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Effect of High Pressure Processing on enzymatic activity for strawberries, sour cherries and red grapes

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Abstract

Color degradation is an important factor that affect the quality and acceptability of fruit juices and purees; several enzymes, as well as the microbial endogenous population are not only responsible for this phenomenon but for changes in flavor and texture. Traditional stabilization methods have been used to preserve these kind of products; however, there is a negative impact on vitamins and bioactive compounds composition. High Pressure Processing (HPP) is a non-thermal alternative that has been applied for the extension of shelf life of fresh products, reducing the adverse effects of classical treatments. The aim of this review is to provide a scientific base on the effect of HPP technology in terms of enzymatic inactivation (peroxidase, polyphenol oxidase, ascorbate oxidase and β-glucosidase) in comparison with a conventional pasteurization process in strawberries, sour cherries and red grapes, and to propose an optimization strategy for the operational parameters to achieve the greatest inactivation.

Keywords: High-pressure processing, juice, enzymatic inactivation, preservation

1. Introduction

Current consumer trends towards highly nutritional value and health promoting food products, in addition to the increase in the demand of minimal processed alternatives, is leading to the use ingredients in non-conventional ways in order to satisfy consumer expectations (Bord Bia, 2019, Di Cagno et al., 2013). Fruits and vegetables are an important source of nutrients such as antioxidants, soluble vitamins, dietary fibers, minerals, organic acids, etc. (Saguir et al., 2019). However, different phenomena are responsible for the deterioration of the quality of these products, reducing their shelf life.

Color degradation is one of the major factors that influence the acceptability of fruit juices and purees. This variation is caused by two different mechanisms: in one side, non-enzymatic browning associated with the degradation of ascorbic acid, Maillard reactions and sugar degradation, producing intermediate compounds responsible for the brown color (Bharate et al., 2014; Teribia et al., 2021). Enzymatic degradation on the other hand is performed by endogenous enzymes, which after the breakdown of the cell structure due to the required mechanical stress to produce the juices, are in contact with specific substrates that produce colored compounds or color fading. In addition, enzymes could also affect the flavor and texture due to the production of flavor active compounds or the degradation of polysaccharides presented in the food matrix (Chakraborty et al., 2014).

Traditional pasteurization technologies had controlled the effect of enzymatic catalyzed reactions by total thermal inactivation of the endogenous enzymes in addition to the reduction of the microbial load. However, this technology has a negative impact in the nutrients and bioactive molecules composition concentration (Summen et al.,2014). Alternative technologies provide effective solutions in terms of microbial reduction but their effect in the enzyme inactivation is not completely understood or documented. High Pressure Processing (HPP) is a non-thermal technology that, using a fluid as a compression medium (generally water or water solutions).

The aim of this review is to compare different HPP treatment conditions and their related effect in enzymatic degradation for selected enzymes (peroxidase, polyphenol oxidase, ascorbate oxidase and β-glucosidase), focused on the processing of juices and purees made of strawberries, sour cherries and red grapes. Additionally, to propose an integrated optimization approach to maximize the enzymatic inactivation of all the enzymes according to the operational parameters for al High Pressure Processing unit.

2. State of the art

2.1 Enzymes and colour degradation

The enzymatic activity and its relationship with color stability in fruit and purees have been extensively studied, in order to understand the specific role of different endogenous enzymes in the degradation of nutritional components. Polyphenol oxidase (PPO) is one of the main responsible enzymes for browning reactions that could significantly affect the color and flavor of fruits (Chakraborty et al., 2014). This enzymatic process is promoted by putting in contact the specific substrates presented in the vacuoles of the fruits with the enzymes located in the membranes, resulting in an oxidation of mono- and diphenols to *o*-quinones which subsequently will polymerize to form melanins, molecules with a characteristic brown color (Guerrero-Beltran et al., 2005).

Peroxidases (POD) are also involved in the modification of flavor and color in fruits; these are responsible for the catalysis of oxidative reactions of phenolic compounds in presence of hydrogen peroxide, producing brown-colored molecules. These enzymes are also capable to use as a substrate other compounds present in the vegetable matrix such as aromatic amines and ascorbic acid among others, to generate these kinds of substances (Vernwal et al., 2006). A synergetic effect between POD and PPO has been suggested by some authors who hypothesized that the hydrogen peroxide required for the POD action, is produced in the oxidation of phenolic compounds catalyzed by PPO (Richard-Forget et al., 1997; Subramanian et al., 1999)

Another mechanism that influence the color degradation is associated with the action of other group of enzymes: β-glucosidases, responsible for catalyzing the hydrolysis of the β-D-glyosidic bonds in aryl and alkyl β-D-glycoside molecules; in the degradation of anthocyanins, these enzymes promote the action of the PPO and POD enzymes, previously restricted due to steric effects (Chakraborty et al.. 2014). Additionally, ascorbate oxidase, responsible for the oxidation of ascorbic acid, is responsible for the generation of specific molecules in its reaction pathway that could interact with anthocyanins promoting the fading of red color or even the formation of brown compounds (Teribia et al., 2021).

