THE ROLE OF BROWNING ENZYMES IN CHERRIES

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Abstract
Cherries contain significant amounts of important nutrients and bioactive food components including fibre, polyphenols, carotenoids, vitamin C, potassium. They are also good source of tryptophan, serotonin, and melatonin. Beside the fact that cherries are considered as an excellent source of numerous nutrients and they also present a low caloric content. These facts lead to their increasing popularity in the human diet. Numerous studies suggest that their regular consumption has a positive effect on health and the well-being of individuals. Another bioactive food components found in cherries are enzymes. The interest in research about enzymes in cherries is not so significant as for other compounds like polyphenols or vitamins. However, number of studies were carried out to characterise enzymes and their function in cherries especially with relation to extending their shelf life. The aim of this work is to give a brief overview of latest research on browning enzymes, softening enzymes and glutathione S-transferase.

Keywords
cherry fruit, browning enzymes, softening enzymes, glutathione S-transferase

Introduction

Cherry fruit is a nutrient dense food with low caloric content. There are more than a hundred cultivars of cherries, which are grouped into two major types: sweet and tart cherries. Both types are grown in North America and in temperate regions of Europe and Asia. Cherries do not contain fat and cholesterol and represent low caloric fruit. They contain many essential minerals such as fibre, vitamin A, vitamin C, iron, calcium, proteins as well as abundant potassium. Red cherries also contain melatonin, which helps to protect against harmful toxins. Another bioactive components present in cherries are phenolics. These compounds can act as a free radical scavenger. They reduce reactive oxygen species, such as hydrogen peroxide and superoxide anion. This is very important because reactive oxygen species are associated with chronic diseases such as cardiovascular disease and cancer. Furthermore, due to its antioxidant properties, cherry fruit has many benefits such as prevention of some types of cancer, reduction of inflammation, prevention of gout and removal of muscle pain. With all mentioned advantages cherries get more popular among consumers [1,2].

One of the main issues with cherry production and fruit production in general is browning, which alters fruit colours, flavours, texture, and thus lowers its marketing value. The browning process can be caused by either enzymatic or non-enzymatic biochemical reactions. Two enzymes involved in the browning process are polyphenol oxidase (PPO) and peroxidase (POD). Several studies were carried out to find a way of treating cherries that would extend their shelf life by inhibition PPO and POD enzymatic activity for example by using chitosan coating or modified atmosphere packaging [3].

This paper gives a brief overview on studies concerning PPO and POD isolation, purification, enzymatic activity during maturation and storage, as well as inhibition of these enzymes by mentioned treatments. Furthermore, research on other enzymes present in cherries such as softening enzymes and glutathionine S- transferase (GST) is discussed.

Cherries

Sweet cherry (*Prunus avium, L.*) and tart cherry (*Prunus cerasus L.*) are the two most important cherry species

commercially. Both species are native to Southeast Europe and Western Asia. Sweet cherries are grouped into two major types. Heart-type cherries, which are ovoid or heart-shaped and have relatively soft flesh, mostly ripening early. Bigarreau type is more commercially important, the fruit is firmer and crisp-fleshed and ripe mid to late season. Eastern Europe, Slovenia, Hungary, and Romania are large producers of tart cherry, while sweet cherry production is concentrated in Western Europe, Italy, Switzerland, France, and Spain. Worldwide the biggest producers of both sweet and tart cherries are Turkey, the United States, Iran, and Russia. Nutrients and phytochemicals can be found in both, sweet and sour cherries. They have low caloric content, as well (63.0 kcal per 100 g for sweet cherry and 50.0 kcal per 100 g for sour cherry) [4].

Fruit chemistry

The best indicators of quality of both types of cherries are soluble solids and fruit colour. Typically, high quality tart cherries have minimum 15 % of soluble solids and sweet cherries have at least 20 % (Table 1) [4].

Titratable acidity (TA) is another attribute, which plays an important role in the acceptability by consumers. It is highly cultivar-dependent parameter. Cherries are considered as slightly acidic fruit with the pH values of sweet cherries ranging from 3.7 to 4.2 and sour cherries ranging from pH 3.1 to 3.6. Significant differences between sweet and sour cherry and among cultivars in TA were observed. One of the main analytical measures of fruit quality is maturity index (TSS/TA ratio), which influences the perception of sweetness and flavour. With the increase of TSS/TA ratio of cherry fruits the consumer perception of sweetness rises, too. The sweet cherry has lower acidity level resulting in higher TSS/TA ratios. Important factors for sensory quality are aroma and flavour, although the contributing compounds represent only 0.001–0.01% of the fruit's fresh weight (FW). Both, free volatile compounds and glycosidically bound aromatic compounds contribute to aroma of cherries. In first mentioned group more than 100 compounds have been identified with hexanal and benzaldehyde as one of the main compounds. Alcohols represent second largest group following by acids, esters, monoterpenes, sesquiterpenes and diterpenes [5].

