# PROCEEDINGS OF II. INTERNATIONAL EDIBLE & OIL SEEDS CONGRESS EDIBOIL 2024

## 31 OCTOBER - 2 NOVEMBER, 2024

## Megasaray Westbeach Hotel, Antalya, Turkey

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## Organized by
## Trakya University
## International Researchers Association

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# WELCOME NOTES

You are welcome to our International Congress Edible and Oil Seeds which is organized by Trakya University and the International Researchers Association. The congress will be held in Megasaray Westbeach Hotel, Antalya, Turkey, on 31 October - 2 November, 2024 with the support of several national and international partners.

The Congress topics will cover Edible and Oil Seeds: Plant Breeding and Genetics, Molecular Genetics and Biotechnology, Biology and Physiology, Genetic Resources, Plant Protection, Agronomy and Production, Animal feeding, Food Science and Nutrients Fats, lipids, and Protein studies, Trade and Economy,

Oil crops are rich sources of oils, proteins, minerals, vitamins, and dietary fibers for both human and animal feeding and provide the raw material for the production of biodiesel. Oil crops are soybean, cottonseed, sunflower, canola, rapeseed, peanut, safflower, flax, sesame, coconut, castor, copra, etc.

Almost 50% of the global food protein supply comes from cereal seeds. Soybean, peanut, common bean, pea, lupine, chickpea, faba bean, lentil, grass pea, cowpea, pigeon pea, etc. are currently the most important legumes for human consumption and animal feed. Because of the edible contents of their seeds; grain legumes, cereals, and other minor crops etc. are edible seeds growing for plant protein for food and feed.

The Congress is intended that the subjects to be kept broad in order to provide opportunity to the science and research community to present their works as oral or poster presentations. The Congress languages will be in English.

Researchers, breeders and others with an interest in the genetics and breeding of oil and protein crops are invited to participate. Among the topics to be discussed are directions of breeding for resistance to abiotic and biotic stresses, improved industrial use, and conventional versus organic production.

As there have been many different scientific meetings around the world, we aimed to bring three different communities together, namely science, research and private investment groups considering practical information sharing that will be of value for breeders, seed enterprises, researchers and scientists, in a friendly environment of Antalya, Turkey to share their knowledge and experience and benefit from each other.

The congress will gather scientists from around the world, and present their recent achievements. The organizers will also invite relevant stakeholders to provide a view on the current situation around the world as well as prospects to overcome the limitation for sustainable crop production to feed the world.

The first meeting has been organized in Lviv, Ukraine in 2019 by Trakya University, with part of more than 100 participants from all over the world with 70 scientific papers. The 2nd congress will gather scientists from around the world, and present their recent achievements.

There are 16 orals and 10 poster presentation in the congress both joining and presenting normal and online from different countries from the world.

With care for our nature and environment, we aim the green congress, meaning that as little as possible papers will be used. Abstract book is published in electronic book and is distributed to the participants by e mail for online participants. All the e-posters are prepared in electronic form and then submit to via the congress e mail and exhibited in electronical poster boards as well as in online e poster hall in our web page during the congress.

We would like to thank all of you for joining this congress and we would like to give also special thanks to our sponsors and collaborators for giving us a big support to organize this event.

Prof Dr Yalcin KAYA
Head of the Organizing Committee

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**CONTENTS**

# INCREASING THE YIELD OF COLD PRESSED POMEGRANATE SEED OIL BY ACID+ENZYME PRETREATMENT

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## ABSTRACT

Seeds are the part of fruits and vegetables with the highest concentration of bioactive molecules. They are therefore important for reuse and valorization in the waste-to-food industry. Pomegranate seeds account for about 60% of the pomegranate juice industry with a volume of 3-6 million tons per year. 22% of the waste consists of kernels with shells.
Processors in the seed oil industry demand the procedures that are cost-effective and also enhance oil recovery. The most preferred method of pomegranate seed oil extraction in terms of quality and bioactive properties is cold pressing. In this study, roasting, enzyme and acid treatments were applied to the seeds to increase the oil yield. In addition, the effects of the treatments on quality indexes of the oil were analyzed. Two different roasting temperatures (100-140 °C) and 1% acid+enzyme (citric acid+pectolytic enzyme mixture) pretreatments were applied. Separate and combined effects of roasting and acid+enzyme pretreatments were analyzed. All of the pretreatments increased the oil yield compared to the non-treated control sample, the highest yield was observed in the batch where roasting at 140 °C and acid+enzyme mixture were applied together (17.8%). Acid+enzyme pretreatment caused slight increase in free fatty acidity, yet the values remained below 0.7% oleic acid. Pretreatments showed slight increment also in the peroxide values compared to that of control samples, even so the highest value in this analysis was measured as 2.9 meq$O_2$/kg. The lowest values for both the parameters (0.25% oleic acid, 0.23 meq$O_2$/kg, respectively) were observed in the samples roasted at 100 °C.

**Keywords:** Pomegranate seed, Cold pressed oil, Citric acid, Pectolytic enzyme, Oil yield

## INTRODUCTION

Pomegranate seed oil has recently attracted attention in terms of human and animal nutrition due to its unique composition in terms of both its fatty acid profile and minor bioactive components. The antimicrobial activity of the minor components of pomegranate seed oil has recently been used as a component of active food packaging. Finally, the presence of secondary metabolites with beneficial effects for human health has encouraged its use in food supplement formulations and the application of pomegranate seed oil as a pharmaceutical ingredient. Pomegranate seed oil constitutes 10% to 25% of the total weight of the seed. Its chemical composition consists mainly of polyunsaturated fatty acids. Punicic acid constitutes 31% to

86%. The unsaponifiable minor part is largely tocopherols, phytosterols and phenols. Pomegranate seed oil is the main source of punicic acid (a conjugated linolenic acid) along with unsaturated fatty acids, tocopherols and phytosterols. It is also known to exhibit antimicrobial, antioxidant, immunomodulatory, anti-carcinogenic and lipid metabolism regulating effects. Solomakou et al. (2024) conducted a study on the evaluation of pomegranate fruit by-products using ultrasound and enzymatic methods. In the study, holistic and sustainable methods for obtaining valuable compounds from by-products of the pomegranate industry were discussed. The optimum conditions for oil extraction were stated as 2.3% pectinase enzyme concentration, 6 mL/g water/seed ratio and 90 min. It was stated that the oil yield under these conditions was 27.53%, which corresponded to 97.8% oil recovery rate. They observed an increase of approximately 50% in oil yield with the pretreatments applied to pomegranate seed oil. Enzymatic pretreatments applied to pomegranate seeds are an alternative way to increase the yield and quality of pomegranate seed oil. Kaseke et al. (2021) tested this study on different pomegranate varieties in 2021. According to the experimental results, they showed that a pomegranate variety with high antioxidant properties and high-quality seed oil was obtained.

Pomegranate seed oil is generally obtained by cold pressing in order not to damage the functional components that are beneficial to health. As a result of the increasing interest in natural and organic foods, cold press oil production and consumption has come to the fore. The cold press method is economical and easy to apply, and it is safe and healthy because it does not involve processes that disrupt the quality parameters of the oil. The important advantages of this method, which is simple and easy to use, are that it requires a very small amount of energy, does not use solvents, does not reach as high temperatures as in other methods, and protects the high polyphenol compounds, which are one of the most important quality elements of the oil, and prevents oxidative deterioration in the oil caused by radicals thanks to the natural antioxidative properties of these compounds. However, the yield obtained in cold press extraction is quite low compared to other methods. The use of cellulosic and pectolytic enzymes that break down the cell walls of oil seeds and seeds to increase oil yield has been scientifically researched, but is a step that has not yet become widespread in terms of implementation.

Previously, enzyme addition and acid applications have been used to obtain oil from various oilseeds and it has been found that they significantly increase the yield. The vast majority of these applications have been carried out alone. The number of studies evaluating the enzyme process together with acid applications is very limited. In this study, combined trials of the above-mentioned pretreatments were carried out and compared with conventional roasting.

## MATERIALS AND METHODS

Pomegranate seeds were supplied by an organic pomegranate juice factory (Elite Naturel Ind. Trade Inc., Ankara, Turkey) (Picture 1). The seeds were washed thoroughly and dried at 50 °C. 16 kg of pomegranate seeds were used in the study. Reagents for measurements were supplied by Sigma-Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany). The enzyme complex was a commercial product consisting of cellulases, pectinases and hemicellulolytic enzymes obtained from *Aspergillus aculeatus* (SEBMax Olive, Advanced Enzyme Techn. Ltd., India).

Oil seeds were ground with a laboratory scale grinder (220 V, 1-30 kg seeds/hour capacity, 1.5 kW, 9000 rpm) to a particle size of 1-2 mm ground. Enzyme preparate was applied via a buffer solution (0.1 M $NaH_2PO_4$, pH adjusted to 6 with 0.5 M NaOH or phosphoric acid) containing 10 g commercial enzyme preparate. This solution (100 mL) was sprayed onto 1 kg of ground seeds layered on an oven tray. The seeds were divided into 4 groups. Roasting was applied at 100 and 140 °C for 30 minutes. For the combined citric acid and enzyme treatment, a 50:50% (v/v) mixture was prepared and after applying to ground seeds, seeds were left for incubation at 60 °C for 15 hours. Control samples were produced without roasting, citric acid or enzyme application. The effect of citric acid+enzyme application following roasting was also investigated as the 4th sample group. After the pretreatments, the seed moisture ratio was adjusted to 8% and oil was extracted with a cold press machine.

750-1000 g of seeds were pressed with a screw press (Karaerler NF 500, Turkiye) (nozzle diameter 5 mm, shaft screw diameter 33 mm, rotation speed 50 rpm) at oil flowing temperatures below 50 °C (Picture 2). Oil extraction was carried out with two replications. The oil yield (%) was calculated as the proportional amount of oil obtained from 100 g of seed (Ezeh et al., 2016). The oil extracted was stored in an amber glass bottle.

Picture 1. Pomegranate seeds used in the assay

**Oil analysis**

Free fatty acids (FFA) and peroxide value (PV) of olive oils were determined following the analytical methods described in Regulation European Economic Commission (EEC, 1992).

FFA, given as % oleic acid, was determined by titration of oil solution dissolved in ethanol/ether (1:1, v/v) with 0.1 mol $L^{-1}$ ethanolic potassium hydroxide solution.

PV was expressed in milliequivalents of active oxygen per kilogram of oil (meq of $O_2$ $kg^{-1}$). The values were determined as following: a mixture of oil and chloroform–acetic acid was reacted with a solution of potassium iodide in dark. The free iodine was then titrated against a sodium thiosulfate solution.

Picture 2. Cold pressed pomegranate seed oil obtained in the assay

## RESULTS AND DISCUSSION

### Effects of acid and enzyme pretreatments on pomegranate seed oil yield

The highest yield was found in the sample where roasting and acid+enzyme treatment was applied together. A significant yield of 17.8% was obtained. This increase meant a 55.73% increase in yield compared to the control.

