Kinetics of hydrolysis reactions and triacylglycerols oxidation in olive oil during prolonged storage

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Abstract. The article presents the results of studies on the effect of the antioxidant beta-carotene and storage temperature on hydrolytic and oxidative processes occurring during storage of extra virgin olive oil obtained from olives grown in the soil and climatic conditions in Syria. The research aims to study the effect of temperature and beta-carotene on the kinetics of the hydrolysis reactions of triglycerides and fatty acids oxidation in olive oil during storage. The object of the study was extra virgin olive oil obtained from olives grown in Syria according to the generally accepted technology in 2019. And beta-carotene, produced by Ekoresurs, has been used as a natural antioxidant. The kinetics of the hydrolysis and oxidation reaction was evaluated by studying the change in the content of saturated and unsaturated fatty acids during storage of the control sample from olive oil (sample No. 1), and experimental samples with the addition of beta-carotene at concentrations of 400 mg / L (sample No. 2) and 600 mg / L (sample No. 3). These samples were stored at 18 ° C. And sample No. 4 was stored without adding beta-carotene at 4 ° C. Upon receipt for storage and during this process, the content of saturated and unsaturated fatty acids in the test samples was determined periodically by gas chromatography on an LC-20 Shimadzu chromatograph, and organoleptic quality indicators were evaluated on a five-point scale. In addition, the content of triacylglycerols, saturated and unsaturated fatty acids were determined based on the change in beta-carotene dose, temperature, and period of storage. Also, the rate constants of the fatty acid oxidation reaction have been calculated. It was shown that the minimum rate of free fatty acids oxidation during storage of extra virgin olive oil for 7 months was in the olive oil sample stored at + 18 ° C with the addition of 400 mg / L of beta-carotene, as well as in the olive oil sample stored in the refrigerator at + 4 ° C without adding antioxidant.

1. Introduction

Extra virgin olive oil is the oil that we obtain from freshly harvested olives without chemical reagents, it has an acidity of not more than 0.8% [1]. As well as, this type of olive oil has a high biological value, as it contains tocopherols, sterols, phospholipids, and other substances that have a curative and preventive effect on the human body [2, 3].

The main reason for the deterioration of the oil quality, the decrease in its nutritional and biological value during storage is the hydrolysis of triacylglycerols (TAGs) with the formation of free fatty acids (FFA),
mono- and di-glycerides, and glycerin, as well as the oxidation of unsaturated fatty acids with the formation of peroxides and carbonyl compounds [4-6].

Various studies have been conducted on the influence of high and low storage temperatures on the biological value and quality of olive oil [5 - 8]. It has been shown that the content of phenolic compounds decreases by 24% at a storage temperature of 15 °C; by 9.5% - at 4 °C and by 7.3% - during storage at -18 °C [7-8].

In work 9 [9] it has been found that decrease in the storage temperature slows down the formation of fatty acids oxidation products in the oil, but is energetically unfavorable.

In solving the problem of preserving the maximum possible quality, nutritional and biological value of olive oil during storage, an urgent and promising direction of research is the use of antioxidants from different structures along with storage temperature, which makes it possible to slow down the processes of triacylglycerols hydrolysis and fatty acid oxidation with prolonged shelf life. Currently, there are conflicting opinions about the effectiveness and feasibility of using antioxidants from various structures, depending on the variety, production technology, and storage temperature of olive oil.

The research aims to study the effect of storage temperature and beta-carotene dose on the processes of hydrolysis and oxidation of extra virgin olive oil and quality indicators, and justify the terms of its validity.

2. Materials and methods

The object of the study was extra virgin olive oil obtained from olives grown in the soil and climatic conditions of Syria according to the generally accepted technology. The olive harvest was picked in August 2019. Also, beta-carotene, produced by Ekoresurs, Saint Petersburg, has been used as a natural antioxidant.

The process of studying the effect of temperature and dose of beta-carotene on hydrolytic and oxidative processes occurring in olive oil during storage, control sample (No. 1) and experimental samples with the addition of beta-carotene in an amount of 400 (No. 2) and 600 mg / 100g (No. 3) was stored at a + 18 °C. And (sample No. 4) was stored without adding beta-carotene at 4 °C. The tested samples were stored in sealed, dark glass bottles.

Upon receipt for storage and during this process, in the tested samples, the following were periodically determined:
- the content of triacylglycerols by the Soxhlet method,
- composition of fatty acids in olive oil by gas chromatography on an LC-20 chromatograph manufactured by Shimadzu,
- acid number - by titration method in an alcoholic medium relative to a standard solution of potassium hydroxide (GOST R 52110-2003),
- organoleptic indicators of oil quality were evaluated on a five-point scale according to the following descriptors: color, smell, taste, and transparency of olive oil.