Table 1. Comparison of characteristic factors in sweet and tart cherries in 100g edible portion. *Source: [5].*

Component	Sweet cherry	Tart cherry
Soluble solids	20 %	15 %
pH	3.7-4.2	3.1-3.6
TA (malic acid)	0.7-1.2 g	1.4-2.9 g
TSS/TA	19.0-40.0	5.8-15.8

Nutritional composition

The main component of cherries is water with its content of 80-83 % in sweet and 81-88 % in tart cherries (Table 2). The most abundant macronutrients in cherries are carbohydrates. Their amount range from 12.2 to 17.0 g per 100 g edible portion for sweet cherry and the average value for tart cherry is 12.2 g per 100 g edible portion. The protein content is between 0.8 and 1.4 g per 100 g edible portion for sweet cherry and below 1.0 g per 100 g edible portion for tart cherry. The main sugars are glucose, fructose, and sorbitol. Regarding organic acids, the malic acid accounts for 98% of the total organic acid content. The main mineral in cherry is potassium. Other minerals observed in cherries in higher amount are calcium, phosphorus, magnesium, and sodium. Cherries contain significant amounts of vitamins especially vitamin C, vitamin E and vitamin K. Sour cherries have higher content of vitamin A than sweet cherries [5].

Table 2. Comparison of nutritional composition in sweet and tart cherries in 100g edible portion. *Source: [5].*

Component	Sweet cherry	Tart cherry
Soluble solids	20 %	15 %
pH	3.7-4.2	3.1-3.6
TA (malic acid)	0.7-1.2 g	1.4-2.9 g
TSS/TA	19.0-40.0	5.8-15.8
Water	80-83 %	81-88 %
Carbohydrates	12.2 -17.7 g	12.2
Protein content	0.8-1.4 g	<1.0 g
Potassium	260 mg	200 mg
Vitamin A	3.0 mg	64.0 mg

Phytochemical composition and antioxidant activity

Sweet cherries contain many phytochemical compounds. The vast of majority are carotenoids, mostly β-carotene, lutein, and zeaxanthin. Another important group of phytochemicals are polyphenols which contribute to colour, taste, aroma, and flavour of cherries and flavonoids. Those compounds take place in antioxidative defence of plants against biotic and abiotic stresses for example high or low temperatures, drought, alkalinity, salinity, UV stress and pathogen attack. The skin of cherry fruits contains the highest content of total polyphenolic compounds, followed by the flesh and the pit. Recent research suggests contribution of polyphenols in the prevention of cardiovascular diseases, cancers, diabetes, insomnia, obesity, and osteoporosis. Another widely abundant type of aromatic secondary plant metabolites are phenolic acids. They affect food quality and organoleptic properties, and are divided into two subgroups; the hydroxybenzoic and the hydoxycinnamic acids. Flavonoids are also present in cherries including anthoxanthins (flavones and flavonols), flavanones, flavanonols, flavans and anthocyanidins. The common anthocyanidins, that give the attractive colour of cherries, are cyanidin, pelargonidin, peonidin, delphinidin, petunidin and malvidin. Flavonols are very important bioactive compounds, crucial for human health. Six flavonols have been quantified in sweet cherry fruit with quercetin being the main one. Cherries contain also indolamine melatonin (MLT), which is an endogenous hormone. The main function of MLT in mammals is regulation of the sleep–wake cycle, but MLT is also potent free-radical scavenger and a broad-spectrum antioxidant [5].

Enzymes in cherries

There are several enzymes found in cherries. This chapter gives brief overview on research about the most important ones such as enzyme PPO and POD which contribute to enzymatic browning in fruit, glutathionine-S-transferase (GST) which acts as detoxification enzyme protecting cellular macromolecules from attack by reactive electrophiles and enzymes that are responsible for fruit softening during maturation and storage.

Enzymatic browning

Enzymatic browning in fruit or vegetable occurs during handling, storage, and processing. It negatively influences the sensory properties and marketability of the product and decreases the nutritional qualities due to associated changes in colour, flavour, and softening. Approximately 50% losses in fruit occur because of enzymatic browning, which is catalysed by PPO and POD [6].

In the presence of molecular oxygen, PPO catalyses the o-hydroxylation of monophenols to o-diphenols (monophenolase activity) and oxidation of the o-diphenols to o-quinones (diphenolase activity), which polymerise into undesirable brown, red and black pigments. These pigments then affect nutritional value in fruit products. PPO is a copper containing enzyme and it is widely abundant in the plant and animal kingdoms. In plants, PPO is present in chloroplasts and its phenolic substrates are mostly located in the vacuoles. If the cell is damaged, the enzyme can be exposed to substrates which leads to rapid oxidation of phenols. PPOs occur in isoforms which were detected for example in apple, banana grape, kiwifruit, lettuce, mushroom, peach, pineapple, potato, spinach, strawberry, and sweet potato. These isoforms differ in their physical, chemical, or enzymatic properties such as electrophoretic mobility, temperature and pH optimum, substrate specificity and pI. Another enzyme involved in enzymatic browning is POD. It is a dicopper-containing enzyme which is found in plants, microbes, and animals. It can reduce diphenols and this enzyme is also involved in lignin production. POD activity is limited by the absence of electron compounds such as superoxide radicals, hydrogen peroxide and lipid peroxides, however it was reported that the enzyme catalyses the browning process of different fruits and vegetables. To extend a shelf life of a fruit or a vegetable, it is necessary to understand the PPO and POD activity and to determine their characteristics to inhibit or control their activity [6,7]

Isolation and Characterization of PPO from White Cherry Fruit

PPO from white cherry fruit was extracted and purified through $(NH_4)_2SO_4$ precipitation, dialysis, and ion exchange chromatography. Using Toyopearl 650 M column the enzyme showed two peaks with PPO activity, which referred to isoenzyme A and isoenzyme B (Table 3). The isoenzymes were further characterised to find pH optima, temperature optima and affinity for catechol [6].