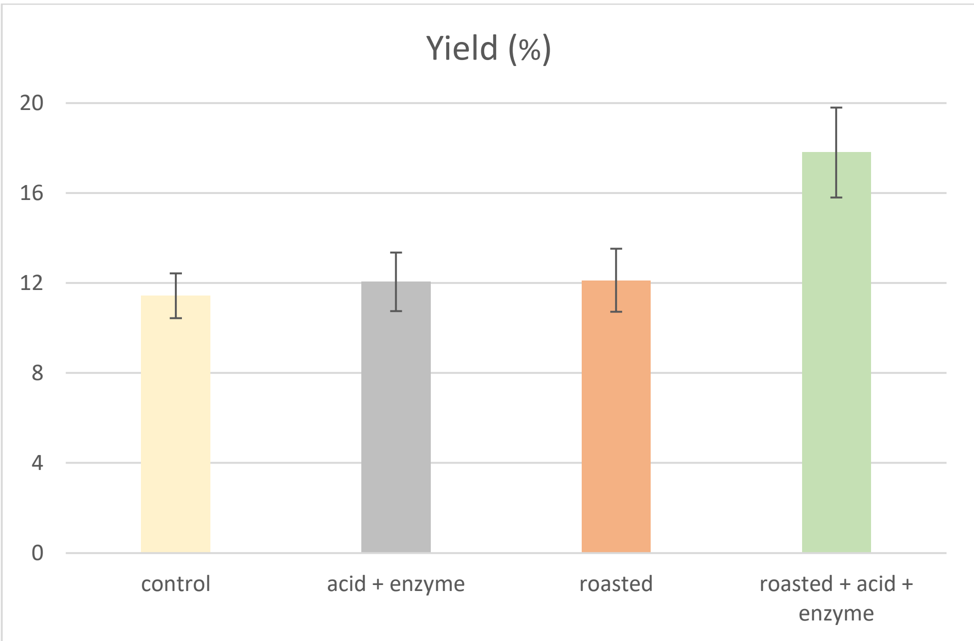


Figure 1. Oil extractability from the pomegranate seeds

There are two main purposes of applying the roasting process to fruits and seeds containing oil; the first is to ensure that the proteins in the seed and fruit coagulate and the oil droplets combine and collect with roasting, and to ensure that the oil in the plant tissue is taken more effectively and easily. The second is to increase the oil yield by reducing the affinity of the oil to the solid surface of the seed. As a result of acid + enzyme application following roasting, these two effects combined and provided the highest yield.

In previous published articles, it has been reported that enzymatic pretreatment significantly increases the oil yield obtained from oilseeds (Passos et al., 2009; Latif and Anwar, 2009; Grasso et al., 2012). The effects of applying enzymes as pretreatment to oilseed vary according to the seed composition. Seed cells are surrounded by a cell wall consisting of cellulose, hemicellulose, pectin and protein, which are included in the group of polysaccharides (Perez et al. 2013). Seed oil bodies are surrounded by phospholipids and a protein membrane (Nikiforidis et al., 2019). Therefore, the extraction of oil is facilitated by the application of enzyme mixtures exhibiting a wide range of activities (Kaseke et al., 2021). Passos et al. (2009) applied cellulase, hemicellulase, protease and pectinase enzymes to grape seeds and thus, 192% increase in oil yield was observed. On the other hand, some fibers can prevent enzymes from accessing the protein (Dominguez et al., 1995).

Treatment of proteins at pHs far from the isoelectric point disrupts the stability of the matrix and facilitates the removal of oil by the solvent (Rosenthal et al., 1996). The partial effectiveness of citric acid in increasing oil yield in the present study may be attributed to this effect.

**Effects of acid and enzyme pretreatments on pomegranate seed oil free fatty acidity**

Acid + enzyme treatment caused a slight increase in free acidity compared to the control. However, despite the increases, acidity values remained below 1% (the highest free acidity value was 0.66 in terms of %oleic acid). The lowest acidity value was determined in oils obtained from roasted seeds with 0.25%. This may be due to the inactivation of lipase enzyme in seed cells due to heat treatment (Keying et al., 2009).

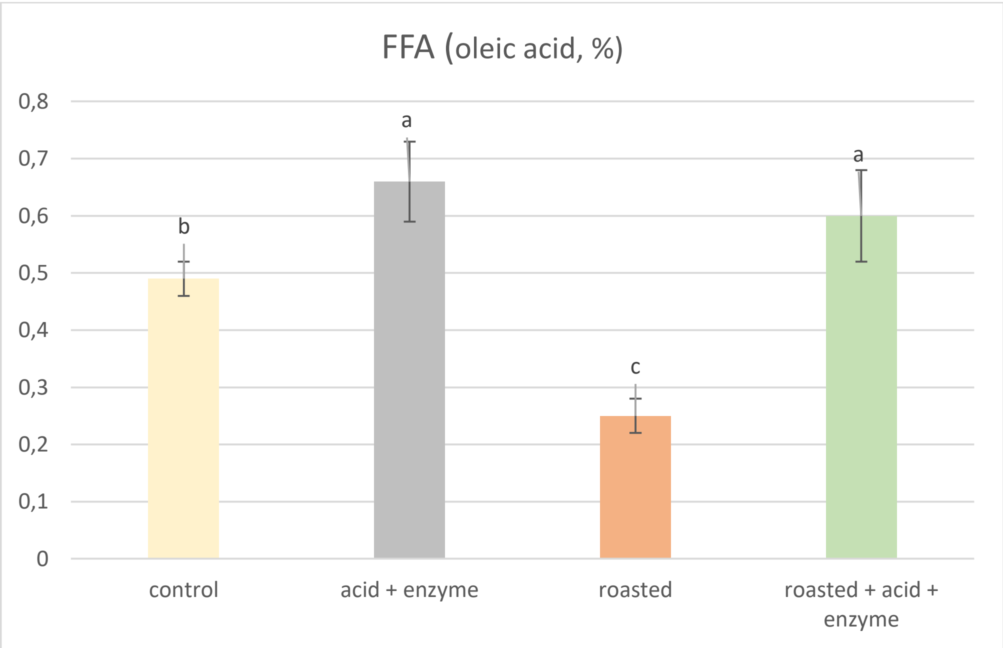


Figure 2. Free fatty acidity of the pomegranate oils

**Effects of acid and enzyme pretreatments on pomegranate seed oil peroxide value**

Among the pretreatments, acid + enzyme treatment caused a significant increase in peroxide values compared to control samples. In this sample, the highest value in this analysis was measured as 2.89 meqO2/kg oil. Peroxide values were measured significantly lower in oils obtained from roasted beans (0.23 and 0.56 meq$O_2$/kg oil). The lowest values for both parameters (0.25% oleic acid, 0.23 meq$O_2$/kg oil) were observed in samples roasted at 100 °C. All applied pretreatments increased the yield compared to the control.

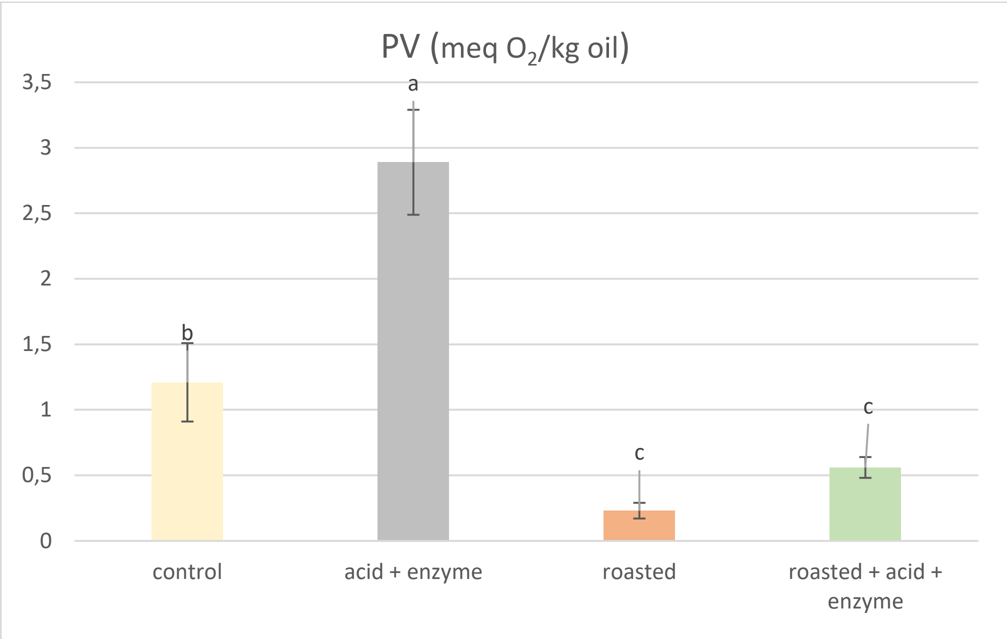


Figure 3. Peroxide values of the pomegranate oils

In the study conducted by Kaseke et al. (2021) on pomegranate seed oil, enzymatic pretreatment showed the highest oil yield (15.40%). It was assumed that this could be due to more damage in the seed matrices and therefore more hydrolytic oxidation of the oil surface area. According to the Codex Alim. Commission standard (CAC, 1999), 15 meq$O_2$/kg oil is allowed as the maximum PV values in unrefined seed oils. Considering the PV values determined in the current study, the quality of the oils obtained is below the limits in the standard. Therefore, the application of pretreatments does not prevent obtaining oils that are acceptable in the international market. Pomegranate seed oil is an oil that is significantly resistant to oxidation during processing due to the strong antioxidant compounds it contains in high amounts (Kaseke et al., 2021).

## CONCLUSIONS

Enzyme + acid application provided a positive increase in yield. There are many examples in the literature that enzymes increase oil yield. It is thought that this is due to the enzyme mixture used, which damages the cell wall and separates the oil more easily. The addition of acid also strengthened this effect. Roasting followed by acid + enzyme pretreatment can be recommended as a prominent process as it does not negatively affect oil quality indices and provides a significant increase in oil yield compared to other applications.

In future studies, the effects of these pretreatments on bioactive components and the effects they will exhibit in the later stages of oxidation can be investigated.

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# SUSTAINABLE AGRICULTURE: GROWING SAFFLOWER OILSEED IN ARID CLIMATES

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## ABSTRACT

Safflower oilseed is a crop native to Egypt and India with arid climates. Global warming in the world requires an alternative approach to well-known oilseeds (sunflower, rapeseed). Many years of research have been carried out to study the culture of safflower oilseed in contrasting natural conditions: Russia, Southern Kazakhstan and Tajikistan. The goal was to study the biological characteristics, productivity, accumulation of oil content, the manifestation of the main disease in arid conditions and to create a variety with adaptive potential for a specific region. Based on the research results, new varieties of safflower oilseed Krasa Stupinskaya (Russia), Akmai, Nurlan, Iirkas and Moldir-2008 (Kazakhstan), Shifo (Tajikistan) were created and recommended for sustainable farming in the above regions.

**Key words:** Safflower oilseed, drought conditions, contrasting ecological regions, global climate warming, variety.

## INTRODUCTION

Due to the ever-increasing diversity and scale of the use of the depleted resources of the natural environment, the future of mankind depends on its ability to harmonize its relations with nature. The claim that man's discord with nature begins with agriculture is indeed justified, as evidenced by hundreds of millions of hectares of eroded, desolate, saline and wetlands, catastrophic destruction of forests in order to increase the area of agricultural land.

