ENZYMES' IMPACT ON QUALITY OF WALNUTS (JUGLANS REGIA L.) AND WALNUT OIL

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Abstract: Quality and safety of walnuts and walnut oil, during the storage, depend largely on the enzyme content and their activity. Lipoxidases catalyze the oxidation of unsaturated fatty acids such as linoleic, linolenic and arachidonic acids, in this way it form esters and peroxides, this process reduces the product's quality. This article provides a bibliographic study of enzymes and their assessment methods in walnut kernels and walnut oil. There were collected and studied different types of walnuts (Juglans regia L.) from Moldova. Nuts were evaluated fresh or after being stored for 1 and 2 years at room temperature. The study presents the changes of enzymes' activity during storage of the walnuts and oil, using titrimetric and spectrophotometric methods.
Key words: enzymes impact, oxidation enzymatic, polyphenol oxidase (PPO), enzymatic degradation

INTRODUCTION

The literature data confirm that walnuts are a rich source of a number of important nutrients that appear to have a very positive effect on human health [9]. Walnuts have a special dietary food value, given their carbohydrate content (11-14%), protein (14 -16%) represented the essential amino acids and lipids (62-65%, of which 44-48% are polyunsaturated fatty acids) [6, 12]. G. Ozkan and M. A. Koyuncu [6] report the chemical composition of 12 genotypes of walnut Juglans regia L.: protein (20.92 – 25.95%); ash (1.68 - 2.06%); fat (66.30 - 74.95%), from 62.4 to 68.7%, the oleic acid content of the oils ranged from 14.3 to 26.1% of the total fatty acids, while the linoleic acid content ranged from 49.3 to 62.3% and the linolenic contents from 8.0 to 13.8%.

Walnuts are also a rich source of bioactive compounds: they contain polyphenols, dietary compounds, tocopherols, folic acid, minerals, and manganese and copper [4, 10].

Walnut and walnuts oil quality depends on many factors, including the presence and activity of enzymes in these products [3, 10] his article provides a bibliographic study of enzymes and their assessment methods in walnut kernels and walnut oil.

1. MATERIAL AND METHODS

Material

The study was conducted on autochthonous walnuts Juglans regia L., harvested in Telenesti, Moldova, during harvest 2010 and 2011. Storage took place at ambient temperature. Also served a object study walnuts kernel, stored in light/dark, walnuts oil industrial and obtained laboratory by cold pressing.

Methods

Polyphenol oxidase (PPO) activity in walnuts kernels and oil was performed in accordance with method exposed in [6, 7].

Acidity value represents KOH quantity in mg that is necessary for neutralization of free fat acids in one of fat (oil) [6].
Water activity plays an important role in the oxidation of walnuts and walnuts oil in storage. Water activity walnuts and nuts oil was evaluated with the device Novasina LabSwift-aw.

RESULTS AND DISCUSSION

Walnut kernels contain substantial quantities of triacylglycerol's and polyunsaturated fatty acids, and thus are susceptible to oxidative and hydrolytic rancidity [10].

Enzymatic oxidation of Unsaturated Fatty Acids

Decomposition of unsaturated fatty acids begins with hydrolysis of various glycerides by lipases, lipolytic acyl hydrolases, and phosholipases, during which the polyunsaturated fatty acids are, freed (Fig. 1).