Table 3. Values of absorbance and white cherry PPO activity on Toyopearl 650 M at the peak. *Source:[6].*

Isoenzyme	Absorbance (280 nm)	PPO activity (units)
Isoenzyme A	3,6	10,000
Isoenzyme B	0,7	10,000

Extraction and purification

Due to the fact, that some PPOs are membrane-bound, detergents are required to solubilize the enzyme. Phenolic compounds interfere with purification of proteins from plants by cross-linked proteins with hydrogen bonds and covalent interactions. Furthermore, homogenization of the plant tissues initiates enzymatic browning which leads into formation of quinones, that may form irreversible covalent linkages. Phenol-absorbing polymers (polyethylene glycol) and reducing agents (ascorbic acid) are commonly applied to overcome these problems [6]. The frozen berries were deseeded, and the pulps were homogenized in cold acetone containing polyethylene glycol. A filter paper was used for slurry vacuum filtration and the residue was re-extracted with cold acetone. This procedure was repeated until a white powder was obtained and then it was dried overnight at room temperature and stored. Acetone powder was homogenized in phosphate buffer containing ascorbic acid, detergents, and other chemicals, stirred and centrifuged. The resulting supernatant was mixed with ammonium sulphate to precipitate and centrifuged again. A small amount of 10 mM phosphate buffer was used to dissolve the precipitate and dialyze overnight in the same buffer. The dialysate was analysed by ion exchange chromatography and PPO activity was monitored using catechol as substrate [6].

Changes in the Chemical Content and Polyphenol Oxidase Activity during Development and Ripening of Cherry Laurel Fruits

Changes in the PPO activity and in the content of other chemicals during the development and ripening of cherry laurel fruits were investigated (Fig.1). Spectrophotometry was used to analyse PPO activity in the fruits harvested every week from the beginning of June to the mid July. It was observed that PPO activity and phenolic content gradually increased during the development of the fruits but decreased in the stage of ripening [8].

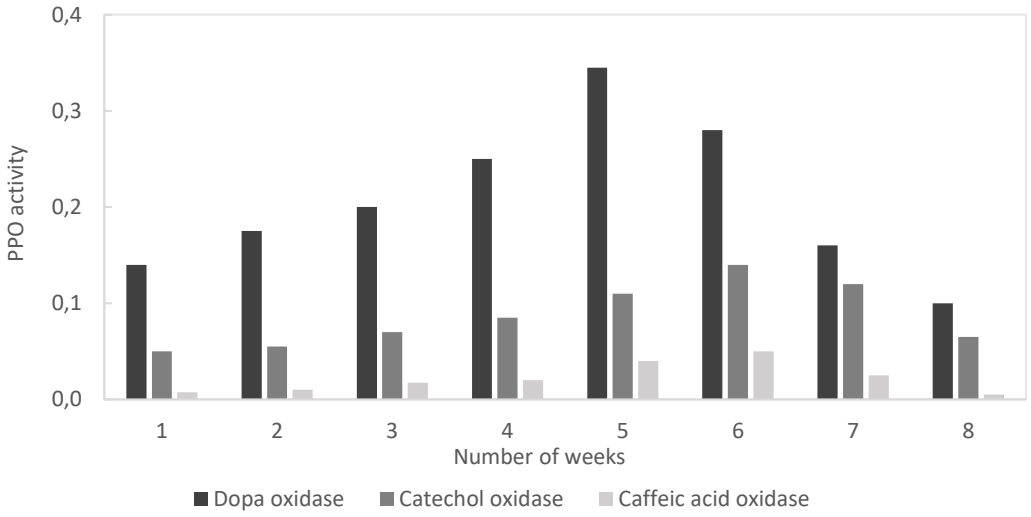

Fig. 1. Changes in the PPO activity during the development and ripening of cherry laurel.
Source: [8].

Polyacrylamide gel electrophoresis was used for further analysis and two isoenzyme bands of PPO were detected. The activities of both isoenzymes increased in the fifth and sixth week, then they decreased in the seventh and eighth week of ripening. The number of PPO isoenzymes did not change during the development and ripening of the fruits [8].

Responses of physiology and quality of sweet cherry fruit to different atmospheres in storage

The storage life of cherries is relatively short. They soften and darken during storage which is unattractive for consumers. Several studies were carried out to find a way of treating cherries that would extend their shelf life. Sweet cherry fruit was stored in modified atmosphere packaging (MAP) and controlled atmospheres (CA) of 5% O_2 plus 10% CO_2; or 70% O_2 plus 0% CO_2 at 1 °C. The effects of different O_2 and CO_2 concentrations on physiological properties, quality attributes and storability during storage periods of 60 days were determined. The inhibition of PPO (Fig. 2) and POD (Fig. 3) and content of malondialdehyde (MDA; the content of MDA indicates lipid peroxidation resulting from oxidative stress) (Fig. 4) were analysed using spectrophotometry and enzymatic activities were defined as an increase in one absorbance unit per minute. It was observed that CA with 5% O_2 and 10% CO_2 more significantly inhibited PPO and POD enzymatic activities and reduced MDA content. Furthermore, more effective was prevented flesh browning, decreased fruit decay, and extended storage life of sweet cherry fruit than did other treatments [9].