Currently, agriculture is the main user of land resources (37% of land), the world's freshwater reserves (about 80%), as well as phosphates, potassium, calcium and other minerals. According to FAO, the annual loss of productive and pasture lands is more than 13 million and the area of irrigated lands with varying degrees of salinity has reached 110 million hectares, that is, more than 40% of their total area. In such situations, sustainable agriculture is required. To ensure food security for their people. In this regard, the purpose of our research was in the cultivation of safflower oilseeds in the growing season (dry and wet years) in the Russian Federation and the acutely arid regions of Kazakhstan and Tajikistan.

There are a small number of countries in the world where safflower is grown [1]. Safflower was mainly grown in Kazakhstan and Russia in 2021. The share of gross safflower harvests in Kazakhstan was 35.5% (of the total collections), in Russia - 24.0%. In 2022, safflower harvests in Russia have increased significantly (**Table 1**.)

**Table 1.** *Areas, harvests and yields of safflower by country, data for 2021*

| № | Country | Area, thousand hectares Collections, thousand hectares Yield, c/ha | Area, thousand hectares Collections, thousand hectares Yield, c/ha | Area, thousand hectares Collections, thousand hectares Yield, c/ha |
|---|---|---|---|---|
| **1** | Kazakhstan | 367.97 | 223.90 | 6.09 |
| **2** | Russia | 242.31 | 151.38 | 6.25 |
| **3** | USA | 54.63 | 61.31 | 11.22 |
| **4** | Mexico | 31.10 | 52.55 | 16.90 |
| **5** | India | 46.47 | 36.00 | 7.75 |
| **6** | China | 22.75 | 33.55 | 14.75 |
| **7** | Türkiye | 14.45 | 16.20 | 11.21 |
| **8** | Tanzania | 25.16 | 13.83 | 5.50 |
| **9** | Ethiopia | 7.52 | 9.65 | 12.83 |
| **10** | Tajikistan | 4.24 | 6.33 | 14.93 |
| **11** | Kyrgyzstan | 7.74 | 6.00 | 7.76 |
| **12** | Argentina | 6.00 | 6.00 | 10.00 |
| **13** | Uzbekistan | 9.83 | 5.45 | 5.55 |
| **14** | Iran | 3.70 | 4.89 | 13.20 |
| **15** | Australia | 6.23 | 3.62 | 5.81 |
| | Other countries | 0.34 | 0.38 | - |
| | Total | 850.43 | 631.05 | 7.42 |

The largest importing countries and their suppliers in 2022

The largest of them in 2022 were: Turkey (62.3 thousand tons), China (60.7 thousand tons), USA (12.2 thousand tons), Belgium (9.1 thousand tons), Poland (5.6 thousand tons).

1. China. Imports of safflower seeds in 2022 were formed only through imports from Kazakhstan.
2. Türkiye - the main volume of purchases is carried out in Russia. Safflower seeds are also imported into the country from Kazakhstan and Egypt.
3. USA - the country makes most of its purchases of safflower seeds in Russia. According to the WTO, the United States purchased 6.1 thousand tons of safflower seeds from Russia in 2022. In addition to Russia, supplies of safflower seeds to the United States in relatively large volumes were carried out by Bulgaria, Mexico, Canada and Argentina.
4. Belgium - the main supplier of safflower seeds is Russia, supplies also come from the Netherlands, Germany, France and other countries.
5. Poland - the main supplier is Russia; smaller volumes of purchases are made from Kazakhstan, Belarus and the Netherlands.
6. The Netherlands - the main volume of purchases is made in Russia. Other suppliers include Spain, Germany, and some other countries [1].

## 2. Growing safflower oilseed in the acutely dry year of 2010 and in contrasting humid conditions in the Central region of the Russian Federation

The problem of drought is acute across large areas of our country. In addition, as the results of research by major Soviet and foreign climate scientists show [2], the likelihood of this unfavorable phenomenon not only will not decrease in the coming decades, but will increase. N. I. Vavilov [3] rightly considered the most important measures to combat drought to be the selection of drought- and heat-resistant crops and the creation of drought-resistant varieties for various ecological and geographical zones of the country based on the widespread use of world collections of agricultural plants of the All-Union Institute of Plant Growing.

The need to carry out such work is associated with the peculiarities of the agricultural conditions of our country, characterized by the widespread and frequent recurrence of such an unfavorable phenomenon as drought [3].

The main goal was not only to study the culture of safflower oilseed under conditions of drought and high humidity, but also to create a variety with adaptive potential for the development of sustainable agriculture in various regions of the Russian Federation.

Long-term studies from 2005 to the present have been carried out in the Moscow, Saratov and Rostov regions. The object of the study was the variety Krasa Stupinskaya (2005) created by us and some samples from the Genbank (St. Petersburg).

Phenological observations and biometric assessment were conducted during the growing season in accordance with the Methodology of State testing of agricultural Cultures [4].

Harvest definition was carried out with using of sample plots in 3 replicates, accounting plot area -10 $m^2$. Determination of oil content in the seeds was conducted in accordance with GOST 10857 «Oilseeds» [5].

Determination of oil content, the fatty acid composition of the oil were made in accordance with GOST 30623-98 «Vegetable oils and margarine. Detection method of falsification» [6].

We present the meteorological weather conditions in the year 2010, which was extremely dry for 50 years of research, and the year 2013, which was atypically overly humid.

The vegetation conditions in 2010 were quite unfavorable. The average temperature of the vegetation season was 6.5°C (22.9°C) above the average long-term norm (16.4°C). In May 2010, weather conditions were favorable for the growth and development of winter wheat plants – the air temperature corresponded to the average annual value (14.2 – 14.5 °C). The last rain fell on June 18 and there was no rainfall until September 3. The air temperature in the Moscow region in June was 33$^o$C, in July – up to 38$^o$C, in August – up to 39.7$^o$C (the average long-term temperature was +18.2$^o$C, +20.5$^o$C, +19.0oC in June, July and August, respectively), the hydrothermal humidification coefficient (HHC) was 0.8. The lack of precipitation, as well as the abnormally high temperature of the air, has created a threat for the normal development and maturation of the agricultural crops.

Average daily air temperatures in the first and second ten days of May in 2013 (13.2 and 19.9 °C, respectively) exceed the long-term average by 3.5 and 8.5 °C, while the figures for the third ten days of May (+17.1 °C) are lower norms by 2 °C. In May, the highest air temperature was recorded (+3.3°C) on May 2, the highest (30.2°C) on May 16.

In the first decade of May, 26 mm of interference fell, which is 12 mm or 46.1% above the norm. In anticipation, rains of varying waves fell daily from May 14 until the end of the month. In total, 131 mm of deviations occurred in a month, which exceeded the long-term average by 2.7 times (the norm was 49 mm).

The average daily air temperature in June (19.9°C) is 4.5°C above normal, with minimum temperatures ranging from +8.2 to +18.3°C, maximum temperatures from +15.1 to +30.7 °C. Precipitation fell regularly as time went on, with 3 days with precipitation observed in each decade; additional monthly fluctuations (34 mm) were lower than the average summer indicators (63 mm) by 29 mm or 46%.

The first ten days of July were characterized by variable air temperatures: sounds - from +14.2 to +17.4 °C, maximum - from +24.1 to +29.5 °C. The daily average (+21.5 °C) exceeded the long-term average by 4.1 °C. In the II and III decades, a decrease in temperature was noted. Minimum air temperatures varied from + 10.5 to +15.3 °C, maximum – from +15.0 to +25.6 °C, average daily air temperatures for the second and third decades (+17.8 °C) – at the normal level (+18 °C). The average temperature of the July month of the week is 19.0 °C, with the norm being 17.7 °C. During the month there were 21 days with precipitation. During the month, 105.5 mm fell, which is 27.5 mm or 35.2% above the norm (78 mm).

In August, the minimum air temperatures varied from +10.0 to +17.9 °C, the maximum - from +21.2 to +30.2 °C, the average daily indicators for the month - +18.1 °C, above the summer average by 2. 1 °C. During the month there were 13 days with precipitation. During the first decade, 36.8 mm fell (+9.8 mm from the norm). In the second decade - 11 mm, in the third decade - 16.6 mm (below the norm by 13 and 6.4 mm, with the norm being 24.0 and 23.0 mm).

The growing season of bright crops was characterized by residual additives exceeding the long-term average. In the spring of 2013, precipitation exceeded the average summer norm by 2 times, and in the summer, it was 1.2 times higher than the norm. Due to the abundant snow cover and heavy rains that occurred in the spring, the soddy-podzolic soil underlying the clayey rock of the village of Mikhnevo was over-compacted and oversaturated with moisture.

Due to waterlogging of the land at the end of April - beginning of May, difficulties arose in preparing the land for spring field work. Safflower sowing was carried out at a relatively late date, May 10-13. Shoots were noted on the fifth or sixth day. On May 14, a period of heavy rains began, which lasted until the end of the month. An excessive amount of interference, combined with a favorable temperature regime in July-August, disrupted the seed filling process. The safflower crop produced a low yield of poor quality. The total temperature above +5 °C in 2013 was - 2264.8 °C, the growing season in 2013 was characterized by wet weather - HHC (hydrothermal coefficient) = 1.6. Agrometeorological conditions in 2013 can be characterized as factorial for all crops.

### 2.1. Biological characteristics of the oilseed safflower variety Krasa Stupinskaya in contrasting soil and climatic conditions

Comparative study of the growth zone influence on the vegetation period and the main economically valuable signs of safflower grown in four regions was made: Central Federal District (Moscow region, Mikhnevo), Volga Federal District (Saratov region) and the Southern Federal District (Rostov region) and Central Tajikistan.

Krasa Stupinskaya is an annual herbaceous plant with well-developed tap root system that penetrates into the soil to 10-20 cm, in the southern regions up to 1.5-2 m (and Central Tajikistan) [7].

Stem is glabrous, erect, branchy, height is about 83-90 cm. Leaves are sessile, lanceolate, oval or elliptical lancet, on the edges with small teeth, ending with small spines. The inflorescences are many-baskets, 1.5-3.5 cm in diameter. The number of baskets on the plant from 5-7 to 20-50 pieces. The flowers are tubular, with five separate corollas yellow or orange color. Fruit - achene, brilliant, reminiscent of sunflower achenes, **Figure 1.** Its hard shell, it is difficult to split, is 40-50 % of the seed weight. The seeds not crumble, can germinate at a temperature of 1-2 ° C, but there are better and friendly germinate when the soil warm up to 5-6 ° C and more at a depth of 10 cm.

**Figure 1.** *Seeds and flowers of safflower oil variety Krasa Stupinskaya*

Sowing in each region annually spent: in Mikhnevo – 7.05-11.05, in the Saratov region – 7.05, in the Rostov region – 26.04, in Central Tajikistan - 20.12-25.12 and 10.03-15.03 (spring planting). Seedlings always have been friendly and appeared in 3-8 days. Period from the beginning of budding until flowering was within 18-23 days. Flowering lasted about 29-35 days. Harvest ingathering was carried out in Mikhnevo – 23.08, in the Rostov region – 12.08, in the Saratov region – 16. 08, In Central Tajikistan – 7.04-10.04 (at the winter sowing) and 28 June - 2 July (during the spring sowing).

