, dried and then ground after being freed from the seed. The resulting carob powder was sieved through a sieve. We added 100 g of carob powder to 500 ml of petroleum ether to remove the lipids. The mixture was centrifuged at 1500 RPM for 48 hours. Then, the liquid and solid phases are separated by filtration. Thereafter, the solid is dried overnight at room temperature

Twenty five grams of the powder obtained is mixed with 100 ml of methanol in a flask. The mixture is stirred for 96 h at room temperature. Then, the solvent is recovered by filtration and the extraction of the PPTs is carried out by the rotary machine.

Determination of phenol compounds by colorimetry

The phenol content of the various extracts obtained from carob bean powder was estimated by the method of Folin-ciocalteu according to SINGLETON and ROSSI (1965). This method is based on the reduction in the alkaline medium of the phosphotungstic and phosphomolybdic mixture of the Folin reagent by the reducing groups of the phenolic compounds, leading to the formation of a blue color reduction product. The latter have a maximum absorption at 765 nm, the intensity of which is proportional to the quantity of the polyphenols present in the sample. The solutions of the different samples to be assayed and the standard range are prepared in the same manner and under the same conditions.

For our assay, 125 µl of each carob extract is taken. 500 µl of distilled water and 125 µl of the Folin-Ciocalteu reagent are then added. The solvent is incubated for six minutes in the absence of light. Then, 1.25 ml of the aqueous sodium carbonate solution (Na 2 CO 3) is added to the reaction medium before adjusting the mixture to 3 ml of distilled water. Then, it is left to stand for 60 minutes incubation at room temperature and in the dark. The absorbance is measured at 765 nm on the spectrophotometer against a blank without extract. The quantification of the phenolic compounds was carried out as a function of a linear calibration curve of the form y = ax made using gallic acid as a reference. The results will therefore be expressed in equivalents of gallic acid. The PPT concentration is calculated from the regression equation of the calibration range established with gallic acid.

Study of oxidation of apples and bananas

For each experiment, the slides of the apple and bananas are placed in sterile and labeled petri dishes. We chose to perform our experiments with apple slides. Indeed, it is a fruit with rapid oxidation once peeled. For each of our experiments, we studied apple slides called "control" (without the addition of any compound) whose oxidation is compared with that of the slides subjected to new experimental conditions (in the presence of salt, sugar , Lemon and different concentrations of carob extract phenol) for four days.

We placed in each petri dish a slice of apple soaked with 3 ml of product (in the presence of salt, sugar, lemon and different concentration of carob extract phenol at different concentrations (100 ml, 500 and 2.6 µg / ml)). Three runs were made for each condition to take the oxidation average.

The study of the oxidation evolution of apple and banana lamellae with extract PPT of carob at different concentrations (100 µg / ml, 500 µg / ml and 2.6 µg / ml) as a function of time showed that the concentration 500 µg / ml is the optimal concentration which shows a significant oxidation compared to the control over time. This concentration will be the concentration used in the experience.

Measurements of the oxidized surfaces are carried out using the measuring software "MESURIM" which gives representative results. We have cut thin slices because the surface measurements on MESURIM require flat surfaces to be able to be realized. Indeed, we used the "surface measurement" option, which allowed us to calculate the oxidized surface of the lamellae at each time t. We then manage to collect these data to create a graph showing the evolution of apple oxidation, so that comparisons can be made.

Statistical Analysis

MESURIM is software designed to do different types of work on digitized images. We used the "surface measurement" option to measure the browning of the apples and bananas slices. At each defined time interval, we measured the surface area of the brown fruit (in cm2) in relation to the total surface of the fruit, in order to plot a curve of the percentage of oxidized surface of the fruit as a function of time comparison. We calculated the percentage (%) of oxidation by the following mathematical formula:

% Oxidation Percentage = Area Brown / Total Area x 100 (%).

3. Results & Discussion

The results of the effect of lemon, salt, sugar and total polyphenol extract from the carob tree polyphenol extract are shown below (Figure 1).

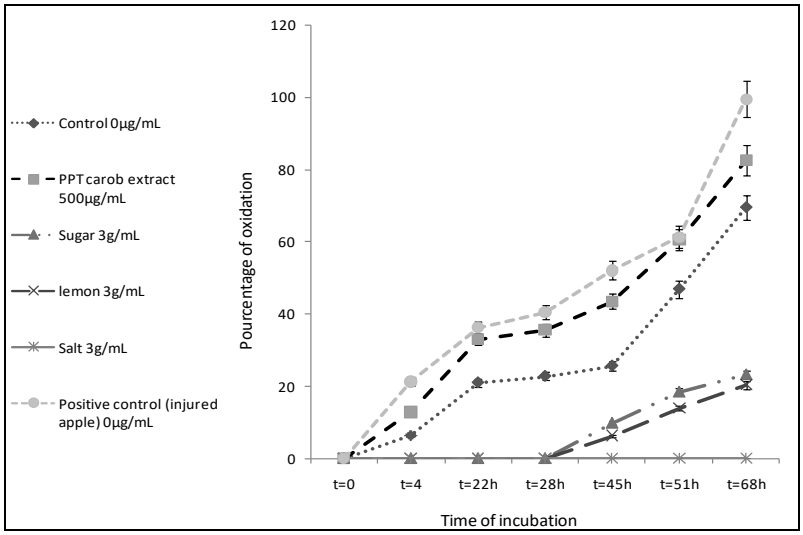

Fig 1: Evolution of the percentage of apple coverslip oxidation under different conditions (control, lemon, salt, sugar and PPT extract).