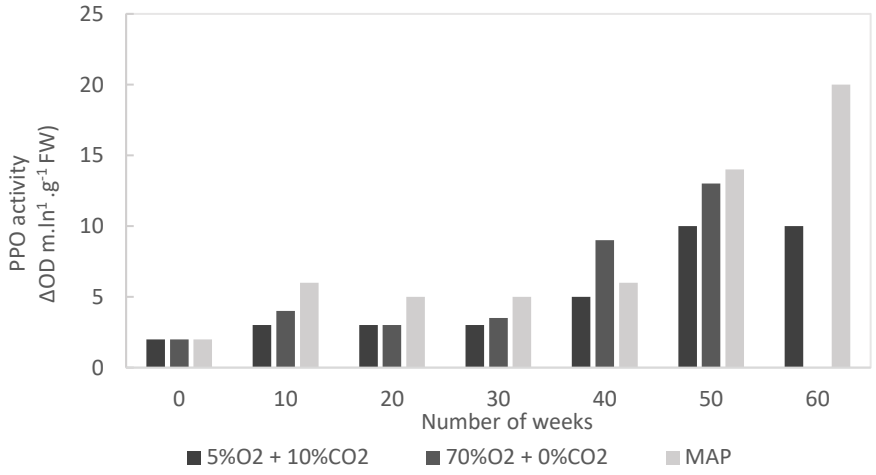

Fig. 2. Changes in PPO activity of sweet cherries stored in different atmospheres at 1 °C during storage periods. *Source: [9].*

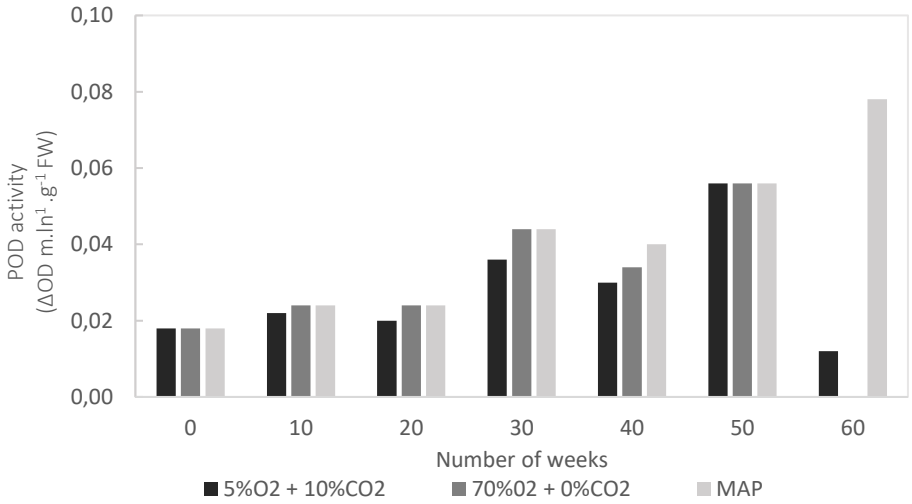

Fig. 3. Changes in POD activity of sweet cherries stored in different atmospheres at 1 °C during storage periods. *Source:[9].*

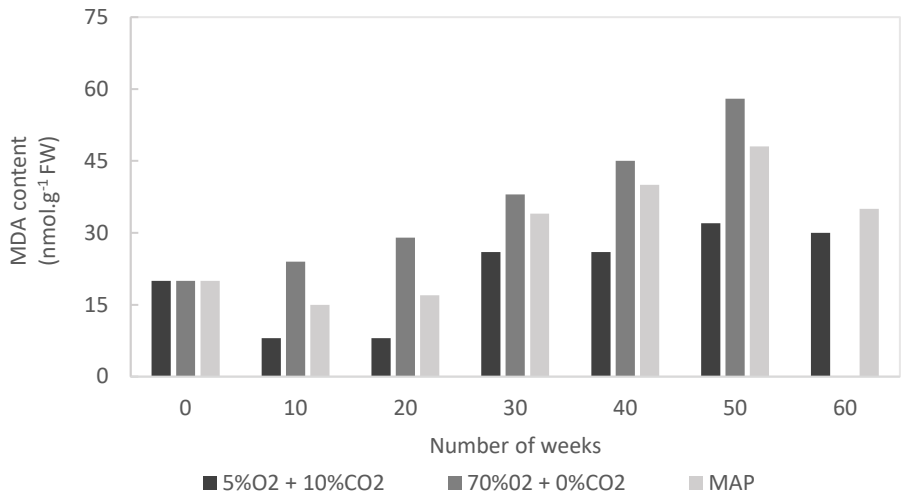

Fig. 4. Changes in MDA content of sweet cherries stored indifferent atmospheres at 1 °C during storage periods. *Source [9].*

The effect of CO_2 concentration on sweet cherry preservation in modified atmosphere packaging

Another study on using different atmospheres for storage of cherry fruits was carried out. Cherries were stored in concentrations of CO_2 at 0%, 5%, 10%, 15%, 20% and 25% (concentration of O_2 was 5% and the rest was filled with N_2). The quality change of sweet cherries was evaluated approximately from –1 to +1 °C and from 80 to 85% relative humidity. Deterioration of sweet cherries was inhibited by all air treatments. The most significant inhibition of reduction of soluble solids and vitamin C and reduction of the PPO and POD activities was obtained in 10% CO_2 group. This treatment reduced rotting rate and maintained firmness, nutrition, and taste of the fruit after 120 days of storage and thus this CO_2 concentration can be used as suitable gas storage conditions of sweet cherries in modified atmosphere packaging [10].