Vegetation period from germination to maturation was 96 days in Moscow region in 2013 (versus 112 days in 2010), 93-95 days in the Rostov region and 89-103 days in the Saratov region and in Central Tajikistan - 110 days. In all regions safflower vegetation period was almost the same.

The calculation of basic safflower harvest indicators gave the following results: the number of plants per 1 m2 (p/m2) was in Mikhnevo – 26 p/m2, in the Rostov region – 30 p/m2 (planted for seeds), in the Saratov region – 62 p/m2 (planted for feeding purposes). Plant height ranged from 63 - 80 cm in all regions. Mass of 1000 seeds were as follows: in Mikhnevo in 2010 – 50.0 g; 2013 – 30.3 g; in the Saratov region in 2013 – 30.9 g, 2010 – 48.1 g; in the Rostov region in 2010 – 42.3 g, in 2013 – 53.4 g. In Moscow region productivity of safflower Krasa Stupinskaya was in 2013 0.4 t/ha, in 2010 – 0.8 t/ha; in Saratov region: 2013 - 0.9 t/ha, 2010 – 2.0 t/ha; in Rostov region - 1.25 t/ha in 2010 and 0.6 t/ha in 2013.

Krasa Stupinskaya is recommended as a green manure, phytosanitary, fodder, ornamental, promising oilseed crop, and also for use in medicine. Safflower performs best as a green manure on soddy-podzolic soils.

### 2.2. The phytosanitary role of safflower

Safflower cultivation for green manure reduces contamination subsequent cereal -spring barley up to 24 pcs/m2 or 62 % (2008-2009), Spelled -11 pcs/m2 or 89 % (2013-2014) after two years of safflower growing. Infestation in barley and spelled crops after mustard white and blue lupine averaged was 17- 20 pcs/m2 or 20.2 %.

### 2.3. Safflower as a fodder crop

At 100 kg of safflower green mass with a moisture content near 76.06% found 22.75 k.e. At 100 kg of silage with the moisture content 82.78% - respectively, 15 k.e. and 1.3 kg of digestible protein. The 100 kg of safflower oil cake contained 75.5 k.e.

### 2.4. Decorative and honey culture

Safflower is also a decorative plant with yellow, orange and red inflorescences that can decorate the gardens. Longer flowering during the month will be pleasing to the eye with its bright colors and fragrant smell. Safflower honey had a golden-orange color, **Figure 2**.

**Figure 2.** *Safflower flowers and honey*

### 2.5. Safflower as an oilseed

At the moment seeds oil content increasing selection have become a major asset of our agricultural production, such as the property becomes a breeding for oil quality change.

It has been shown that each variety and even the shape of the population are composed of a larger or smaller number of biotypes differing by number of features, including the concentration of the fatty acid oil [8].

The basis in the selection to the quality of oil for technical and food use is the knowledge of genotypic variability of the fatty acid's composition in the range of cultivated species and wild relatives.

N.I. Vavilov [3] attached great importance to the study of differentiation within the species for chemical signs of quality grades, repeatedly emphasizing need to identify genetic differences that can be seen in the study in the same conditions of different varieties in different geographical locations.

We conducted a comparative analysis of the oil content determination in the safflower seeds (Krasa Stupinskaya) for three years, obtained from the Rostov region. Mass fraction of fat in the seeds was 19.02% in excessively wet 2013, while the fat content in seeds was 23.7% in severely dry 2010. In the Moscow region, in the excessively humid year of 2013, the oil content was 6.4%, in the extremely dry year of 2010 - 31.2%.

We noted that the accumulation of oil content depends not only on the amount of precipitation, but also on the temperature factor. Moderate amounts of precipitation and temperatures above 18 °C (flowering and filling phases) have a positive effect on the formation of oil content.

In 2013, when 335.8 mm of precipitation fell during the growing season (the norm was 264 mm) and the temperature was 18.4°C, the mass fraction of fat was only 6.4%. In 2010, an acutely dry year, with an increased air temperature of 18.8 °C (average temperature 15.1 °C) and a reduced amount of precipitation of 154.4 mm during the growing season, the accumulation of the mass fraction of fat was 31.2%.

According to the content of linoleic acid, that is not synthesized in the human body, this variety (Krasa Stupinskaya) is not inferior to the southern variety Mahalli 260, **Table 2**. According to the content of oleic acid - 16.89 %, responsible for preserving the freshness of the oil over a long period, it exceeded other varieties. The higher content of saturated fatty acids, particularly palmitic characterized sort Krasa Stupinskaya. Krasa Stupinskaya has oil yield near 240 kg/ha (at plant density of 250-300 thousand/ha and seed yield - 0.8 t/ha). In Central Tajikistan oil output amounted near 940 kg per hectare (at plant density of 160 thousand plants per hectare and crop seeds 1.7 t/ha).

**Table 2.** *Fatty acid composition of safflower oil in 2013*

| **Fatty acids** | **Mass fraction of fatty acids, % to total content of fatty acids** | | | |
|---|---|---|---|---|
| | Mahalli 260 (Tajikistan), 2013 | Krasa Stupinskaya, 2010 | Krasa Stupinskaya, 2013 | Norms in accordance with GOST 30623-98 |
| **$C_{14:0}$ (myristic)** | 0.1 | 0.1 | 0.1 | < 1.0 |
| **$C_{16:0}$ (palmitic)** | 7.6 | 9.94 | 7.7 | 2.0-10.0 |
| **$C_{16:1}$ (palmitoleic)** | 0.2 | 0.55 | 0.1 | < 0.5 |
| **$C_{18:0}$ (stearic)** | 2.6 | 2.48 | 2.0 | 1.0-10.0 |
| **$C_{18:1}$(oleic)** | 13.2 | 16.89 | 13.6 | 7.0-42.0 |
| **$C_{18:2}$ (linoleic)** | 75.6 | 65.88 | 75.7 | 55.0-81.0 |
| **$C_{18:3}$ (linolenic)** | 0.2 | - | 0.1 | < 1.0 |
| **$C_{20:0}$ (arachidic)** | 0.3 | - | 0.4 | < 0.5 |
| **$C_{20:1}$ (gondoinovaya)** | 0.2 | - | 0.3 | < 0.5 |

The productivity increase and safflower product quality depend onfarming practices of cultivation. It is necessary to adhere to morphobiological features of crops and varieties, keeping the complex soil-climatic conditions of the region, a specific agricultural production, hydrothermal regime during the growing season. What matters is technical equipment, financial condition and agronomic management frames.

Therefore, the potential yield and economic effect of the new culture introduction will largely depend on the use cultivation technology adapted to local conditions, taking into account all these factors. All agricultural practices that are recommended for the cultivation of crops should be carried out at one time, because the omission or wrong application of one of the elements will affect the yield and quality of seeds.

## 2.6. Place in crop rotation and tillage

The best safflower precursors are crops. Safflower is not demanding to soil. It grows well even on poor (on qualitative structure) sod-podzolic soils of Moscow region. Culture is particularly demanding to the heat in the phase of flowering and ripening. Seedlings can withstand frosts to 3-5 ° C.

The fat content in seeds of safflower depends on temperature. Under optimum standards rainfall during the growing season (average 255.4 mm, normal 264 mm) and moderate temperatures - at a rate of 18,2 15,1 ° C, oil content in seeds is up to 30.5%, in the dry 2010 - 31.2 % (on dry substance).

At high humidity like in 2013 (not typical), reduces the yield and oil content in seeds and also enzyme-mycotic exhaustion developing with an abundance of empty seeds.

### 2.7. The results of the survey in 2010, an acutely droughty year with late harvesting

The list of the most common diseases of safflower is quite extensive. Among the harmful bacterial diseases is bacterial spotting of leaves and stems, caused by the bacteria *Pseudomonas syringae*. Phytoplasmas (mycoplasma-like organisms - MLO organisms) can cause growth and phyllodes of safflower inflorescences.

Among fungal diseases, the most common are *Alternaria* leaf spot, caused by a specific species *Alternaria carthami* (Rodigin) Chowdhury, as well as other leaf spots caused by anamorphic fungi, incl. cercospora - *Cercospora* sp., septoria - *Septoria* sp., ascochyta - *Ascochyta carthami* M. Chochr., phyllosticta - *Phyllosticta carthami* Tropova, ramularia - *Ramularia carthami* Zapr. Safflower plants can be affected by sclerotial rot caused by the fungi *Macrophomina phaseolina* Ashby., downy mildew - *Bremia lactucae* Regel, powdery mildew - *Oidium carthami* Jacz., and several species of rust pathogens from the genera *Puccinia* and *Aecidium*. Of the pathogens with a wide phylogenetic specialization, the causative agents of carbonaceous hard botrytis rot - *Botrytis cinerea* Pers and white rot - *Scleritinia sclerotiorum* Fuck.; Plant wilting can be caused by soil-dwelling fungi *Fusarium oxysporum* Schl. and *Verticillium dahlia* Kleb; The causative agents of late blight root and stem rot are oomycetes of the genus *Phytophthora*, most often *P. cactorum* (Lebert & Cohn) J. Schröt.; In the early stages of ontogenesis, root rot of seedlings is caused by *Pythium* spp. [9].

Among viral diseases, the most common are: *Alfalfa mosaic virus*, *Cucumber mosaic virus*, *Lettuce* mosaic *virus*, *Turnip mosaic virus*.

Based on the results of visual diagnostics of the variety samples, it was revealed that the seeds had no external signs of damage. On the leaves, 40.5% of the lesion was found in the form of black-gray and white-gray fungal plaque on necrotic areas of round or irregular shape. On inflorescences, similar external signs of damage were common at the level of 50%.

The results of assessing the infestation of aboveground organs of safflower plants using the wet chamber method are presented in Table 3 and Figure 3. Fungal infestation was assessed by the presence of plaque - mycelium structures and sporulation. Only on inflorescences were fungal and bacterial microorganisms found in almost equal proportions. And on the seeds and inflorescences, bacteria clearly dominated in prevalence, determined by the presence of bacterial exudate in the form of droplets of mucus or liquid.

**Table 3.** *Average infestation of above-ground organs of safflower plants, identified by the wet chamber method*

| **Test samples** | **Contamination**, % | | |
|---|---|---|---|
| | bacteria | fungi | total |
| Leaves | 25,0±1,5 | 12,0±2,1 | 40,5±1,2 |
| Seeds | 60,0±1,7 | 40,7±2,0 | 50,0±2,4 |
| Inflorescences | 20,5±2,0 | 20,5±1,5 | 60,4±1,7 |

Subsequently, the plaque and exudate structures were transferred under sterile conditions to a nutrient medium, where pure cultures of microorganisms were obtained. Based on the appearance of the colonies and the color of the exudate, bacterial pathogens, presumably *Pseudomonas* syringae, were dominant, **Figure 4.** According to microscopy results, the main component of the fungal microbiota, both on seeds and on leaves and inflorescences, were fungi of the genus *Alternaria*; zygomycetes, presumably of the genus *Rhizopus*, were less common, **Figure 5**. At the same time, there were no differences in the presence of microorganisms on the surface and inside the seeds, except for damage by *Rhizopus*, which was isolated only from the surface. The high overall contamination of seeds - 50% - may have been associated with the increased moisture content of the examined seeds.