The data were processed by the method of mathematical statistics with finding a confidence interval at a probability of 95% using standard computer programs; the tables and figures show the arithmetic mean values of the studied indicators.

3. Results and discussion

3.1. Dynamics of the fatty acid composition in olive oil during storage

Upon receipt of olive oil for storage, the content of triacylglycerols was 97.6%. Table [1] shows data on the saturated and unsaturated fatty acid content in olive oil. As follows from the data presented, of saturated fatty acids, the percentage of palmitic acid reaches 137 mg / g, while the content of stearic acid is 9.60 mg / g. Among the monounsaturated fatty acids, oleic acid predominates and it has a value of 68.6 mg /g, of polyunsaturated fatty acids, linolenic acid in an amount of 48.64 mg / g. Here there are the following
polyunsaturated fatty acids: linoleic, eicosatetraenoic, eicosadienoic, docosahexaenoic, pentadecanoic, and heptadecanoic present in (1 - 2)%.

Table 1. Content of saturated and unsaturated fatty acids in olive oil before storage

| Carboxylic acid methyl ester | Free fatty acid concentration, mg/g |
|-----------------------------|-----------------------------------|
| Myristic acid               | 0.18                              |
| Pentadecanoic acid          | 0.49                              |
| Palmitic acid               | 137                               |
| Heptadecanoic acid          | 1.80                              |
| Stearic acid                | 9.60                              |
| Eicosanoic acid             | 2.80                              |
| Proven acid                 | 0.75                              |
| 9-hexadecenoic acid         | 0.94                              |
| Oleic acid                  | 686                               |
| Linolenic acid              | 48.64                             |
| 5, 8, 11, 14 - eicosatetraenoic acid | 0.12 |
| 11, 14,17 - eicosatrienoic acid (omega 3) | 0.04 |

Tables [2] and [3] show the results of a study about the effect of the antioxidant beta-carotene, temperature, and shelf life of olive oil on the fatty acid composition in the oil.

Table 2. Composition of fatty acids of olive oil samples No. 1 - No. 4 after 5 months of storage

| Carboxylic acid methyl ester | Free fatty acid concentration, mg/g |
|-----------------------------|-----------------------------------|
|                            | No. 1  | No. 2  | No. 3  | No. 4  |
| Myristic acid               | 0.09   | 0.102  | 0.102  | 0.120  |
| Palmitic acid               | 71.08  | 97.14  | 97.00  | 90.38  |
| Stearic acid                | 8.40   | 9.36   | 9.45   | 9.49   |
| Docosanoic acid             | 0.35   | 0.45   | 0.48   | 0.45   |
| Oleic acid                  | 250    | 303    | 309    | 341    |
| Linolenic acid              | 21.38  | 37.05  | 37.92  | 45.78  |
| Cis-11,14-eicosadienoic acid (omega 6) | 0.10 | 0.51 | 0.61 |

| Linolenic acid              | 21.38  | 37.05  | 37.92  | 45.78  |
Table 3. Composition of fatty acids of olive oil samples No. 1 - No. 4 after 7 months of storage

| Carboxylic acid methyl ester | Free fatty acid concentration, mg/g |
|----------------------------|----------------------------------|
|                            | No. 1   | No. 2   | No. 3   | No. 4   |
| Myristic acid              | 0.059   | 0.086   | 0.089   | 0.120   |
| Palmitic acid              | 70.35   | 92.43   | 91.78   | 90.14   |
| Stearic acid               | 7.46    | 9.06    | 9.41    | 9.31    |
| Docosanoic acid            | 0.29    | 0.30    | 0.29    | 0.35    |
| Oleic acid                 | 162     | 301     | 300     | 326     |
| Linolenic acid             | 25.35   | 34.71   | 35.11   | 38.45   |
| Cis-11,14-eicosadienoic acid (omega 6) | 0.59 | 1.18 | 1.25 | 1.89 |

As follows from the data presented in tables [2] and [3], the amount of saturated and unsaturated fatty acids decreases during the storage of all studied oil samples, which explains their oxidation. However, the kinetics of fatty acid oxidation reactions depends on the antioxidant dose, acid saturation, period, and storage temperature, as follows from table [4], which shows the rate constants of oxidation reactions of essential saturated and unsaturated fatty acids (K). The constants are calculated based on the data given in tables 1 - 3, according to the formula $K = 1 / \tau \ln C_0 / C$.