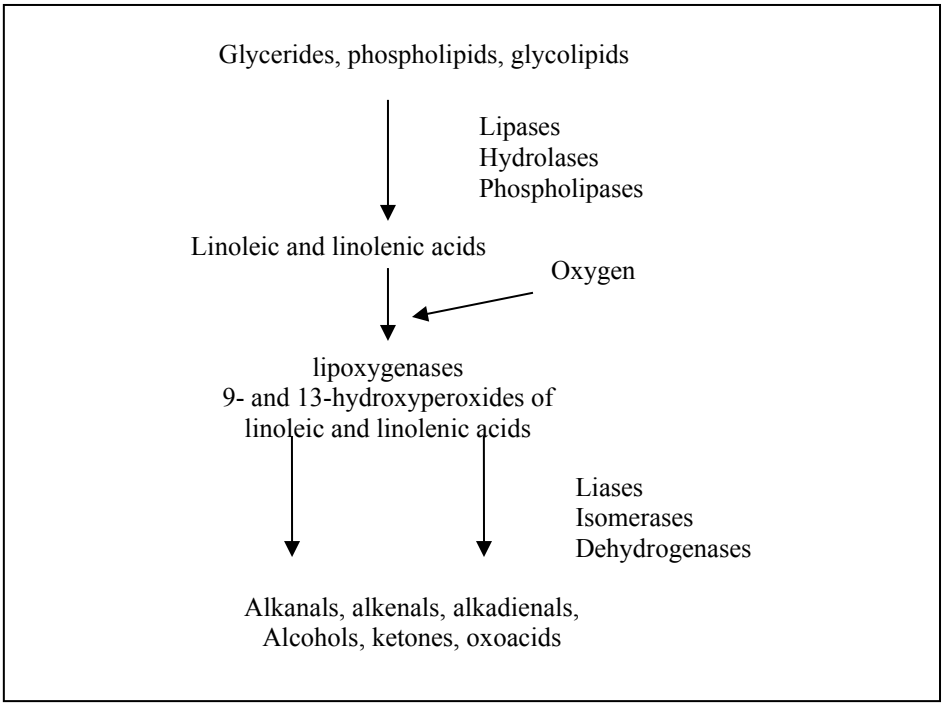

Fig. 1. Enzymatic oxidation of unsaturated fatty acids [1]

Lipoxygenases then convert unsaturated fatty acids intro hydroperoxides, mainly 9 and 13 isomers, which are unstable. In last step, lyases, isomerases and dehydrogenases transfer hidroperoxides into a variety of volatile and nonvolatile products. The flavor components formed, such as aldehydes and alcohols, can be directly responsible for off-flavor [3].

Free unsaturated fatty acids, particularly linoleic and linolenic acids in plant are the preferred substrate for oxidation by lipoxygenases. Certain lipoxygenase is enzymes can

also catalyze the oxidation of unsaturated fatty acids when they are esterified in lipids. Presence of oxygen and light accelerates enzymatic activity [3, 10]. Lack of oxygen does not halt oxidation because some forms of lipoxygenases can oxidize fatty acids without the presence of oxygen, thus forming free radicals.

The presence of trace amounts of hydroperroxides accelerates the oxidation of unsaturated fatty acids by lipoxygenase, particularly under anaerobic conditions. Free radical formed from the decomposition of hydroperoxides can elevate further oxidation, which causes earlier than expected off-flavor formation and results in lower oil stability during storage [3].

The oxidation of oil is influenced by the fatty acid composition of the oil, oil processing, energy of heat or light, the concentration and type of oxygen, and free fatty acid, mono - and diacylglycerols, transition metals, peroxides, thermally oxidized compounds, pigments, and antioxidants. These factors interactively affect the oxidation of oil and differentiation of the individual effect of the factors is not easy [2].

Minor unsaponifiable compounds present in Oil

Edible oil consists of mostly triacylglycerols, but it also contains minor components such as free fatty acids, mono- and diacylglycerols, metals, phospholipids, peroxides, chlorophylls, carotenoids, phenolic compounds, and tocopherols. Some of them accelerate the oil oxidation and others act as antioxidants: phospholipids, color compounds, tocopherols, phenolic compounds. Free fatty acids act as prooxidants in edible oil [5].

Polyphenols and tocopherols are the main groups of phenolic compounds acting as primary antioxidants to inhibit oxidation in virgin olive oils. They mainly act as chain breakers by donating radical hydrogen to alkylperoxyl formed during the propagation step of lipid oxidation and subsequently forming a stable radical (A^{*}) through the well-known reaction [11]:

$$ROO^{\bullet} + AH \rightarrow ROOH + A^{\bullet} \quad (1)$$

Most of the groups of minor compounds are reported to have either a beneficial or detrimental effect on oil stability although the positive contribution of the primary antioxidants present in the unsaponifiable fraction is the major determinant in the resistance of virgin olive oil to oxidation [11].

Probably the same thing occurs in walnuts oil, because according to studies [4, 10] oil nuts also contain minor unsaponifiable compounds. This minor substances thrives enzymes in walnut oil. Quality and safety of walnuts and walnut oil, during the storage, depend largely on the enzyme content and their activity.

Buranasompob A. et al. [2] hypothesized that short time heat treatments inactivate lipoxygenase (LOX) or lipase enzymes and extend the shelf-lives of walnut and almond kernels (Figure 2 and 3).