When assessing the frequency of occurrence of fungal and bacterial species on the nutrient medium (based on CFU), it was found that bacterial pathogens predominated, presumably *Pseudomonas syringae* (P=60%), fungi of the genus *Alternaria* (P=35%) and *Rhizopus* (P=35%) were less common. P=5%).

**Figure 3.**
*Fragments of affected leaves and safflower seeds in a humid chamber*

**Figure 4.** *A mixture of colonies of microorganisms isolated from the surface of safflower seeds (left), colonies (reseeding with streaks) of bacteria (Pseudomonas sp.) on a CHA (right)*

**Figure 5.** *Sporangia and mycelium of Rhizopus sp. (left); conidia and mycelium of Alternaria sp (right) from safflower seeds (x400)*

According to the results of the phytosanitary examination, it was revealed that on the seeds of safflower oleaginous varieties, with a total seed infection of 50%, bacterial pathogens, presumably *Pseudomonas syringae* (P=60%) dominate; Fungi of the genus *Alternaria* (P=35%) and *Rhizopus* (P=5%) were less common.

For more precise identification, the use of molecular research methods is additionally required.

Conclusions. As a result of many years of research on the safflower crop in the Central region of the Russian Federation, the influence of arid and humid climate conditions on yield and accumulation of oil content, as well as disease damage, has been established.

The genotype of the safflower crop showed its advantages in arid conditions. Consequently, with an annual increase in air temperature, i.e. With climate change, for sustainable agriculture in the Central region, we can recommend the Krasa Stupinskaya safflower variety we created for cultivation in agricultural production.

## 3. Cultivation of safflower oilseed in the southern arid climate of Kazakhstan

Kazakhstan is among the top producers of wheat in the world, and a lot of farmers there also grow crops like safflower and flax that can withstand drought. This is made possible by the great profitability of producing these crops, which allows one to diversify risks associated with farming traditional crops.

In the south of Kazakhstan, due to a decrease in precipitation during the growing season, there is a need to select drought-resistant crops, one of which is safflower. Also, a biological feature of safflower is the ability to grow on saline salt marsh lands and produce crops in extremely dry years, when other grain crops die. In addition, the cost of safflower production is much lower and the costs are economically justified compared to sunflower. Safflower also showed resistance to pests and diseases compared to sunflower in the south of the country.

Safflower is mostly grown in Kazakhstan for its seeds, which are then processed into edible vegetable oil and used for breeding and seed production.

The production of plant food products is carried out using environmentally friendly cultivation technology.

Kazakhstan led the world in safflower seed production in 2018, accounting for 34% of the total with 627,653 tonnes produced worldwide. Other significant producers were the United States and India, 26% of world production combined.

In Kazakhstan, safflower selection is carried out at the Kazakh Research Institute of Agriculture and Plant Growing (KazNIIZiR), the Krasnovodopadskaya Agricultural Experimental Breeding Station (SHOSS) and the Aktobe Agricultural Experimental Station (ASC). These scientific institutions have created many safflower cultivars which have good productive and economic qualities. Drought-resistant varieties. Huskiness – 38-45%. Oil content 36-38%. The yield, depending on the rainfed zone, averages 7-12 c/ha.

The introduction of a new safflower cultivar into production will correspond to the main direction of diversification of the crop growing industry in the region - expanding the range of drought-resistant oilseed crops.

Safflower is not a commodity crop, and the price is based on an analysis of production and consumption in the world. The most important price parameter is the quality of edible safflower oil. Of course, the price depends on many factors, in particular on the balance of supply and demand. But over the past 4-5 years, safflower supplies to China have been the most profitable.

Safflower is adapted to the conditions of a sharply continental climate and, due to its demands on moisture, is one of the most drought-resistant plants.

Safflower seeds germinate at soil temperatures at a seeding depth of 1-2ºC, and seedlings tolerate frosts down to – 6-8ºC. The growing season in the conditions of the South and South-East of Kazakhstan is 110-120 days.

In addition, safflower is a honey plant. As a honey plant, safflower is very unstable, but is a good pollen bearer. In the South Kazakhstan region, where its crops cover 80-105 thousand hectares, it produces nectar in rare years and usually for no more than 5-10 days. At this time, the control hive gains weight from 0.5 to 2.5 kg per day. It has been noticed that at the slightest change in weather or wind, the function of the honey plant is interrupted. Beekeepers use safflower crops only in combination with other honey plants, so that in extreme cases the bees can stock up on pollen.

Safflower honey is usually light in color, with a characteristic yellowish tint, without any particular aroma.

The yield of safflower seeds in the unsecured rainfed areas of the southern regions was 8-10 hectares, and in the semi-secured rainfed conditions of the South-East of Kazakhstan - 12-14 c/ha.

**Place in crop rotation:** Leguminous crops, perennial grasses, and clean fallows are some excellent predecessors during cultivating safflower for seeds.

Basically, when safflower is cultivated for oil and feed purposes, the predecessors for safflower can be grains, as well as other crops.

Safflower in crop rotations is cultivated as a trailing crop for the purpose of clearing weeds and is a good precursor for spring crops.

**Soil cultivation:** The biological characteristics of safflower determined a number of agrotechnical methods for its cultivation. Preparing the soil for safflower sowing begins in the fall. The soil is plowed to a depth of 20-22 cm on heavy soils, and on light soils to a depth of 10-12 cm with flat-cutting subsoilers.

In dry autumn conditions, as a rule, the soil is dry, so the plowed land is left unfenced for the winter. Tilling dry soil with harrows in the fall leads to its spraying, which causes severe wind erosion.

In early spring, it is necessary to monitor the readiness of the soil, and as it dries, harrow with needle harrows in an active position with a low angle of attack.

**Sowing time:** The influence of sowing timing on the yield of cultivated crops is well known. Particularly important is the choice of time for sowing safflower in unsupplied rainfed areas, where in the spring there is a rapid increase in positive temperatures, causing increased evaporation of soil moisture.

The optimal time for sowing safflower in the rainfed conditions of the Turkestan region is before the first half of March (March 15). In Almaty and Zhambyl regions, the optimal sowing time is considered to be early April (before April 5), the period one week after the start of spring field work, i.e. after early spring harrowing in order to seal off moisture and carry out pre-sowing cultivation with harrowing and rolling. Later sowing dates, as well as excessively early sowing, lead to a decrease in safflower yield.

**Sowing methods:** Safflower is sown with a vegetable seeder SON-4.2 with a row spacing of 45 cm. If there are no special seeders on the farm, safflower is sown with ordinary seeders SZ-3.6; SZP-3.6; SZT-3.6 with setting the specified row spacing.

The continuous or row (15 cm) method is used when cultivating safflower for green fodder or silage.

**Seeding rates:** The optimal seeding rate when sowing with a row spacing of 60 cm is 160 000 seeds or 7.5-8.0 kg per 1 ha.

When sowing in a row method, the seeding rate increases 3-4 times - 0.5-0.7 million seeds or 25.5-32.5 kg per 1 ha.

In almost all areas of the region, farmers prefer to sow zoned cultivars of safflower: “Akmai”, “Nurlan”, “Iirkas” and “Moldir-2008”. They have proven their worth more than once, withstanding droughts, 50ºC heat and dry winds. Judging by the first cuttings, the safflower yield in 2021 was not bad, on average 9-10 centners per hectare. This is approximately 0.5 centners per hectare more than the yield of the previous 2020 year. Safflower harvesting in the region traditionally ends in early September (September 5).

The eastern and central regions of the South Kazakhstan (now Turkestan) region have been growing safflower from 1996 to the present time. This is a common agricultural crop, often found in the southern regions of Kazakhstan. In the structure of areas cultivated on rainfed lands in the Tulkubas, Kazygurt, Baidibek, Ordabasin, Sairam, Keles and Tolebi regions, safflower occupies about 35%. The average yield in 2021 was 10 c/ha.

Farms in the region use seeds bred by the Krasnovodopadskaya Agricultural Experimental Breeding Station.

In the southern regions of Kazakhstan, there is a tendency to reduce the amount of precipitation during the growing season for this oilseed crop, hence the lack of moisture, so there is a need to select drought-resistant crops, one of which is safflower. Also, a biological feature of safflower is the ability to grow on saline lands and produce crops in extremely dry years when other grain crops die.

In 2014 an oil plant for processing safflower seeds was launched. Raw materials are mainly sold to China, since there is a stable demand for these products in that country. At the same time, part of the safflower is processed into oil, which is sold both for export and on the domestic market of the country.

The prospects for safflower in Southern Kazakhstan are high. Firstly, this is a highly profitable production, even with a yield of 3 c/ha it gives a profit, **Table. 4.** Secondly, innovative areas of safflower use in the world in the form of flower petals in medicine are being developed, especially in the treatment of cardiovascular diseases. Therefore, there will always be a demand for this crop.

**Table 4.** *Safflower yield in the Turkestan (South Kazakhstan) region*

| | ***2016*** | ***2017*** | ***2018*** | ***2019*** | ***2020*** |
|---|---|---|---|---|---|
| **ha** | 70 546 | 109 287 | 92 827 | 88 055 | 80 732 |
| **Tons** | 59 258,64 | 93 986,82 | 81 687,76 | 72 205,10 | 75 080,76 |
| **c/ha** | 8,4 | 8,6 | 8,8 | 8,2 | 9,3 |

**“Akhram” - a new cultivar for conditions of hot dry winds.** It is a promising and adapted safflower cultivar for Western and Northwestern Kazakhstan. It was created by KazNIIZiR (Almaty region).

The plants have a compact shape, the number of productive baskets on one plant is 10-16 pieces, the height of the plants is 45-65 cm. The leaves are entire, sessile, the lower ones are oblong-oval without thorns. The flowers are orange-red, the achenes are yellowish-white.

The weight of 1000 seeds is 44.0-45.2 grams, which exceeds the standard by 10-15%. Seed oil content is 37-38%, which is 13.8% higher than the Akmai standard cultivar.

The average yield in the conditions of the Aktobe region is 9.5-10.5 c/ha, which is 30% higher than the zoned cultivars.

The following productivity structure element accounts for the high oilseed yield when compared to the "Akmai" standard cultivar: on a single plant, there are two or more times as many productive baskets with large seeds than the standard.

Cultivation of a new adapted safflower cultivar “Akhram” for oilseeds is profitable. The oil yield per hectare is 286 kg, which is 90 kg higher than that of the Akmay standard.

The introduction of this cultivar into production will contribute to the sustainable development of agriculture in the acutely arid regions of Kazakhstan.

Conclusions. The southern region of Kazakhstan is an acutely arid region. Many years of research have shown the successful cultivation of safflower oilseed in the fields. Scientists have created varieties “Akmai”, “Nurlan”, “Iirkas” and “Moldir-2008” that form a yield of 7 to 12 c/ha and an oil content of 36-38% even at 50ºC heat. Currently, a new variety, Akhram, has been created for the Central regions with an arid climate. The average yield is 9.5-10.5 c/ha, seed oil content is 37-38%. The varieties are recommended for the sustainable development of agriculture in the region.