Influence of postharvest chitosan treatment on enzymatic browning and antioxidant enzyme activity in sweet cherry fruit

A study focusing on extending shelf life of cherry used chitosan as a treatment for cherries. The effect of chitosan fruit coating on enzymatic browning and antioxidant enzyme activity in three sweet cherry cultivars was evaluated. Cherries were dipped into 0.5% chitosan solution, stored at 2°C for 14 days (Fig. 5) and sampled at harvest, 7 and 14 days of cold storage followed by storage at 24 °C for 3 days (Fig. 6) to evaluate the shelf life of the fruit. Cherries were homogenized in sodium phosphate buffer containing polyvinylpyrrolidone (PVPP). Crude enzyme extract was incubated with a buffered substrate (catechol in sodium phosphate buffer) and monitored by measuring the increase in absorbance. Cherries dipped in distilled water were used as a control. The treatment inhibited PPO and POD activities and as a result flesh-browning was prevented. Therefore, storage life of sweet cherry fruit was extended [11].

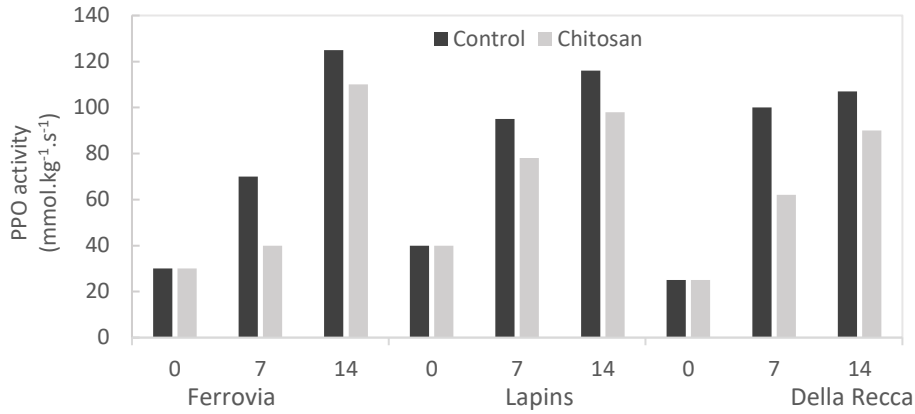

Fig. 5. PPO activity of the three sweet cherry cultivars at harvest (0), after 7 and 14 days of cold storage on chitosan-coated (chitosan) and uncoated fruit (control). *Source: [11].*

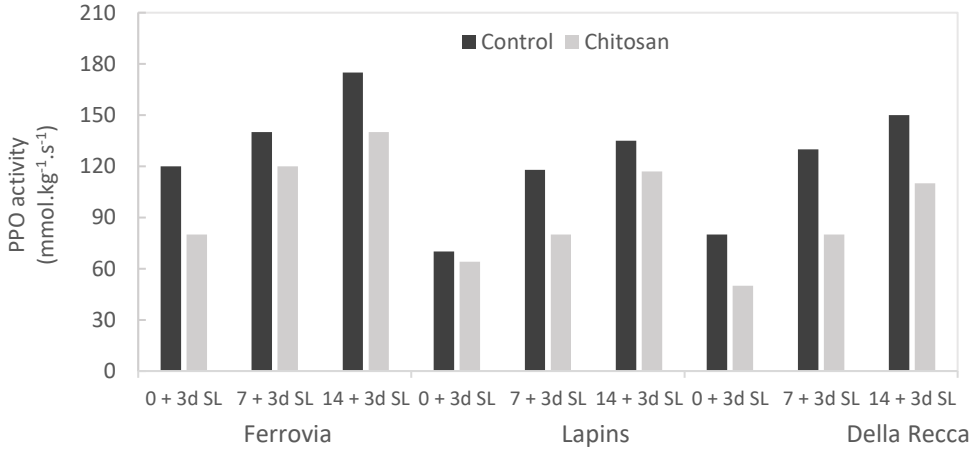

Fig. 6. PPO activity on the three sweet cherry cultivars at harvest after 3 days of shelf life on chitosan-coated (chitosan) and uncoated fruit (control). *Source: [11].*

Effect of β-aminobutyric acid on cell wall modification and senescence in sweet cherry during storage at 20 °C.

An experiment investigated how the postharvest β-aminobutyric acid (BABA) treatment affects fruit firmness, pectin degrading enzymes, cell wall constituents and microstructural alterations of pericarp in sweet cherry fruit. The results showed that BABA treatment slowed down fruit senescence and softening, probably due to depressed membrane permeability and malondialdehyde content. Activities of polygalacturonase (PG) and pectinmethylesterase were significantly decreased by BABA treatment. Furthermore, the treatment enhanced cell wall polysaccharides content and maintained subepidermal cell structure in sweet cherry.