The graph analysis clearly show that the control apple placed in the light and at ambient temperature knows at first (0 to 45 h) a slow oxidation of 25% and then at the end of 68 h the oxidation becomes faster reaches 69.39%.

Our results show that the presence of lemon on the apple slices significantly slows down its oxidation compared to the control (the browning does not appear until after 28 h during the period studied and does not exceed 20%). Lemon is a powerful antioxidant, vitamin C or ascorbic acid, limiting the production and oxidizing effects of free radicals under experimental conditions (free air, room temperature).

In the presence of sugar or salt on the surface of the fruit, the browning is very low (sugar) or nonexistent (salt). Sugar and salt, in contact with the apple, represent hyperosmotic solutions more concentrated than the cells of the apple.

A movement of water will thus be created from inside the fruit cell to the outside. These solutions are so-called depressant agents, reducing the cellular content in water. Enzymes need some water activity to ensure normal function. Salt and sugar, reducing the water content of the cell, will cause a drop in enzyme activity and therefore a major reduction in the oxidation of the fruit. The phenomenon of "osmosis" explains its results.

We used as a positive control a tapped or damaged apple that oxidizes more quickly. Since, the damage created by the shock on the apple causes a rupture of the membranes separating the phenols and the PPO. Thus, PPO acts faster and more efficiently to accelerate the oxidation of phenols. Similarly, O2 is more directly in contact with phenols and the intracellular medium, further accentuating its role in the biochemical process.

The graphical analysis shows that the carob tree TPP accelerates the oxidation of the apple compared to the control because the polyphenol curves are always below the control for the duration of the experiment.

The observations of the microscopic view (figure 2) and the photos (figure 3) of the surface of the apples treated with 500 µg / ml of the PPT extract clearly show that the treated apple placed in the light and at ambient temperature undergoes oxidation at 68 h presented by a browning of the color.

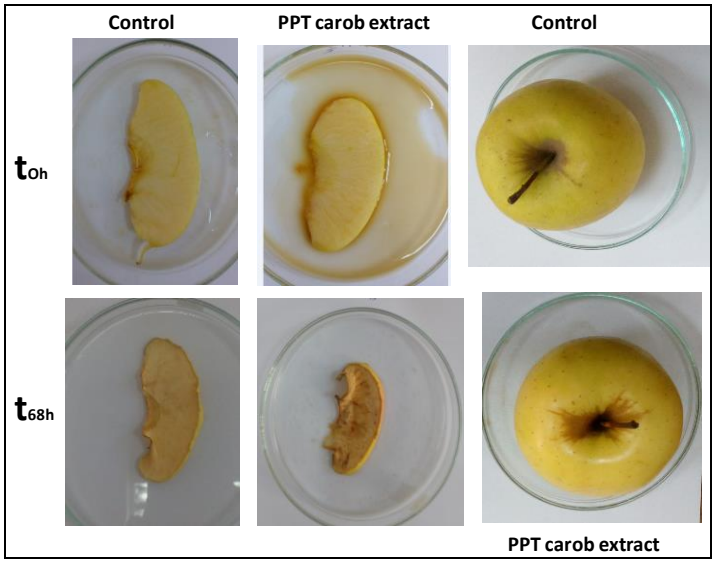

Fig 2: Evolution of lamella oxidation and peel of the apple at 500 µg / ml for the polyphenol extract at 68h.

After treatment of the apple wall with the total polyphenol extract of the carob tree for 4 days (the image above), there is a change in color and an increase in the lightening of the color (shine). This is a criterion of fruit quality that can be explained by the stimulation of ethylene that induces maturation.

Fig 3: Observation of maturation evolution of apple, banana and lemon (polyphenol and control case) after t = 70h.

The fruits soaked with the carob tree's total polyphenols (Figure 3) were more clear and attractive than the control fruits. our results suggest that carob PPT has a major role in the acceleration of browning and fruit oxidation under the following experimental conditions (free air, ambient temperature).

4. Conclusions

Our work is based on the PPT extract of carob and their relationship with the oxidation of the fruits, after the completion of their extractions and evaluate the effect of PPT on lamellae (apple, banana and lemon) results are obtained which suggest that carob PPT has a major role in the acceleration of browning and fruit oxidation under the following experimental conditions (free air, ambient temperature).

Our results show that the PPT of the carob tree pod play a role in apple, banana and lemon maturation. It is then suggested that carob PPT has a major role in accelerating fruit oxidation that the PPT of the carob tree pod play a role in fruit maturation.

We suggest that our product can be used to replace chemicals that are used in the acceleration of fruit maturation.

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