## 4. Key findings from research of safflower cultivation in Tajikistan

The search for innovative agricultural technologies is one of the most important problems of agricultural production. This is especially important in the arid climate of Tajikistan.

With the help of new, highly productive, drought-resistant crops that can tolerate the climatic conditions of rain-fed areas, the yield of fodder and oilseed crops in zones should be increased using cutting-edge technologies. These requirements are met by safflower, which, due to its biological characteristics, is able to produce quite high yields of green mass with good feed qualities in conditions of dry-farming lands. It is also a universal culture that may be grown on seeds to produce oil [10].

Safflower is currently grown throughout the republic as an oilseed crop. Up to 37% of the edible oil is found in its seeds. The absolute fat content in the purified seeds reaches 60%. It is quite similar to hemp and poppy seed oils chemically, refers to semi-drying oil, the iodine number is 126-151. Safflower oil serves a variety of technical functions, including the creation of drying oils, the production of soap, the creation of linoleum, the preparation of margarine, and other uses in the paint and flooring industries.

The waste of oil production - oilcake and meal – is an excellent feed for animals.

### 4.1. Productivity of different safflower cultivars

More than 10 safflower varieties have been studied and the most productive of them for producing green mass have been identified. The goal was to investigate and pinpoint the plant cultivars that, under the bogara conditions of Tajikistan, produce the most seeds with a high oil content.

It is well known that the number of baskets on each plant, the number of seeds inside each baskets, and the weight of 1000 seeds are the three most significant components of the structure of the safflower grain harvest.

The Shifo cultivar has the highest indicators and produces an average of 27.0 baskets, each containing 33.2 of the largest seeds, on a single plant. 1000 seeds mass was 32.7 grams total. The highest seed productivity was also the highest -26.3g per plant. The biological crop of seeds has a yield of 31.6 c/ha (**Table 5**).

**Table 5**. *Crop structure elements of various safflower varieties*

| **Cultivars** | **Quantity, pcs** | | **Mass, g** | | **Biological yield, c/ha** |
|---|---|---|---|---|---|
| | Baskets on one plant | Seeds in one basket | Seeds from one plant | Mass of 1000 seeds | |
| **Milutinsky 114** | 18,8 | 24,3 | 18,2 | 31,5 | 22,8 |
| **VIR-489** | 25,2 | 31,6 | 23,7 | 31,8 | 28,4 |
| **Mestnaya 498** | 21,6 | 30,9 | 19,4 | 29,2 | 23,3 |
| **VIR 454** | 25,7 | 32,0 | 25,2 | 32,3 | 30,2 |
| **VIR 483** | 20,5 | 28,6 | 18,7 | 30,0 | 22,4 |
| **Shifo** | 26,9 | 33,2 | 26,3 | 35,7 | 31,6 |
| **Mestnaya 492** | 19,9 | 30,0 | 17,6 | 27,8 | 21,1 |
| **Mestnaya 260** | 24,5 | 30,4 | 25,0 | 32,1 | 30,0 |
| **VIR 376** | 17,7 | 24,5 | 17,8 | 29,7 | 21,4 |
| **Mestnaya 505** | 23,3 | 30,0 | 22,6 | 31,0 | 27,1 |
| **VIR 487** | 21,4 | 26,9 | 22,2 | 30,3 | 26,6 |

The number of baskets per plant, which in these cultivars is 24.5; 25.2; and 25.7 pcs, is also very interesting. Each basket contains 30.4; 31.6; and 32.0 seeds, weighing 25.0; 23.7; and 25.2g, respectively.

**Table 6** shows that throughout the years of research, the Shifo cultivar produced the highest seed yield. The average yield of seeds from one hectare of this cultivar's crops in three years was 24.3 c/ha. The seed yield of the VIR-464 was slightly lower (23.2 c/ha).

**Table 6.** *Safflower seed yield, c/ha*

| **Cultivars** | **Years** | | | **Average** | **Deviation from the standard** |
|---|---|---|---|---|---|
| | 2018 | 2019 | 2020 | | |
| **Milutinsky 114 (standart)** | 16,7 | 20,7 | 17,8 | 18,4 | - |
| **VIR-489** | 20,6 | 26,2 | 22,2 | 23,0 | +4,6 |
| **Mestnaya 498** | 16,8 | 20,9 | 18,1 | 18,6 | +0,2 |
| **VIR 454** | 21,9 | 25,9 | 21,8 | 23,2 | + 4,8 |
| **VIR 483** | 25,7 | 21,0 | 18,5 | 18,4 | ± 0 |
| **Shifo** | 22,4 | 27,5 | 23,0 | 24,3 | + 5,9 |
| **Mestnaya 492** | 16,1 | 20,2 | 17,1 | 17,8 | - 0,6 |
| **Mestnaya 260** | 20,5 | 24,2 | 21,3 | 22,0 | +3,6 |
| **VIR 376** | 14,9 | 19,3 | 16,2 | 16,8 | -1,6 |
| **Mestnaya 505** | 19,6 | 23,4 | 20,9 | 21,3 | +2,9 |
| **VIR 487** | 18,0 | 22,7 | 20,5 | 20,4 | +2,0 |

Therefore, it was found that the Shifo cultivar of safflower is promising for cultivation in conditions of rainfall-rich bogara of Tajikistan based on the results of three-year tests of various safflower varieties.

This cultivar was created using the individual selection method from the VIR catalog number 494's source material. Its plants stand out due to their height; in rain-fed bogara, stem lengths can reach 145–150 cm.

The leaves are large, completely extreme, almost sessile, oblong-lanceolate, and arranged in the following order.

The number of first order branches is large, ranging from 14 to 18 and each ending in a basket. On the branches of the first order, 4-5 branches of the second order frequently form and carry seed baskets as well. On one plant, 20–30 baskets are formed. The tubular, five-part, orange or yellow flower has five distinct petals. In the basket, between 30 and 65 seeds are formed. 1000 of their pieces weigh 34–40 g each. The white, glossy shells that make up the seeds' weight range from 34 to 39 percent. The core tightly fills the shell. The fat content in the dry kernel is 55-60%. The oil content of the whole achene ranges from 30-34%. The variety belongs to the medium-ripe group. With spring sowing, the duration of the growing season is 100-120 days, with winter (December) sowing 170-180 days.

Conclusions. In the arid climate region of Tajikistan, several safflower varieties were evaluated for yield and oil content. The Shifo variety was isolated with a maximum yield of 24.3 c/ha (average for three years of research), oil content of 55-60% (in dry kernel). The variety belongs to the mid-ripening group with a growing season of 100-120 days.

## CONCLUSIONS

The development of stable, sustainable agriculture during severely dry years in the Russian Federation, Kazakhstan and Tajikistan has highlighted the benefits of the safflower oilseed crop, which is native to Egypt and India. The biological characteristics, productivity, accumulation of oil content and disease development on safflower plants were studied. Oilseed safflower varieties with adaptive potential have been created, combining a stable yield and sufficient oil content. In the context of impending climate warming, the varieties will ensure food security in the above-mentioned countries.

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# INVESTIGATION OF CHANGES IN GERMINATION AND SEEDLING CHARACTERISTICS OF SOYBEAN SEEDS WITH DIFFERENT PACKAGING MATERIAL AND STORAGE PERIOD

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## ABSTRACT

Soybean seed quality is affected by pre- and post-harvest periods. Especially germination parameters are affected by post-harvest conditions and generally decrease until sowing time. In this study, changes in seed viability and seedling characteristics of soybean seeds stored in different packaging materials were investigated during storage. Soybean seeds were stored at room temperature (24oC±1) in plastic containers, plastic bags, glass jars, airless packages, paper bags and cloth bags for 12 months. At 2-month intervals, some germination and seedling characteristics of soybean seeds were examined. Germination rate, germination index, average germination time, vigor index, dry matter content, sprout and root lengths of soybean seeds decreased with increasing storage period. The decrease in germination and seedling characteristics of soybean seeds during storage increased especially after the sixth month of storage. According to the packaging materials, the least decrease in germination and seedling characteristics of soybean seeds was determined in glass jars and airless packages, while the highest decrease occurred in seeds stored in plastic containers and bags. In the study, it was determined that packaging materials are very important for soybean seeds to be stored without losing their viability. Considering the characteristics examined in the study, it was determined that soybean seeds can be stored in glass jars and airless packages with high quality.

**Keywords:** Soybean, packing materials, storage period, seed vigor

## INTRODUCTION

Soybeans are an important member of the legume family with high levels of protein and oil. Due to this feature, it is of great importance in both human and animal nutrition worldwide. Soybeans are used as many different products such as animal feed, soy flour, soy milk, soy sauce, lecithin, primarily vegetable oil (Liu, 1997). In addition to these, it is grown for energy agriculture and biodiesel production. The homeland of soybean is considered to be the Far East countries (China and Korea). It spread from China to Asia and then to the Americas in the 17th century (Öner, 2006). In Turkey, it is reported that it was first introduced from the Black Sea Region during World War I and a limited amount was cultivated in Ordu province (Cinsoy and Dizdaroğlu, 1994; Kınacı, 2011).

Soybeans are grown both as a main crop and as a second crop. For the main crop, depending on the regions and soil temperature, it is planted in late April - May, and for the second crop, it is planted in June immediately after the harvest of the pre-crops. Soybeans require a temperature of 2400-30000C during the growing period. A soil temperature of at least 8-100C is required for germination. As the development progresses, the temperature should be between 16-300C (Tuğay, 2007). It is sown at a distance of 50 x 5 cm and a depth of 3-5 cm.

Bradyrhizobium japonicum bacteria and 3 kg/da pure N and 6 kg/da P2O5 fertilizers are applied. In addition, soybean is a hoe plant and it is necessary to hoe 1-2 times during its vegetation. In addition to this, it needs 700 mm of water throughout its vegetation. This water requirement should be met especially during flowering, pod formation and filling periods. Soybean plants are harvested and threshed when the leaves are completely yellowed and the pods dry and the grains harden. Depending on the genotype and environmental conditions, yield normally varies between 300-400 kg for the first crop and 250-350 kg for the second crop (Nazlıcan, 2024)

Soybean seeds have a dicotyledonous structure, which requires care in harvesting and threshing. In addition, preventing seed deterioration in storage, reduction of respiratory metabolic activity and the occurrence of browning under the influence of biotic factors such as insects is the first priority in guaranteeing the quality of the seeds. For this reason, harvested seeds should be harvested and stored when they have a moisture content of 13-14%. Seeds are stored in advanced modern silos or indoors (Tuğay, 2007). However, the maintenance of seed viability depends on storage conditions and duration (Balešević-Tubić et al., 2005), and the reduction of germination power is much more severe under tropical conditions. Such unfavorable storage conditions and environmental conditions make it very difficult to maintain seed viability during storage (Shelar et al., 2008). Different seed storage periods and aging conditions negatively affected seed viability (Tatić et al., 2012). Arif (2005) concluded that seed viability of soybean seeds gradually decreased from 64.5% to 39.2% as the storage period increased from 2 to 12 months. Khaliliaqdam et al. (2012) suggested that differences in seed viability and deterioration patterns occurred among soybean varieties. In addition, differences in seed storage periods and storage conditions negatively affected seed viability (Sharma et al. 2007; Vijay et al. 2009; Khaliliaqdam et al. 2012). Considering such situations, it is very important for the seed sector and agricultural production to minimize losses in terms of seed viability and seedling characteristics by storing seeds in different packaging materials. In this context, regarding packaging materials, Padma and Muralimohan Reddy (2001) determined that the storability of soybean varieties can be increased by four months after storage of seed in a polyethylene bag compared to a cloth bag. Singh et al. (2003) concluded that soybean seeds stored in cloth bags were able to maintain their germination characteristics only for 4 months of storage. On the other hand, wheat seeds stored in sacks, cloth and plastic bags retained moisture content and had higher germination than seeds stored in metal and earthen drums (Chattha et al., 2012). It is understood that the losses in seed viability are quite high according to the storage conditions of soybeans. In addition, packaging materials have been used in different studies to minimize the viability of soybean seeds. However, there is still a lack of information about the changes in germination and seedling characteristics of soybean seeds during storage according to different packaging materials. Considering these conditions, this study aimed to determine the effects of storage of soybean seeds in different packaging materials for 12 months on seed viability and seedling development.