PG activity was measured using spectrophotometry and pectin methyl esterase (PME) activity using acid-base titration. BABA treatment inhibited PG activity (Fig. 7) after 3 days of storage and PME activity after 2 days of storage (Fig. 8). On the 5th day, activity of PME was 25% lower than activity in control fruit [12].

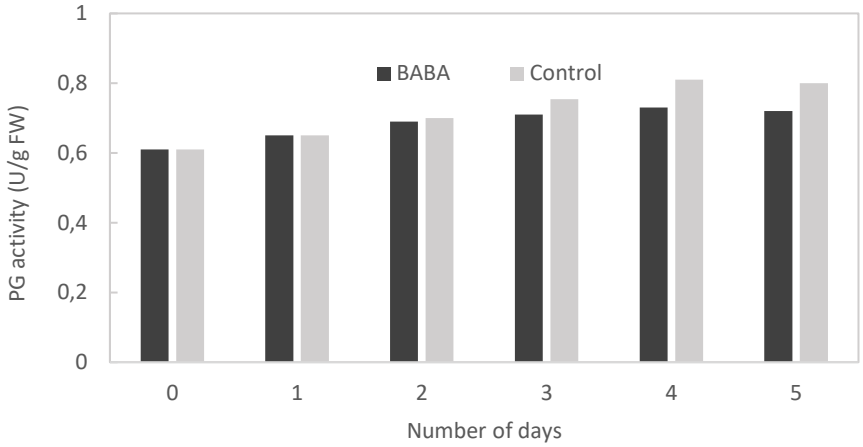

Fig. 7. Effect of BABA treatment on PG activity in sweet cherry fruit during storage at 20 °C. *Source:*[12]

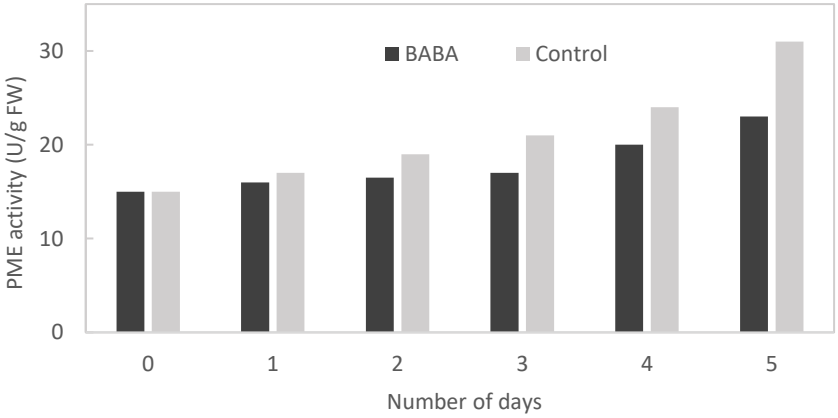

Fig. 8. Effect of BABA treatment on PME activity in sweet cherry fruit during storage at 20 °C. *Source:* [12].

Purification and Characterization of Glutathione S-Transferase from Cherry Laurel

Another enzyme found in cherry fruit is GST. It is a multifunctional enzyme that removes a high number of electrophilic xenobiotics in living organisms by binding to tripeptide glutathione (GSH) and plays important role in the detoxification system by various mechanisms. GST removes some toxic compounds from the system by covalent or non-covalent bonding and by incorporation into phase II reactions in the detoxification of xenobiotics. Furthermore, this enzyme produces antioxidant activity against the stress caused by organic hydroperoxides with peroxidase activity. GST is found in various organisms as in microbes, insects, fungi, fish, birds, mammals and plants [13].

GST was obtained from the cherry laurel flesh fruit. Two separate processes were used for enzyme purification: gel filtration and affinity chromatography. The sodium dodecyl sulphate electrophoresis method was used to determine enzyme purity. GST was analysed to obtain optimum pH, optimum temperature, optimum ionic strength, stable pH, and KM and Vmax values for glutathione and 1-chloro-2,4-dinitrobenzene. Furthermore, the inhibitory effects of metal ions and organic compounds were studied. It was found that Cd^{2+}, Ni^{2+}, Cu^{2+}, Mg^{2+}, and Ca^{2+} metal ions did inhibit activity of the enzyme, Ca^{2+} ions with the highest rate and Cd^{2+} with the lowest. Concerning organic molecules, ethylenediaminetetraacetic acid (EDTA), sodium dodecyl sulphate (SDS), benzoic acid, ascorbic acid, ethanol, and tocopherol (vitamin E) were used as the inhibitors and SDS was found to have the fastest inhibition rate and EDTA the slowest [13].

Activity of Softening Enzymes during Cherry Maturation

Important quality factor in cherries is the texture. During the mature the firmness of the fruit decreases due to structural changes in cell wall and middle lamella, which lead to cell separation and softening tissue. These changes occur due to the enzymes such as PG (Fig. 9), PME (Fig. 10), β-galactosidase (β -gal) (Fig. 11), cellulose

and others. This study analyses activity of the four mentioned enzymes during the cherry maturation and storage in two cultivars [14].

Cellulase activity was not detected at any time during maturation or storage, but activity of PG, PME and β-gal was changing during maturation. The level of PG activity in cherries (0.32 units/g fresh weight) was relatively low compared to other fruits: avocado 0.8, peaches 4 to 6, pears 20 to 70 units/g fresh weight. PME activity was detected at earlier stages than PG activity and it was increasing continually during maturation and storage and reached a maximum at harvest in both cultivars. β-gal activity was also detected at the early stages of cherry ripening and the maximum was reached later in both cultivars than the peaks in either PG or PME [14].

