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Inhibitory effects of some additives on LOX activity in Santa Maria pear puree

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Abstract: Lipoxygenase (EC 1.13.11.34.; LOX) enzymes; They are iron carrier dioxygenases that oxidize fatty acids with two or more unsaturated bonds in their structure and do not contain heme groups in their structure. LOXs are found in plants, animal tissues, and cyanobacteria. In this study, the change of LOX enzyme activity in Santa Maria pear puree depending on different concentrations of fumaric acid, syringic acid and rosmarinic acid and cooking process was followed for 7 days. It has been observed that fumaric acid increases LOX activity. This increase was not prevented by the cooking process. It was observed that syringic acid and rosmarinic acid decreased LOX activity day by day, and a faster decrease in activity was observed in the samples that were additionally cooked. At the end of 7 days, it was observed that rosmarinic acid caused approximately 60% inhibition, while syringic acid caused approximately 80% inhibition. Based on these results, we can say that the cooking process with the addition of rosmarinic acid or syringic acid to the medium in order to prevent the loss of taste, smell and flavor of the pear, which is frequently used in baby complementary foods and fruit juices, extends the shelf life.

Keywords: LOX, rosmarinic acid, syringic acid, fumaric acid, shelf life.

Santa Maria armudu püresinde LOX aktivitesi üzerine bazı katkı maddelerinin inhibisyon etkileri

Özet: Lipoksigenaz (EC 1.13.11.34.; LOX) enzimleri; yapısında iki ya da daha fazla doymamış bağ bulunduran yağ asitlerini oksitleyen, yapılarında hem grubu bulundurmayan demir taşıyıcı dioksigenazlardır. LOX'lar bitkiler, hayvan dokuları ve siyanobakterilerde bulunurlar. Bu çalışmada Santa Maria armudu püresindeki LOX enzimi aktivitesinin fumarik asit, sirinjik asit ve rosmarinik asitin farklı konsantrasyonlarına ve pişirme işlemine bağlı olarak değişimi 7 gün boyunca takip edilmiştir. Fumarik asitin LOX aktivitesini artırdığı gözlenmiştir. Bu artışa pişirme işlemi de engel olamamıştır. Sirinjik asit ve rosmarinik asitin LOX aktivitesini gün geçtikçe azalttığı, ek olarak pişirme uygulanan örneklerde daha hızlı bir aktivite azalışı olduğu görülmüştür. 7 gün sonunda rosmarinik asitin yaklaşık % 60'lık inhibisyona sebep olduğu, sirinjik asitin ise yaklaşık %80'lik bir inhibisyona sebep olduğu görülmüştür. Bu sonuçlardan yola çıkarak bebek ek gıdaları ve meyve sularında sıkça kullanılan armudun tat, koku ve lezzet kaybının önlenmesi için ortama rosmarinik asit ya da sirinjik asitin eklenmesiyle birlikte pişirme işlemi uygulanması raf ömrünü uzatmaktadır diyebiliriz.

Anahtar Kelimeler: LOX, rosmarinik asit, sirinjik asit, fumarik asit, raf ömrü.

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1 INTRODUCTION

Lipoxygenases (LOXs) are a family of monomeric proteins that produce hydroperoxide by oxidation of polyunsaturated fatty acids (PUFA) such as linoleic, linoleic, and arachidonic acids [1]. LOXs are found in plants, animals, fungi and cyanobacteria [2]. 5-LOX is ubiquitous in the mammalian body, its job is to oxidize the number 5 carbon atom. 9-LOX and 13-LOX are found in plants and they catalyze the oxidation of linoleic and linolenic acid [2, 3]. Lipoxygenases play an important role in stimulating inflammatory reactions in the human body. The excessive reactive oxygen species formed in the body first stimulates the release of cytokines and then the activation of the LOX enzyme, the result may be inflammation. Inflammation in the body is associated with many diseases such as cancer, stroke, cardiovascular and neurodegenerative diseases. LOXs are involved in the synthesis of prostaglandins and leukotrienes, therefore they are associated with diseases and their inhibition is considered important for disease treatment [4].

