Study of the Biochemical Potential of Wild Fruit of the Caucasus Medar (*Mespilus caucasics L.*) in the Post-Harvest Period

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The carbohydrate complex (fructose, glucose, sucrose, pectin substances), the main organic and fatty acids, which are important food functional ingredients, were studied in the wild fruits of the Caucasian loquat during their storage. It has been established that during 40 days of storage there are significant changes in the biochemical potential of fruits for almost all the studied food ingredients, the level of their final content is 5-10% of the original content.

Keywords: biochemical potential (carbohydrates, organic acids, fatty acids), wild medlar fruits, post-harvest period

Wild fruits of the Caucasian medlar (Mespilus caucasica L., fam: Rosaceae), widely distributed in Azerbaijan, are a unique source of food functional ingredients [1]. In particular, the carbohydrate complex, consisting of water-soluble carbohydrates and pectin substances, as well as organic acids, largely determines the nutritional value of fruits and their storage capacity [2], and the lipid complex of fruits largely determines the aroma and color [3]. Organic acids, especially citric and malic acids in medlar fruits are important components that, along with mono- and disaccharides, form their taste. Pectins are polyfunctional food ingredients and have antioxidant activity [4]; therefore, extracts of wild medlar fruits can be used for the production of functional foods. One of the main features of wild medlar fruits is their limited shelf life, therefore, the study of the biochemical potential of fruits during their storage is an important task of preserving their quality for industrial processing.

The fruits of the medlar reach the stage of technical maturity in early October. They differ significantly in shape and size: there are oval and pear-shaped with a size of 2.5 to 4.5 cm, weighing from 15 to 50 gram. The skin of the fruit is initially green, then, as it ripens, becomes light yellow or orange-yellow, quite dense and easily removed from ripe fruits. The pulp of the fruit is juicy and tender from white to orange in color. Due to the high initial humidity, the fruits of wild medlar, according to various literature data [5-6], quickly deteriorate, so the purpose of our research was to determine the biopotential of fruits in the climatic conditions of Azerbaijan in order to optimize industrial use.

Materials and methods

For biochemical studies, wild-growing medlar fruits were collected during their mass ripening in a state of consumer and technical maturity. Freshly harvested fruits were stored in wooden boxes at a controlled temperature of 2-4 ^{0}C for 40 days; the pulp with skin accounted for an average of 60 % tw.

Reagents and standarts. All reagents were of analytical grade. The calibration and internal standart of fatty acid were purchased from Nu-Check (GLC-68, USA), and the solvents (hexane, chloroform, methanol) were purchased from Burdick & Jackson (USA). The water used for analyses was treated in a Millipor water purification system (Millipore, USA).

Sugar and organic acid extraction. For the ethanolic extraction of sugar and organic acid fruit fresh without seeds was blended in the dark with 95 % ethanol for 3-5 min. The homogenate was vacuum-filtered through Watman N 1filter paper and the residue washed twice with 80 % ethanol. The filtrates were combined and adjusted to 5 ml/g of fresh weight (FW) with ethanol. This is considered as the ethanolic extract.

HPLC condition for sugars and organic acids. Sugars and organic acids were analyzed in a Hewlett-Packard 1090 liquid chromatograph equipped with a photodiode array detector and a Waters 410 differential refractometer connected in series. Date was processed by means of Hewlett-Packard 85-B computing system and a Beckman Analogue Interface Module 406. Isocratic separation of the compounds was made on a stainless-steel Ion-300 (300 mm x 7.8 mm, 10 μm) column containing a cation-exchange polymer in the ionic hydrogen form with a IonGuard GC800 guard column and thermostated at 25 ^{0}C. The mobile phase utilized for the elution consisted of a filtered and degassed solution of 0.0085 N H_2SO_4 and a flow rate of 0.4 ml/min. UV detection was selected at 195 and 245 nm the refractive index detector was used at sensitivity 16x and the injection volume was 20 μl [7].

Pectin extraction. Pectin extraction was performed according to [8] freshly pressed pulp of medlar was mixed with water in the proportion of 1:8 (w/v). The pectin extraction process was performed at 65-70 °C during 15-30 min using rotor-pulsation device MT-1500 (Kinematica, Switzerland). This resulted in disintegration of solid phase and high-rate pectin extraction into liquid phase. The sum of hydropectin and protopectin content were determined by approved AOAC methods [9].