2.2 HPP technology

HPP technology has been introduced as a technology for the treatment of packaged food many years ago as well summarized by Rastogi et al., 2007. A typical system is formed by a high-pressure pump that inject water in a container that can support high pressures and that contain the product that will be treated. For this application, the product should be previously packaged in a flexible packaging material without headspace in the case of liquid products or vacuum-sealed for solid foods. (Gómez-López et al., 2021)

In the last twenty years, many companies design industrial systems improving them year by year as far as the treatment and above all the cost and the environmental impact are concerned (Cacace et al., 2020). However, as preliminary described in the introduction section, the discussion about the enzymatic inactivation remains as partially unsolved. In fact, since the first series of tests with this technology, the aspect related to the limited enzymatic activity reduction appeared as significant (Hendriks et al, 1998), and many evaluation tes have been carried out to understand the kinetics and the effect of this treatment for specific substrates (Roobab et al., 2021). Based o the exisiting knowledge on this technology only application o specific fruts have been considered in this work, without evaluating other interesting sector such as that of Milk and derivatives (Stefanini and Vignali, 2020) or Meat (Campus, 2010).

2.3 Strawberry purees and HPP

Strawberry is a good representative of red fruits that suffer a significant color degradation; being an interesting subject of study for alternative technologies that could prevent it. Several researchers have evaluated the impact of High Pressure Processing for preservation of nutrients and bioactive compounds in strawberry juices and purees in comparison with classic pasteurization and their effect on enzyme inactivation.

In a study performed by Cao et al. in 2011, a comparative evaluation of the enzymatic inactivation of PPO, POD and β-glucosidase for HPP and thermal treatments was made; samples of strawberry pulp were tested at three different pressure conditions (400, 500 and 600 MPa) for a maximum time of 25 min, measuring the activity each 5. Additionally the heat treatment was carried out at 70 °C. The results show that in the case of PPO, under all HPP conditions there was a reduction of the residual activity (RA) with the increase in time and pressure, achieving the lowest value (51%) with the conditions 600 MPa for 25 min. These results are in agreement with other studies: García- Palazón et al. in 2003 found complete inactivation after 15 min in samples treated at 400, 600 and 800 MPa while Sulaiman et al. in 2013 found minimal RA of 18% with 600MPa for 15 min. In this study 200 MPa was also evaluated. For this condition, an increase in the activity after 5 min treatment was observed, followed by a decrease after 15 min (110% and 87% respectively). These results are in agreement with other authors who also observed increase in the activity (Terefe et al., 2010); associating this particular behavior with a possible change in the 3-dimensional structure of the enzyme after HPP treatment. The variability observed in between studies could be attributed not only to the particular characteristics of the equipment but also to the strawberry varieties used in each experiments.

POD inactivation presented very similar results: a significant effect in the RA was found with 400 and 500 MPa with a minimal value in 25 min (56.5% and 74.6% respectively), an increase in the RA for the 600 MPa condition was observed after 10 min treatment to exhibit a regular trend after this point reaching 54.5% RA after 25 min. Similar results were found by García-Palazon et al in 2003 where 176% of RA was measured after 15 min under 400 MPa, despite that for the other conditions tested in this study (600 and 800 MPa) no increase was observed. Once again, the variation in between studies could be associated with the cultivar and variety dependency of the strawberries used. Studies conducted by Teribia et al. in 2021, where the thermal stability of enzymes in strawberry purees from different cultivars was evaluated after thermal treatment under 35°C and dark conditions for 14 days as storage conditions, demonstrated that despite that the variability in PPO activity was less compared to POD, there is a high dependency of the enzymatic activity respect to cultivars. This also suggests that differences in inactivation curves might be found when different varieties or cultivars are tested.

In the case of β-glucosidase at low pressure conditions (400 MPa), an increase trend in the RA was observed with a maximum value of 116.7% after 25 min. Regular inactivation trends were found for 500 and 600 MPa; results that confirm the findings of García-Palazón et al. in 2003 who observed an increase of 176% after 15 min at 400 MPa and regular inactivation behavior for 600 and 800 MPa conditions. With respect to the results obtained after thermal treatment, there was no enzymatic activity reported for any enzyme. Despite the quantity of ascorbic acid available in strawberries, no studies have evaluated the activity of ascorbate oxidase (AOX) in this substrate.