## MATERIAL AND METHOD

In the study, soybean Batem-Erensoy variety seeds were obtained from the Western Mediterranean Agricultural Research Center and grown in the experimental fields of Isparta University of Applied Sciences, Faculty of Agriculture in 2022. The plant height of Batem-Erensoy variety was 76 cm, the number of pods in the plant was 114, 100 grain weight was 11.82 g and yield was 2056 kg ha-1 under Isparta conditions. Harvested soybean seeds were cleaned and taken into the study without any spraying. In the study, soybean seeds were stored in various packaging materials and at a certain temperature (24oC±1) for different periods of time. Plastic containers, plastic bags, glass jars, airless packages, paper bags and cloth bags were used as packaging materials. The study continued for 12 months (start October 20, 2022; end October 20, 2023) and seed viability and seedling characteristics were determined every 2 months. In the study, 200 seeds and a total of 21600 soybean seeds (200 seeds x 6 packaging materials x 6 storage periods x 3 replicates) were used for each packaging material and for each two months considering insect damage or negative situation.

Germination and seedling characteristics were analyzed every 2 months. At the end of storage, 30 seeds for each replicate were left to germinate for 8 days at 20±1°C according to ISTA (2012) rules for soybean seeds in petri dish. During this period, seeds with rootlets reaching 2 mm in length every day were considered germinated (Murillo-Amador et al., 2002; Karaman and Kaya, 2017). When the germination period was completed (at the end of the 8th day), the number of germinated seeds was proportioned to the total number of seeds and multiplied by 100 to determine the germination rate. The germination index (Wang et al., 2004) was calculated by dividing the germinated seed rate by the number of counting days, and mean germination time (Ellis and Robert, 1980) was calculated by multiplying the number of germinated seeds per day by the number of germination days and dividing the sum by the total number of germinated seeds. While determining germination index and mean germination time, seeds were counted every day and the day when germination stabilized was determined as the last day of counting. At the end of germination, shoot and root length of 10 randomly germinated seedlings in each treatment material were determined. Vigor index (VI) was calculated according to Eq.1 using the obtained root and seedling lengths (Hu et al., 2005).

VI = Germination percentage x (root length + seedling length) (Eq.1)

Fresh and dry weights (g) were determined by weighing the fresh weights of 10 selected seedlings on a 0.01 precision balance and then the seedlings were dried in an oven at 65˚C for one day and weighed on a precision balance and their dry weights were determined. The dry matter ratio was calculated according to the formula Eq. 2.

DMO (%)= ((shoot fresh weight - shoot dry weight)/ shoot fresh weight )*100 (Eq.2)

The study was established according to the completely randomized design with 3 replications. The data obtained from the study were analyzed in Minitab (Minitab v.17.2.1) statistical package program and the differences between the treatments were determined according to Tukey multiple comparison test ($P<0.05$).

## RESULTS

In the study, the effects of packaging materials and storage times on germination and seedling characteristics of soybean seeds were found to be statistically significant as a result of storage of soybean seeds in different packages and for different periods of time. When the interactions of packaging materials x storage time were examined, it was determined that the characteristics other than average germination time and germination index had statistically significant effects on germination and seedling characteristics of soybean seeds (Table 1; 2).

The germination rate, which is one of the most important indicators of seed viability, varied between 94.44-98.06% according to the packaging materials. According to the packaging materials, the highest germination rate was determined in airless bag and this was followed by glass jar, cloth bag, plastic container and paper bag materials respectively and there was no statistical difference between them. The lowest germination rate was determined in plastic bag. On the other hand, storage time caused the germination rate of soybean seeds to decrease. According to the storage period, the highest germination rate was determined in the 2nd and 4th months and the lowest germination rate was determined in the 12th month (Table 1). When the interactions of packaging materials x storage time were examined according to germination rate, the germination rate values of soybeans were in the same group statistically until the 10th month of storage time. It is seen that germination rate values emerged especially at 12 months according to packaging materials. In this context, it was determined that the packaging materials were effective on the germination rate and airless bag, cloth bag and glass jar materials were able to protect the seed viability of soybean seeds relatively better than other packaging materials during the storage period (Figure 1).

Table 1. Averages of germination characteristics of soybean seeds according to packaging materials and storage time

| | | Germinate rate (%) | Mean germination time (day) | Germination index | Vigor index |
|---|---|---|---|---|---|
| Packaging materials | Airless bag | 98.06 a | 4.47 ab | 7.97 ab | 1520.67 a |
| | Cloth bag | 97.22 a | 4.54 ab | 8.02 ab | 1383.99 b |
| | Glass jar | 97.50 a | 4.41 b | 8.41 a | 1536.47 a |
| | Paper bags | 96.11 ab | 4.48 ab | 7.49 b | 1386.91 b |
| | Plastic bags | 94.44 b | 4.86 a | 7.54 b | 1247.99 c |
| | Plastic containers | 96.94 a | 4.80 ab | 7.27 b | 1329.95 b |
| | F- Value | 5.97** | 3.82** | 5.34** | 41.46** |
| Storage Times | 2 month | 100.00 a | 3.67 d | 11.57 a | 1956.56 a |
| | 4 month | 100.00 a | 4.25 c | 9.35 b | 1769.70 b |
| | 6 month | 97.78 b | 4.64 bc | 7.70 c | 1540.60 c |
| | 8 month | 96.39 bc | 4.79 b | 6.34 d | 1254.85 d |
| | 10 month | 95.28 c | 4.98 ab | 6.10 d | 1058.15 e |
| | 12 month | 90.83 d | 5.20 a | 5.65 d | 826.13 f |
| | F- Value | 43.05** | 7.82** | 156.56** | 628.05** |
| Interaction | F-Value | 2.33** | $0.53^{ns}$ | $1.17^{ns}$ | 5.47** |

[1] The difference between the means in the same column and starting with the same letter was statistically insignificant according to the Tukey–HSD test, **: P ≤0.01, ns: not significant

The average germination time of soybean seeds during storage varied between 3.67-5.20 days. The shortest average germination time was determined in the 2nd month and the longest average germination time was determined in the 10th and 12th months. It is an expected result that the average germination time of soybean seeds increases during storage. According to the packaging materials, the shortest average germination time was determined in glass jar (4.41 days). The longest average germination time was 4.86 days in plastic bag, while plastic container (4.80 days), cloth bag (4.54 days), paper bag (4.48 days) and airless bag (4.47 days) were in the same statistical group. When the interaction of packaging materials x storage time was examined, it was found that the average germination time was prolonged according to the storage time (Figure 1), and there was no statistical difference between packaging materials and storage time.

In the study, the highest germination index of soybean seeds stored in different packaging materials was determined in seeds stored in glass jar (8.41), followed by seeds stored in cloth bag (8.02) and airless bag (7.97), respectively. The lowest germination index was determined in soybean seeds stored in plastic container. However, soybean seeds stored in plastic container, which had the lowest germination index, and soybean seeds stored in paper bag and plastic bag packaging materials were in the same statistical group. Germination index of soybean seeds varied between 5.65-11.57 according to storage time. The highest germination index was determined at 2nd month and the lowest germination index was determined at 12 months. There was no statistical difference between month 12, which had the lowest germination index, and months 8 and 10 (Table 1). According to different packaging materials and storage period, the highest germination index values of soybean were determined in airless bag and glass jar (Figure 1). In the study, it was determined that the germination index values of soybeans decreased during the storage period and airless bag and glass jar, which are packaging materials, may be effective in decreasing the germination index values.

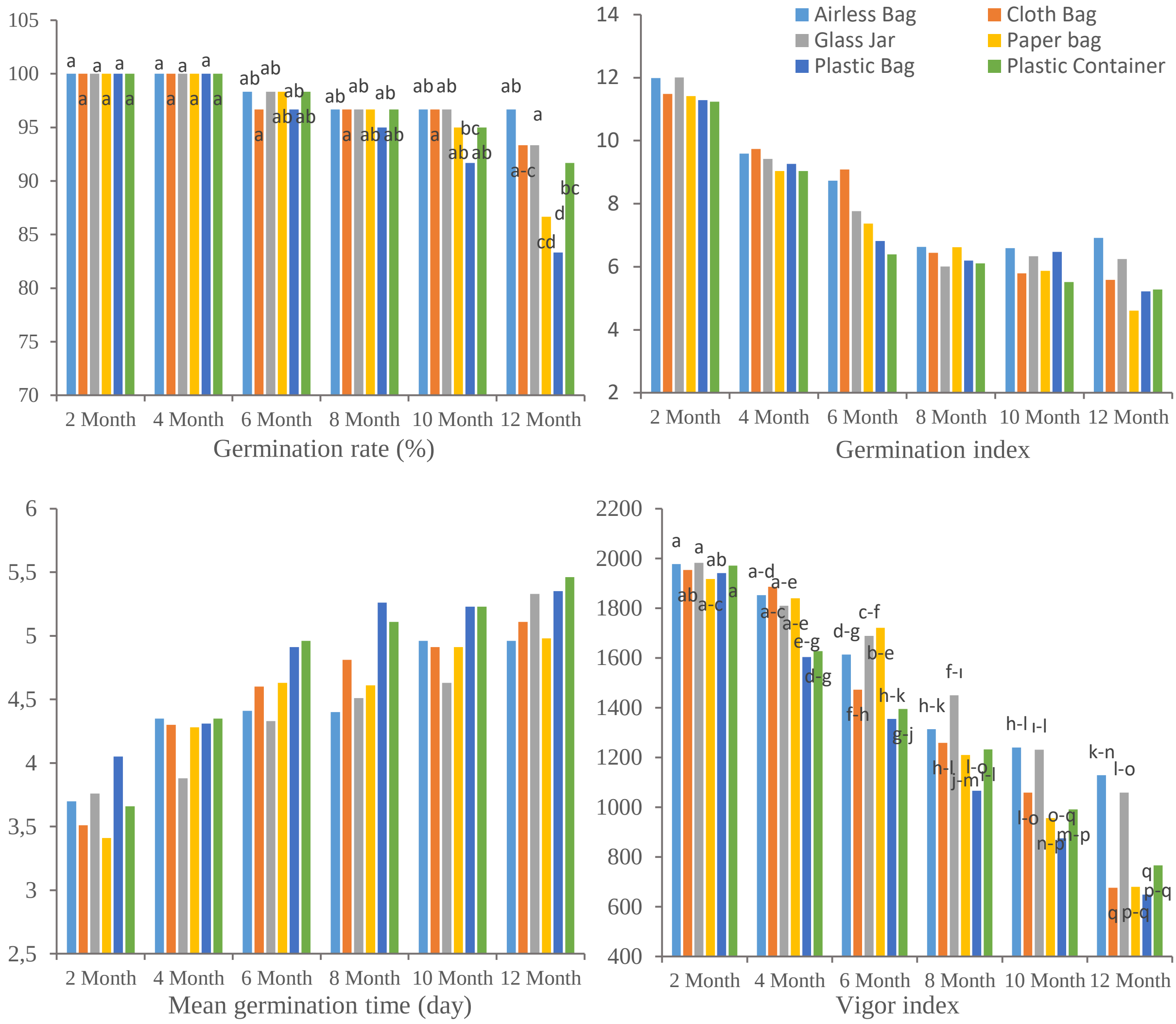


Figure 1. Interaction plots of packaging materials x storage time for germination characteristics of soybean seeds.