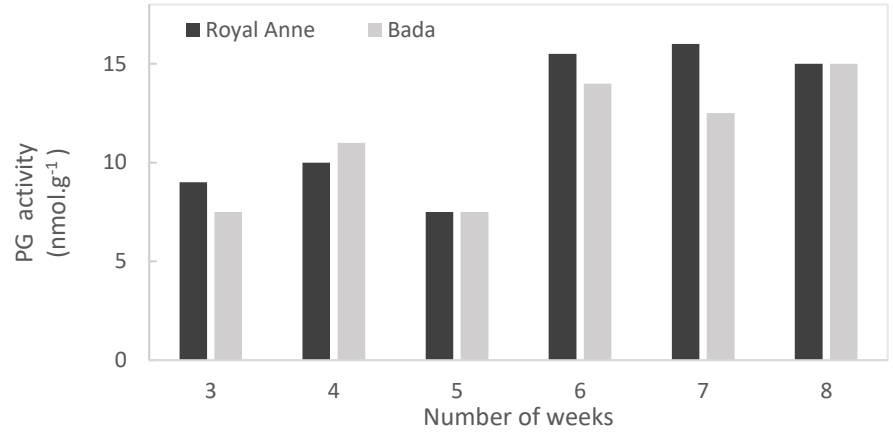

Fig. 9. Polygalacturonase activity in Bada and Royal Anne cherries. *Source: [14].*

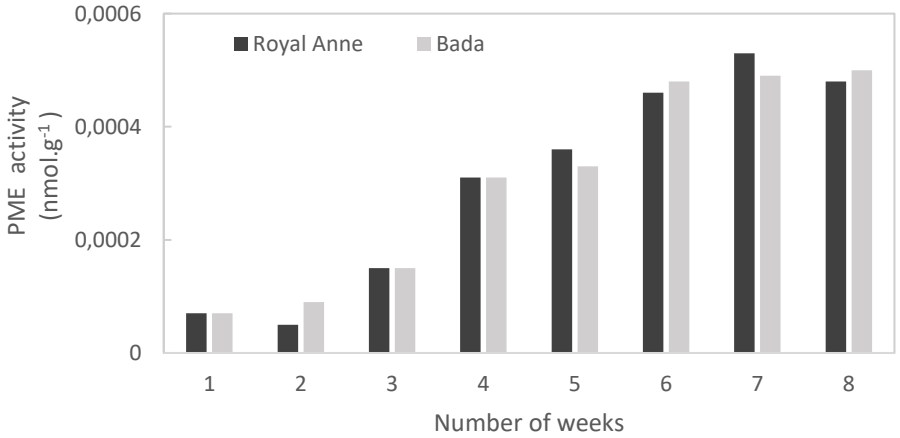

Fig. 10. Pectin methyl esterase activity in Bada and Royal cherries. *Source: [14].*

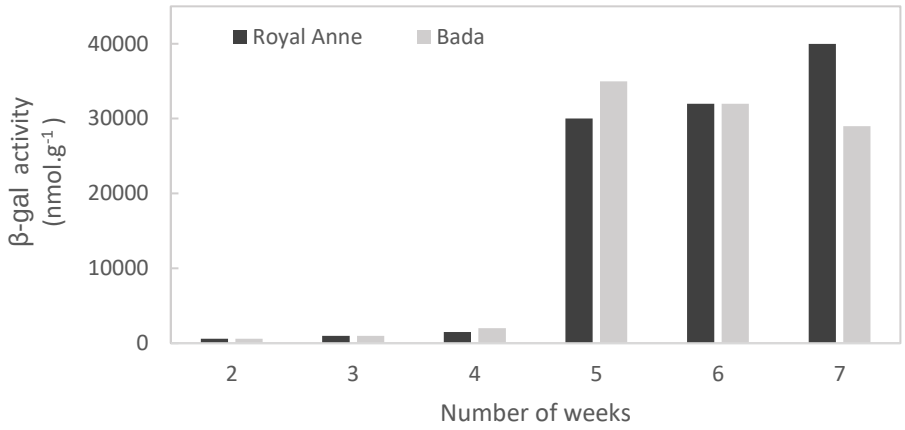

Fig. 11. β-galactosidase activity in Bada and Royal cherries. *Source: [14].*

Impact

As well as cherries, different kinds of fruit are also beneficial for human health because they contain vitamins, antioxidant compounds and fibre. During the harvest, preparation, and storage the fruit undergoes changes and loses both microbial and antioxidant properties. Following microbial contamination, oxidation is the second most significant cause of food deterioration inducing changes in flavour, odour, and nutritional value. The development of various methods and treatments for fruit preservation and extension of postharvest life of fruit is nowadays a high priority in the food industry [15].