HPP and Thermal Treatment

Considering that the inactivation achieved by TT is much more effective than that with HPP (Teribia et al., 2021), different researchers have evaluated the combined effect of high pressure and thermal inactivation. Sulaiman et al. in 2013 also evaluated the PPO inactivation with a combined treatment: 200 and 600 MPa for 5 min, under 40, 50, 60ºC, these temperatures varied from the set point during the holding pressure stage exceeding in all cases the proposed conditions and varying in an interval during the treatment. The results obtained show that there is a significant reduction in the residual activity in comparison with the samples without thermal assistance: RA from 65% to 9% was measured in a temperature interval of 34 to 71 ºC at 600 MPa, in the case of the low pressure condition, an increase of the activity was observed (same as the non-heated sample), achieving a final reduction from 110% to 22% in the temperature range of 29 to 65ºC. A similar study conducted by Marszałek et al., in 2014, strawberry puree was tested under 300 and 500 MPa for 5, 10 and 15 min, with two controlled temperatures (0 and 50ºC), to evaluate the effect on different parameters including PPO and POD activity. The results support the previous finding, showing a direct relationship between the increase of the temperature and the increase of the inactivation. At 0 ºC, no significant inactivation at any time-pressure combination was observed for PPO and POD. In contrast, a significant reduction in the activity was measured for both PPO and POD, with the increase of time and pressure at 50 ºC.

2.3 Sour cherry, red grapes and HPP

The use of high pressure technology for the cherry processing has been already studied, considering microbial reduction, phenolic compounds, anthocyanin content, vitamin C, sensory characteristics among others, even combining thermal treatment and HPP (Dalla Rosa et al., 2019; Garcia-Parra et al., 2017; Bayındırlı et al. 2006). Nevertheless, none of them has evaluated the effect of the treatment in the enzymatic activity. Jia et al. in 2011 studied the properties of PPO present in sour cherry, evaluating the effect of pH, temperature, substrate, substrate concentration and inhibitors in the enzymatic activity, unfortunately nothing referred to pressure effect was evaluated.

The case of red grapes is very similar, the evaluation of the differences in anthocyanins, polyphenols, antioxidant capacity and enzymatic activity (PPO) of different red grape cultivars has been conducted by Orak et al. in 2007, finding significant differences in between cultivars respect to PPO activity. About the use of HPP technology, Morata et al. in 2015 evaluated its effect on the microbial population and phenolic extraction towards the production of wine, no mention about the enzymatic inactivation measurement during the study.

This study is focused on the evaluation of the effect of HPP technology on the inactivation of endogenous enzymes (PPO, POD, β-glucosidase and AOX), optimizing the operational conditions to achieve the greatest inactivation, for strawberries, sour cherries and red grapes, fruits with high sensitivity to color degradation who can be highly influenced by the action of these protein structures.

3. HiStabJuice PROJECT

3.1 Overview

HiStabJuice, is an international, intersectoral and interdisciplinary network that combines the scientific expertise of 5 universities and 2 research institutions with the technological experience of 10 industrial partners in 7 European countries. The project is focused on the evaluation of different factors that influence the color stability in fruit juices, emphasizing in raw materials and processing techniques, also considering some associated effects that could compromise the health benefits associated with the final products.

The contribution to color stability and nutritional value of fruit variety, harvest time, ripeness, thermostable enzymes and preservation techniques (pasteurization, freezing, PEF, Ohmic heating, HPP), will be evaluated in a 4D approach (microbes, enzymes, nutrients and physico-chemical properties) (HISTABJUICE, 2021)

3.2 Experimental design

To optimize the operational conditions of HPP treatment, a response surface methodology is proposed to evaluate the simultaneous effect of time, temperature and pressure on the inactivation of PPO, POD, β-glucosidase and AOX. The experimental conditions will be selected according to the maximum and minimum achievable conditions for the available HPP equipment, this to describe in a more effective way the design space beyond the regular operational conditions. A face centered central composite design is the selected experimental design with three independent variables and two levels per variable, this selection will allow to identify possible curvatures in the response surface. A quadratic polynomial equation (1) will be used to describe the response for each response variable.

$$Y = b_0 + \sum_{i=1}^{n} b_i x_i + \sum_{i=1}^{n} b_{ii} x_i^2 + \sum\sum_{i<j} b_{ij} x_i x_j \qquad (1)$$

where Y is the response variable, n the number of independent variables, b_0, b_i and b_{ij} are the coefficients and x_i and x_j the independent variables. Lack of fit, R^2 and R^2 adjusted are the selected indicators of the adequacy of the model. ANOVA will be evaluated to identify the significance of the terms presented in the model and the final version of the model will only include the significant terms according to this criteria. Finally, contour plot will be necessary to analyze the entire design space and fin an optimal solution that maximize the inactivation of all enzymes in the system.

4. Challenges and Conclusions

The implementation of the experimental proposal present some challenges: the randomization of the trials to guarantee the significance of the statistical conclusions drawn after the study, this because the some parameters like temperature (usually in case of drastic changes) require some time to stabilize in between trials, creating downtimes in the operation.

Respect to the characteristics of the samples there is an intrinsic variation in the enzymatic availability for different varieties and cultivars making possible that some enzymes will be present in a low concentration even out the range of detection of the analytical techniques, making impossible to stablish a correlation in between the operational variables and the response.

In conclusion, despite the exposed challenges, this study represents an interesting opportunity to optimize the equipment parameters to achieve greater enzyme inactivation and subsequently better color stability for strawberry, sour cherry and red grape juices.

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