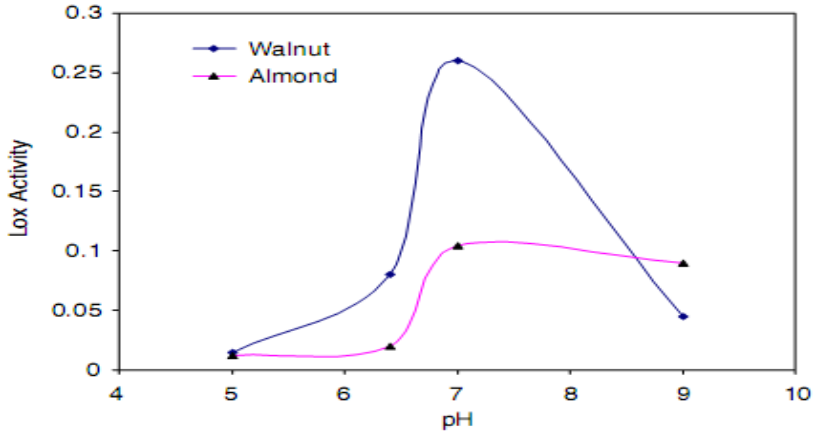

Fig. 2. Lipoxygenase activity (mMO_2/l s) in the homogenates of untreated almond of walnut kernels (1.0 g/ml) in pH range of (5.0–9.0)

( ) Walnut, ( ) Almond

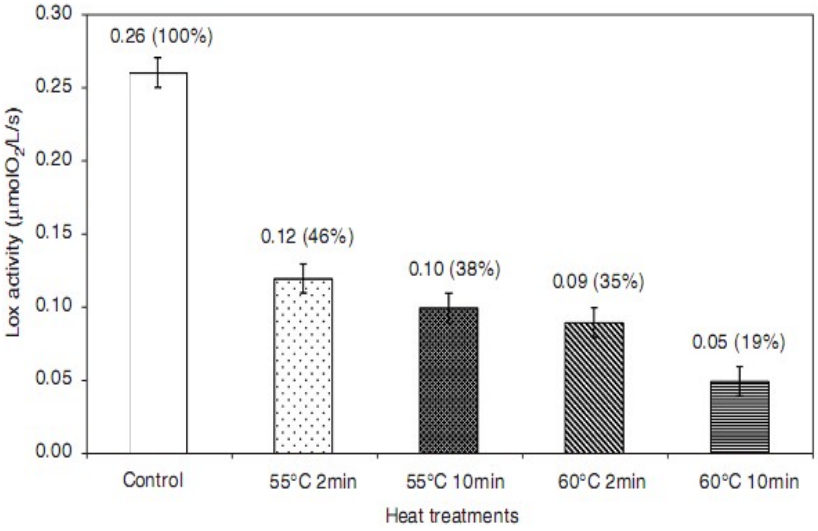

Fig. 3. Lipoxygenase activity in homogenates (0.1g/ml, pH 7.0) of control and heat treated walnut kernels

The enzyme polyphenol oxidase (PPO) is present in almost all plants, and catalyzes the oxidation of monophenols and o-diphenols to quinones. PPO in walnut (Juglans regia L.) has been little studied since the early 1900s [4, 5 and 10].

In Table 1 we present experimental results recorded on walnuts harvested in Moldova during the years 2010 and 2011, stored at ambient temperature, and walnut kernels packed in polietelen bags, stored in light and dark conditions. Following indicators were investigated: PPO activity, total acidity (TA) and water activity (Aw).

Table 1. Change indicators PPO, TA and Aw Walnut Juglans regia L during storage

Parameters investigated	Harvest year		During storage (days)	Storage conditions	
	2010	2011		light	dark
PPO activity, units of activity	2	1	0 15 30	1 1.6 2.0	1 1.1 1.3
TA,%	0.4	0.3	0 15 30	0,3 0.46 0.5	0.3 0.4 0.4
Aw,%	0.500	0.495	0 15 30	0.495 0.481 0.479	0.495 0.477 0.475

Figure 4 we present changes activities PPO in walnut kernels Juglans regia L. packed in polietelen bags, stored the dark and light (15 and 30 days).

Fig. 4. Changes in PPO activity of walnuts kermels samples during 30 days storaje period

Light and darkness have a different effect on enzyme activity in storage. The flow of light increases enzyme activity. It is necessary to examine the factors that contribute to increased enzyme activity in order to minimize enzymatic degradation walnut oil. Thus we can partially solve the problem of keeping the oil.

CONCLUSION

This paper presents a bibliographic and experimental study of enzymes' impact on quality of walnuts (Juglans regia L.) and walnut oil. Quality and safety of walnuts and walnut oil, during the storage, depend largely on the enzyme content and their activity.

Enzyme activity in walnuts depends on storage conditions: temperature, humidity, *p*H, light access, packaging.

Sunlight increases enzyme activity. The core of walnuts should be stored in waterproof packaging to oxygen, water vapor, light, to avoid chemical and enzymatic degradation of walnuts and walnut oil.

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