This paper summarizes different methods used in treatment of cherry fruits developed in past years. The results showed that all mentioned treatments, particularly chitosan coating, BABA coating and storage in different atmospheres improved the postharvest life of cherry fruit. These findings are very interesting for possible real-life applications. The agricultural industry is and will benefit, first economically, able to maintain the freshness of the fruit and longer shelf-life and secondly, by introducing sustainable technology in food processing. Furthermore, the methods for isolation and purification of several enzymes found in cherries are discussed. This paper presents a review of studies published in past years concerning enzymes in cherries. Because we know that enzymes are important for the healthy functioning of the organism as well as on an industrial scale, we can expect the effects in different areas of human activity. With knowledge of the importance and content of enzymes in a particular type of fruit, an individual can plan an appropriate healthy diet. On the other hand, an impact on the development of the industry is expected, not only in the food industry but also in the production of fine chemicals.

Conclusion

Cherries are low caloric fruit with high content of important bioactive compounds and nutrients including fibre, polyphenols, carotenoids, various vitamins and minerals, and hormones. Nowadays, cherry fruit becomes more and more popular among consumers due to its health benefits. One of the main issues with production of cherries is their short shelf life as they darken and soften when stored. Several enzymes contribute to these changes and they have attracted attention of numerous researchers. Some experiments such as chitosan and MAP treatment successfully inhibited browning enzymes and slow down deterioration of the fruit. However, more studies should be carried out to find a way how to extend shelf life of this highly nutritious fruit even more and thus prevent its loss.

Conflict of interest

There are no conflicts to declare.

Acknowledgments

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References

[1] D.S. Kelley, Y. Adkins, K.D. Laugero, A review of the health benefits of cherries, Nutrients. 10 (2018) 368. https://doi.org/10.3390/nu10030368.

[2] T. Gür, F. Karahan, H. Demir, C. Demir, Investigation of Some Antioxidant Enzyme Activities in Cherry Fruit, Biosci. Biotechnol. Res. Asia. 16 (2019) 725–729. https://doi.org/10.13005/bbra/2788.

[3] P. Nokthai, V.S. Lee, L. Shank, Molecular modeling of peroxidase and polyphenol Oxidase: Substrate specificity and active site comparison, Int. J. Mol. Sci. 11 (2010) 3266–3276. https://doi.org/10.3390/ijms11093266.

[4] W. Loescher, Cherries (Prunus spp.): The Fruit and Its Importance, in: Encycl. Food Heal., Academic Press, 2015: pp. 10–13. https://doi.org/10.1016/B978-0-12-384947-2.00138-0.

[5] M.J. Serradilla, M.F. Aksic, G.A. Manganaris, Ercisli, Fruit chemistry, nutritional benefits and social aspects of cherries, in: J. Quero-García, A. Lezoni (Eds.), Cherries Bot. Prod. Uses, Cabi, 2017: pp. 420–441.

[6] M.Ü. Ünal, Ö. Gökkaya, A. Şener, Characterization of Polyphenol Oxidase from White Cherry Fruit (Starks gold), GIDA. 36 (2011) 255–262.

[7] X. Zhang, X. Shao, Characterisation of polyphenol oxidase and peroxidase and the role in browning of loquat fruit, Czech J. Food Sci. 33 (2015) 109–117. https://doi.org/10.17221/384/2014-CJFS.

[8] A. Kadioglu, I. Yavru, Changes in the Chemical Content and Polyphenol Oxidase Activity during Development and Ripening of Cherry Laurel Fruits, Phyt. - Ann. Rei Bot. 37 (1998) 241–251.

[9] S.P. Tian, A.L. Jiang, Y. Xu, Y.S. Wang, Responses of physiology and quality of sweet cherry fruit to different atmospheres in storage, Food Chem. 87 (2004) 43–49. https://doi.org/10.1016/j.foodchem.2003.10.014.

[10] S. Xing, X. Zhang, H. Gong, The effect of CO2 concentration on sweet cherry preservation in modified atmosphere packaging, Czech J. Food Sci. 38 (2020) 103–108. https://doi.org/10.17221/255/2019-CJFS.

[11] M. Petriccione, F. De Sanctis, M.S. Pasquariello, F. Mastrobuoni, P. Rega, M. Scortichini, F. Mencarelli, The Effect of Chitosan Coating on the Quality and Nutraceutical Traits of Sweet Cherry During Postharvest Life, Food Bioprocess Technol. 8 (2015) 394–408. https://doi.org/10.1007/s11947-014-1411-x.

[12] L. Wang, P. Jin, J. Wang, L. Jiang, T. Shan, Y. Zheng, Effect of β-aminobutyric acid on cell wall modification and senescence in sweet cherry during storage at 20°C, Food Chem. 175 (2015) 471–477. https://doi.org/10.1016/j.foodchem.2014.12.011.

[13] F. Türkan, A. Aygun, H. Şakiroğlu, F. Şen, Glutathione S-Transferase: Purification and Characterization of from Cherry Laurel (Prunus laurocerasus L.) and the Investigation In Vitro Effects of Some Metal Ions and Organic Compounds on Enzyme Activity, Bionanoscience. 9 (2019) 683–691. https://doi.org/10.1007/s12668-019-00636-w.

[14] D.M. Barreit, C. Gonzalez, Activity of Softening Enzymes during Cherry Maturation, J. Food Sci. 59 (1994) 574–577. https://doi.org/10.1111/j.1365-2621.1994.tb05565.x.

[15] L. Vámos-Vigyázó, Prevention of Enzymatic Browning in Fruits and Vegetables, Eur. Sci. J. 9 (1995) 49–62. https://doi.org/10.1021/bk-1995-0600.ch004.