LOXs are also abundant in grains, legumes and potato tubers [5, 6]. The products produced in the LOX metabolic pathways have various effects. LOX acts as a storage protein in vegetative growth and also mobilizes storage lipids during germination [7]. LOX protects plants from pathogens and insects. It also plays a role in the production of substances such as jasmonates, divinyl ethers, leaf aldehydes that help protect during abiotic stress [8, 9].

On the other hand, the reaction between unsaturated fatty acids and LOX can cause bad taste and odour, resulting in food spoilage. Therefore, investigation of LOX inhibition has gained importance [10]. Plant phytochemicals play an important defensive role in preventing diseases caused by oxidative stress. Plants have been used as medicine for centuries. Today, many drugs are isolated from plants and there is increasing interest in plant-derived therapeutics [11].

Fumaric acid ((E)-2-butenedioic acid or trans-1,2-ethylenedicarboxylic acid) (Fig. 1) is a naturally occurring organic acid originally obtained from the plant Fumaria officinalis. Since it is synthesized as a key intermediate in the citrate cycle, it is produced in many living things, albeit in small amounts [12]. Fumaric acid esters have been used for many years in the treatment of chronic plaque psoriasis [13] and multiple sclerosis [14] diseases.

Syringic acid (SA) (Fig. 1) is a phenolic compound found in many natural foods (pumpkin, grapes, dates, various spices, olives, acai palm, honey and wine) [15, 16] with important effects such as antioxidant, antiinflammation, antimicrobial, anticancer, antidiabetic, heart, liver and brain / CNS (central nervous system) protection [17].

SA is also used in industry. Syringic acid is among the many phenolic compounds that contribute to the structural integrity of lignin. Syringic acid in lignin acts as a good substrate for laccase, an important enzyme in the pulp and bioremediation industry [18].

Although rosmarinic acid (Fig. 1) got its name because it was first obtained from rosemary, it is also found in a wide variety of herbs such as sage, mint, thyme, lemon balm, basil and thyme [19, 20]. Studies show that herbal medicines containing RA do not have serious side effects and have many positive effects on the body. In addition to in vitro studies, it has been reported to be effective in the treatment of metabolic syndrome, increasing cognitive performance, osteoarthritic disorders, various otolaryngological treatments and dermatological treatments in clinical studies. It has been targeted in many studies that RA is also used for long-term treatments by prolonging the elimination process from the body [21-28].

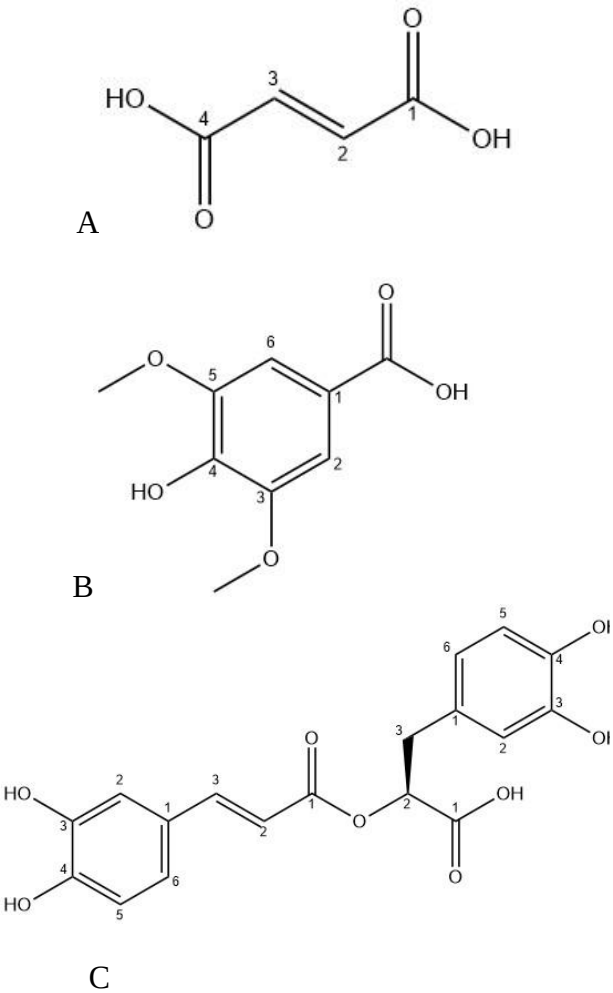

Fig. 1. Structure of fumaric acid (A), syringic acid (B) and rosmarinic acid (C).