Lipid extraction. Samples of finely ground powder of medlar mesocarp (1 g) in triplicate were weighed and extracted with chloroform: methanol (2:1 v/v) and soline (NaCl 0.9%) were added at rate of 20 % of the extraction volume. The mixture was shaken and centrifuged, organic layer was collected and filtered. The samples were dissolved in anhydrous chloroform, then 16 µg of triheptadecanoin (containing three molecules of heptadecanoic acid 17:0) and a 0.1-ml aliquot of the lipid sample was transferred to a 15-ml Teflon-lined tube. Fatty acid methyl esters were obtained using 14 % (w/v) boron trifluoride (BF_3) in methanol. After removing the solvent by nitrogen gas, the sample was mixed with 0.5 ml of the BF_3 reagent and placed in a warm bath at 100 ^{0}C for 30 min. After cooling saline (NaCl, 0.9%) and extracted into the hexane. A mixture of known methyl esters standart was used to calibrate the gas chromatograph and to identify ester peaks.

Gas chromatography. Aliquots (1-2 µl) of the hexane solution containing the fatty acid methyl esters were analyzed using a Hewlett-Packard 5890 gas chromatograph equipped with a fused-silica capillary column and flame ionization detector. Capillary column HP-Sms (5 % diphenyl and 95 % dimethylpolysiloxane, phase thickness 0.25 µm) size 30 m x 0.25 mm; the temperature of the evaporator and detector interface -250 °C; the initial temperature of the column thermostat is -150 °C; exposure - at the initial temperature - 5 min; temperature programming from 150 to 210 °C at a speed of 5 °/min, exposure at the final temperature - 10 minutes. The carrier gas was helium (99.999 %) and the flow rate was approximately 50 ml/s. The fatty acids are reported as the average of three determinations conducted of three independent assays. The internal standart (heptadecanoic acid 17:0) and a calibration mixture of fatty acid standarts (GLC-68, USA) were used to identify and quantify the fatty acids in the various lipid extracts.

Results and discussion

The dynamics of changes in the biochemical potential of wild-growing medlar fruits (carbohydrates, pectin, organic and fatty acids) was studied during 10-40 days of their storage and the results are given in Table 1 and 2.

As follows from the data in Table 1, the level of free carbohydrates (fructose, glucose and sucrose) gradually decreases as the fruit storage time increases. At the same time, it should be noted that in the first decade of storage of fruits, the process of biotransformation of the disaccharide proceeds especially intensively, this fact is indicated by the data on the increase in the same period of storage of monosaccharides - glucose and fructose. In the next three decades of fruit storage, there is a consistent quantitative decrease in free carbohydrates. Pectin substances of medlar fruits are represented by a water-soluble fraction (940 mg/100 g) and protopectin (1600 mg/100 g) and, as can be seen from the data obtained, during storage, the ratio of pectin fractions increases towards a significant increase in the water-soluble fraction. This fact largely explains the decrease in the commercial quality of fruits due to a decrease in the mechanical strength of their skin.

It should be noted that the obtained data on the decrease in the level of free carbohydrates during storage of wild-growing medlar fruits of Azerbaijan correlate well with the literature data for medlar fruits growing in Turkey, Spain and the USA [10].

Table 1. The content of saturation, pectin poisoning and acid poisoning (mg/100g) in wild medlar products during storage.

Substances	Days after harvest			
	10	20	30	40
Fructose	2355.5±5.1	2480.3±0.4	180.7±2.1	35.8±1.8
Glucose	854.3±4.1	920.2±2.3	510.8±0.8	34.5±1.2
Sucrose	350.6±4.8	188.6±2.1	35.8±1.4	7.5±0.2
Hydropectin	940.4±3.8	1120.0±2.3	1310.5±3.5	1480.2±2.3
Protopectin	1600.8±4.3	1212.0±2.2	870.4±2.1	458.8±1.6
Malic acid	561.4±1.1	673.8±1.2	288.5±0.8	48.3±0.3
Citric acid	452.5±1.1	310.8±0.8	118.2±0.8	28.5±0.1
Ascorbic acid	9.3±1.1	7.1±0.7	4.3±0.3	1.1±0.1

Values, means of three independent extractions and determinations for each sampling (n=5).

Organic acids of wild medlar fruits are represented mainly by free malic and citric acids, as well as ascorbic acid, the concentration of which is 9.3 mg/100g, which is the average level among most fruits eaten. Ascorbic acid performs a number of important functions in the human body: as a strong reducing agent, it takes part in many biochemical processes; it participates in the synthesis of collagens, the degradation of tyrosine, and the synthesis of catecholamines and bile acids [11]. As can be seen from the presented data, the concentration of ascorbic acid greatly decreases on the 40th day of storage of fruits, which should serve as a guide both for their direct use in food and for industrial processing.