Here $C_0$ and $C$ are the concentrations of saturated or unsaturated fatty acids at the beginning of storage and at any given time $\tau$ during storage, respectively.

Table 4. Reaction rate constants of oxidation of FFA in olive oil TAG during storage, K (-), day-1

| Acid name                     | $0 \leq \tau \leq 150$ | $0 \leq \tau \leq 200$ |
|-------------------------------|-------------------------|-------------------------|
|                               | No. 1      | No. 2      | No. 3      | No. 4      | No. 1      | No. 2      | No. 3      | No. 4      |
| Palmitic acid                 | 0.0054     | 0.0022     | 0.0021     | 0.0028     | 0.0033     | 0.0019     | 0.0020     | 0.0016     |
| Stearic acid                  | 0.0009     | 0.0001     | 0.0001     | 0.0001     | 0.0013     | 0.0001     | 0.0001     | 0.0001     |
| Oleic acid                    | 0.018      | 0.0054     | 0.0055     | 0.0046     | 0.0072     | 0.0041     | 0.0041     | 0.0037     |
| Linolenic acid                | 0.0054     | 0.0017     | 0.0016     | 0.0004     | 0.0032     | 0.0017     | 0.0016     | 0.0012     |

The amount of stearic acid practically does not change during the entire storage period and does not depend on the dose of the antioxidant. It should be noted that the maximum changes in the content of oleic and linolenic acids are typical for control oil samples stored at +18 °C for more than 5 months, minimum for oil samples stored at +18 °C for 7 months with the addition of an antioxidant. No significant differences
were found in the change in the content of saturated and unsaturated fatty acids depending on the dose of the antioxidant. In addition, the minimum change in the fatty acids was observed in the oil sample stored at 4° C for 7 months.

Thus, to slow down the oxidation of fatty acids in extra virgin olive oil and to increase its stability, it is recommended to add beta-carotene at a concentration of 400 mg/l, store the oil at a +4 °C without adding antioxidants.

3.2. Organoleptic indicators of the olive oil quality during storage
An organoleptic assessment of the quality indicators was carried out on the studied oil samples according to the following descriptors: taste, color, smell, and transparency. The sensory assessment was done upon receipt of control samples of oil for storage, then control and experimental samples during storage for 7 months.

It was shown that before the beginning of storage, the olive oil was distinguished by its delicate taste, mild pleasant aroma, greenish-yellow color, and transparency, and had a maximum organoleptic assessment of excellent.

After 5 months of storage, the smell and taste of sample No. 1 deteriorate as a result of the oxidation of unsaturated fatty acids and the formation of peroxides, the other three samples retained good taste and smell. But after 7 months of storage, the control sample had a rancid taste and unpleasant odor due to the oxidation of unsaturated fatty acids and the formation of apparently low molecular weight aldehydes and ketones (score 3.78).

At the same time, oil samples to which antioxidants were added (No. 2, No. 3) and sample No. 4 stored in a refrigerator at 4 °C had higher scores on sensory assessments of taste and smell (score 4.56). No change in oil color was noted in all of the test samples. It should be noted that the oil stored at 4 °C crystallized, the consistency changed and was not transparent, but after keeping at a temperature of 20 °C, the crystals collapsed and the oil acquired its characteristic transparency.

4. Conclusion
Based on the analysis of the research results, the following conclusions were made. It has been shown that extra virgin olive oil obtained from olives grown in the soil and climatic conditions of Syria contains 97.6% of triacylglycerols, is characterized by a high content of unsaturated fatty acids, especially oleic (68.6%), linolenic (12.7%); palmitic (13.7%) and stearic (9.6%).

It was found that during the storage of experimental oil samples, hydrolytic and oxidative processes of triacylglycerols are significantly slowed down when the antioxidant beta-carotene is added. It has been shown that minimal changes in the content of TAG hydrolysis products and FFA oxidation during storage of olive oil at a temperature of + 18 °C are characteristic for samples containing 400 mg / l of beta-carotene and for oil samples stored at + 4 °C without the addition of antioxidants.

It has been shown that during storage of olive oil, the amount of saturated and unsaturated fatty acids, especially oleic, decreases, which is explained by the oxidation of free fatty acids of the oil. The minimum rate of their oxidation during storage of extra virgin olive oil for 7 months at a temperature of + 18° C was observed with the addition of 400 mg / l of beta-carotene and at a storage temperature of + 4 °C without the addition of an antioxidant.

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