In this study, fumaric acid, syringic acid and rosmarinic acid were added to pear puree and activity measurement was made for 7 days to examine the effects of these chemicals on LOX activity. The effects of this chemicals on the bitterness of pear puree provide important ideas for the shelf life of the puree.

The inhibitors used in the study were chosen because they are natural substances obtained from plants. so its use as an additive in the food industry will also be beneficial for human health. The use of such natural products in the sector is more preferred than artificially produced chemicals.

We think that it would be beneficial to add natural inhibitors that reduces LOX activity to the literature.

2 MATERIALS AND METHODS

2.1 Materials

The Santa Maria pear used in the study was obtained from greengrocers. The chemicals used were purchased from Merck, Sigma-Aldrich. (Fumaric acid CAS Number: 110-17-8, syringic acid CAS number: 530-57-4, roamarinic acid CAS number: 20283-92-5, linoleic acid CAS number: 60-33-3). In the study, activity measurements were made with the Beckman coulter DU730 UV-Vis spectrophotometer.

2.2. Preparetion of pear puree

Santa Maria pears washed and grated. The seed zone was not used in the study. Purees were taken into 10 mL glass jars. Weighings were made to correspond to 1mM, 2mM, 3mM, 4mM and 5 mM concentrations for each inhibitor and added to the jars and mixed.
Some of the grated pears were cooked at 100°C for 15 minutes and placed in 10 mL jars. After cooling, 3 mM inhibitory substance was added to each jar (Figure 2). Here, 3 mM was chosen so that the inhibitor concentration was not too high or too low. Jars were stored at 4˚C for 7 days. For each measurement, 500 µL of sample was taken from the jar, centrifuged at 1000 x g for 10 minutes, and activity with the supernatant was measured.

Fig. 2. Pear puree jar

2.3. Activity measurement procedure of LOX

For the activity measurement, the substrate solution was first prepared by dissolving 0.04 mM linoleic in 5 mL of methanol.
3 mL of 5mM phosphate buffer (pH 6.5) was added to 90 µL of substrate solution. It was incubated at 20˚C for 5 minutes. After incubation, 30 µL of enzyme was added and the change in absorbance was measured at 234 nm for 3 minutes [29].
Enzyme unit was defined as the amount of enzyme that converts 1 µmol of substrate to product in 1 minute under optimal conditions.

3 RESULTS

In this study, the effects of rosmarinic acid, fumaric acid, syringic acid and cooking process on the shelf life of pear puree was investigated. Activity measurement of LOX enzyme was performed for 7 days.

When the graph of rosmarinic acid is examined (Fig.3), the first thing to notice will be that the cooking process negatively affects the LOX activity and decreases the activity rapidly. Although there is no change in activity in the first 4 days at 5 mM concentration, a rapid decrease is observed in the following days. In other cases, no effective reduction in LOX activity was observed. This will cause the pear puree to deteriorate and deteriorate its taste. Based on these results, it can be said that 5 mM concentration of rosmarinic acid in raw pear puree and 3 mM concentration in cooked pear puree can be effective on LOX activity and prolong shelf life.