The level of change in the concentration of malic and citric acids also tends to decrease with the shelf life. As a rule, at the stages of ripening and the initial period of storage of fruits, the transformations of organic acids are associated with oxidation reactions, and at the stage of fruit aging, they are associated with decarboxylation [12]. It should be noted that the concentration of malic acid slightly increases in the second decade of storage from 561.4 mg/100g to 673.8 mg/100g, which does not quite fit into the biochemical process of decarboxylation of malic acid with the release of CO_2 [13]. A fairly rapid decrease in the content of malic and citric acids in medlar fruits after 30 days of storage leads to a sharp decrease in the commercial quality of fruits due to enzymatic reactions of darkening of the pulp and a decrease in their mechanical strength, which leads to a sharp increase in their microbial spoilage. This is due to the fact that organic acids are actively involved in metabolic processes, being respiration substrates, and can also be precursors for the synthesis of fatty acids of phenolic and other compounds [14].

As can be seen from the data in Table 2, the fatty acid composition of the pulp in wild-growing medlar fruits is quite diverse, 18 fatty acids were found at all stages of storage. At the same time, capric acid (C_{10}:0) and tridecanoic acid (C_{13}:0) were quantitatively determined only at the initial (first decade) storage period, and after the second decade they were practically not identified. The level of such fatty acids as palmitic (C_{16}:0), stearic (C_{18}:0), oleic (C_{18}:1), linolenic (C_{18}:2) and lipoletic (C_{18}:3) acids consistently decreased sharply during storage. It was also found that the level of such fatty acids as arachidonic (C_{20}:0), eicosadienoic (C_{20}:2), behenic (C_{22}:0), erucic (C_{22}:1) and lignoceric (C_{24}:0) in the first 10 days of storage decreases by 3-5 times, and then gradually increases within 30 days, but the level of increase does not exceed 10-15 % of their initial content. In general, the total level of fatty acids in medlar fruits during 40 days of storage does not exceed 5 % of their initial content.

Table 2. Fatty acid composition (μg/g dry wt) of wild medlar fruit at different stages of post-harvest period.

Fatty acid	Days after harvest			
	10	20	30	40
C 10:0	5.3±0.1	n.d.	n.d.	n.d.
C 12:0	5.7±0.1	2.7±0.1	3.1±0.1	2.3±0.1
C 13:0	5.1±0.1	n.d.	n.d.	n.d.
C 14:0	10.2±0.2	2.3±0.2	2.8±0.2	3.1±0.2
C 14:1	9.8±0.3	1.9±0.1	2.8±0.1	3.3±0.1
C 15:0	4.2±0.2	1.5±0.1	1.8±0.2	1.9±0.2
C 16:0	510±1.8	88.5±0.4	85.2±0.4	64.3±0.4
C 16:1	10.4±0.2	7.3±0.2	5.1±0.2	2.2±0.2
C 18:0	75.4±1.4	21.7±1.1	15.8±1.2	8.3±0.3
C 18:1	331.2±4.1	28.8±1.2	11.7±0.4	3.3±0.4
C 18:2	1562±6.0	210.8±4.0	88.4±1.4	43.7±0.2
C 18:3	462.8±3.3	104.5±1.5	54.2±1.6	23.8±1.1
C 20:0	45.8±1.5	10.3±0.3	14.1±0.4	14.9±0.6
C 20:1	5.5±0.4	2.4±0.2	1.4±0.1	0.8±0.1
C 20:2	n.d.	0.4±0.1	0.8±0.1	1.1±0.2
C 22:0	52.3±2.6	11.4±0.6	14.5±0.8	17.3±0.8
C 22:1	5.5±0.8	1.8±0.4	2.1±0.4	2.3±0.4
C 24:0	31.4±2.2	1.1±0.2	4.5±0.4	6.1±0.4

Values, means of three independent extractions and determinations for each sampling (n=5)

Decreases in the chemical constituents in fruits during ripening can be explained by two possible mechanisms. First, as a result of senescence rapid metabolic changes occur during fruit ripening. Ripening is considered to be an early stage in the senescence of climacteric fruits and ethylene plays an important role in this process. Sometimes ripening proceeds in parallel with fruit softening in which case ethylene appears to be involved in tissue softening during ripening and in de-greening and color formation that occurs in many fruits [15]. A second possible mechanism for the marked decline in a fatty acid content of fruit ripening could involve degradative lipolytic enzymes (phosholipipase, phosphatidic acid phosphatase, lipolytic acyl hydrolase and lipoxygenase).

These enzymes are capable of degrading endogenous lipids in senescing membranes and causing many chemical changes in the lipid bilayer including loss of lipid phosphate and acids an increase in the sterol: fatty acids.

Conclusions

Our results suggest that a rapid and marked decrease in total lipid fatty acid in wild medlar may also be due to an increase of fatty acid β-oxidation during the ripening process.

In summary here we reported the changes in important chemical constituents (such as sugars, pectin, organic acids, and fatty acids) of widely sold and consumed wild medlar during the post-harvest period. Thus, the desirable shelf life of wild medlar fruits for direct consumption and for their industrial processing should not exceed 30 days.

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