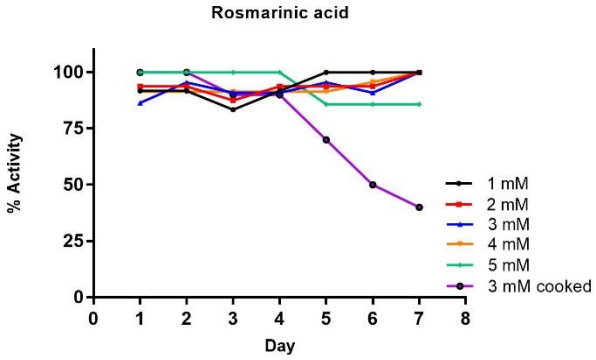

Fig. 3. Effects of rosmarinic acid on LOX

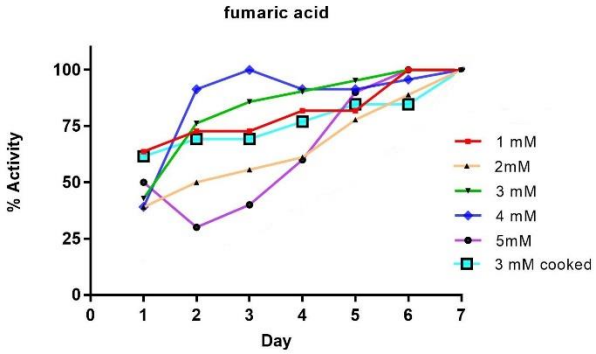

Fig. 4. Effects of fumaric acid on LOX

When Figure 4 is examined, it is seen that all concentrations of fumaric acid increase the LOX activity in pear puree for 7 days, even when cooked. Based on these results, it can be said that fumaric acid does not inhibit the LOX enzyme, but activates it and shortens its shelf life.

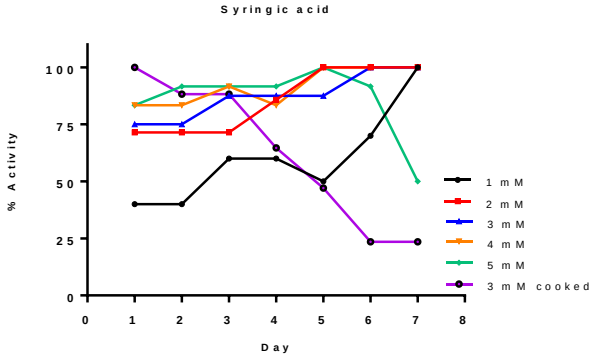

Fig. 5. Effects of syringic acid on LOX

Figure 5 shows that syringic acid has a similar effect to rosmarinic acid. It is seen that the LOX activity in the cooked pear puree containing 3 mM syringic acid experienced a rapid decrease from the first day. At the end of 7 days, approximately 75% of activity loss is observed. Pear puree containing 5 mM syringic acid appears to have half as much LOX activity as at the end of 7 days. Looking at the results obtained in the study, it can be said that the best inhibitor of LOX enzyme is syringic acid. rosmarinic acid also appears to be effective, but fumaric acid appears to increase LOX activity continuously. In addition, it is seen that the cooking process is very effective in extending the shelf life. A longer shelf life can be achieved by adding less additives.

4 DISCUSSION

For this reason, there are studies investigating LOX inhibitors in the literature. Yashanswinj et al. investigated the effect of sesame derivatives on LOX inhibition and reported that 51.84 μM amount of sesamol reduced LOX activity to half of the initial activity [30].

The effects of salicylic acid, eupatorin, eupatilin, gardenin A substances on LOX activity isolated from bovine liver in 2019 were investigated and the highest inhibition effect was seen in Eupatilin (IC_{50}=0.46 mM), and the lowest inhibition effect was seen in Gardenin A (IC_{50}= 5.31 mM) has been reported [22].

There are studies indicating that the correlation between lipoxygenase activity and quality changes such as color change and off-flavor formation in vegetables may be better [31-33] In a study carried out, it was determined that boiling at 100°C for 20 minutes was sufficient for LOX inactivation and no LOX regeneration was detected during the storage period. The tomato puree produced without the boiling process was stored at two different temperatures, -7 and -18°C. It was reported that the activity of the enzyme decreased gradually during 130 days of storage in these samples and no activity was observed when this period was extended [34]. This study with tomatoes could be explored for other foods as well.

Studies show that LOX is a very important enzyme for food quality and storage, and its inhibition can be done by various methods such as additives, heating and cold storage. It is thought that extensive studies should be carried out on LOX, especially in the food and health industry.

5 CONCLUSIONS

LOX is a very important enzyme for the health and food industry, which can be obtained from plants, animal tissues and microorganisms. In this paper we measured LOX activity in pear puree for 7 days. We saw that the chemicals added to the environment and the applied heat were quite effective on the LOX activity. The LOX activity in the pear, which is widely used especially in fruit juices and baby supplements, causes a great change in the color, smell and taste of the food. The important results we obtained as a result of our study are that adding syringic acid or rosmarinic acid to the medium or cooking the puree is beneficial in maintaining the shelf life and therefore the product quality for a long time. We think that LOX inhibition and longer shelf life can be achieved by developing different additives, cooking times or methods.

Conflicts of Interest: The authors declare no conflict of interest.

Ethical Approval: No experiments were performed on any living creature in this study, it does not need ethical approval

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