The vigor index, which is an indicator of seed germination and seedling strength, varied between 1247.99-1536.47 according to the packaging materials. According to the packaging materials, the highest vigor index was in glass jar and there was no statistical difference between this packaging material and airless bag (1520.67). The lowest vigor index was determined in plastic bag (1247.99). According to the storage period, the vigor index of soybean seeds varied between 826.13-1956.56. The highest vigor index was determined in the 2nd month and the lowest vigor index was determined in the 12th month (Table 1). When the interaction of packaging materials x storage time was examined according to the vigor index, it was determined that the vigor index values of soybean seeds decreased according to storage time and packaging materials. However, it was concluded that the packaging materials with the lowest decrease in vigor index during storage were airless bag and glass jar (Figure 1).

The sprout length of soybeans varied between 4.38-9.98 cm during storage. The shortest seedling length was measured at the 12th month and the longest seedling length was measured at the 2nd month. The decrease in the seedling length values of soybean seeds during storage was in parallel with the vigor index results. According to the packaging materials, the longest seedling length was determined in airless bag (8.06 cm) and was in the same statistical group with soybean seeds stored in glass jar (8.01 cm). The shortest seedling length was 6.47 cm in the plastic container (Table 2). When the interaction of packaging materials x storage time was analyzed, similar to the vigor index values, airless bag and glass jar packaging materials were determined to be the best packaging for the preservation of soybean seeds during storage (Figure 2).

Table 2. Means of seedling characteristics of soybean seeds according to packaging materials and storage time

| | | Seedling length (cm) | Root length (cm) | Dry matter rate[1] (%) |
|---|---|---|---|---|
| Packaging materials | Airless bag | 8.06 a | 7.40 ab | 12.59 a |
| | Cloth bag | 7.04 b | 7.11 bc | 12.47 a |
| | Glass jar | 8.01 a | 7.68 a | 12.42 a |
| | Paper bags | 7.01 b | 7.23 ab | 11.73 b |
| | Plastic bags | 6.98 b | 6.54 d | 11.02 c |
| | Plastic containers | 6.47 c | 6.64 cd | 11.36 bc |
| | F- Value | 38.35** | 13.01** | 40.30** |
| Storage Times | 2 month | 9.98 a | 9.59 a | 13.62 a |
| | 4 month | 9.17 b | 8.52 b | 13.23 a |
| | 6 month | 8.22 c | 7.52 c | 12.53 b |
| | 8 month | 6.35 d | 6.67 d | 11.73 c |
| | 10 month | 5.46 e | 5.63 e | 10.61 d |
| | 12 month | 4.38 f | 4.66 f | 9.88 e |
| | F-Value | 460.21** | 221.96** | 202.38** |
| Interaction | F-Value | 7.23** | 2.81** | 2.41** |

[1] The difference between the means in the same column and starting with the same letter was statistically insignificant according to the Tukey–HSD test, **: P ≤0.01

In the study, the highest root length of soybean seeds stored in different packaging materials was determined in seeds stored in glass jars (7.68 cm), followed by seeds stored in airless bags (7.40 cm) and paper bags (7.23 cm). The lowest root length was found in soybean seeds stored in plastic bag (6.54 cm). According to the storage period, the root length of soybean seeds varied between 4.66-9.59 cm. The highest root length was determined at 2nd month and the lowest root length was determined at 12 months (Table 2). According to different packaging materials and storage period, the highest root length values of soybean seeds were determined in seeds stored in airless bag and glass jar (Figure 2). In the study, it was determined that the root length values of soybean seeds decreased during storage and airless bag and glass jar, which are packaging materials, can be the packaging materials that can minimize the decrease in root length values.

In the study, the dry matter rate varied between 11.02-12.59% according to the packaging materials. According to the packaging materials, the highest dry matter rate was determined in airless bag, followed by cloth bag (12.47%) and glass jar (12.42%) packaging materials, respectively, and there was no statistical difference between them. The lowest dry matter content was determined in plastic bag (11.02%). According to the storage period, the highest dry matter content was determined in the 2nd and 4th months (13.62% and 13.23%, respectively) and the lowest dry matter content was determined in soybean seeds stored for the 12th month (9.88%) (Table 2). When the interactions of packaging materials x storage period were analyzed according to dry matter content, in general, the dry matter values of soybeans were in the same group statistically until the 6th month of storage period. Dry matter content decreased during the storage period and it was determined that airless bag and glass jar materials had higher dry matter content of soybean seeds (Figure 2).

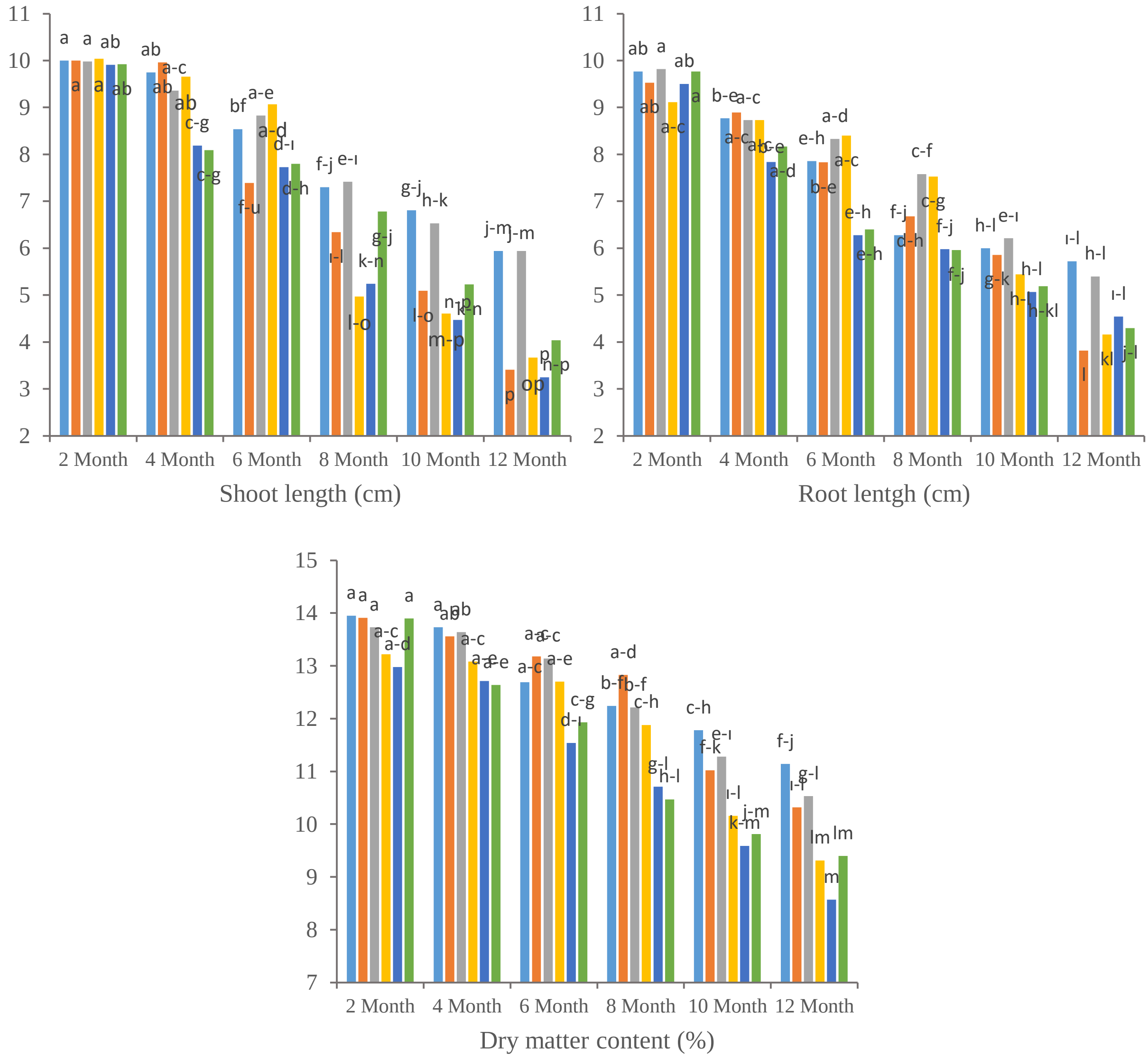


Figure 2. Interaction plots of packaging materials x storage time for seedling characteristics of soybean seeds

## DISCUSSION

In the study, the changes in soybean germination and seedling characteristics were determined under the same temperature and humidity conditions in different packaging materials and during storage. Germination characteristics (germination rate, germination index, average germination time and vigor index), which are one of the best indicators of seed viability, showed significant differences according to storage period. The germination rate, germination index and vigor index decreased with increasing storage time, while the average germination time increased (Table 1). Packaging materials were also found to be highly effective on germination characteristics. Especially airless bag and glass jar packaging materials were found to preserve the germination rate, germination index, average germination time and vigor index values of soybean seeds in the best way during storage (Table 1 and Figure 1).

Seed aged is usually characterized by a decrease in vigor. In addition, seed aged increases as the storage period of seeds increases (Chuansin et al., 2006). In this context, Arif (2005) determined that the germination rate of soybean seeds is inversely proportional to the storage period and that germination gradually decreases from 64.5% to 39.2% as the storage period increases from 2nd to 12th month. Tatić et al. (2008) stored soybean varieties for 6 and 12 months (under normal and controlled conditions) and found that the germination rate decreased with increasing storage time and this difference varied according to varieties and storage conditions. In addition, Balešević-Tubić et al. (2005) reported that the differences in germination characteristics depending on storage periods may be due to lipid changes of the seed during storage and that the decrease in phospholipids and polyunsaturated fatty acids led to a significant decrease in seed viability and germination index. On the other hand, seed deterioration led to a decrease in germination percentage and rate and a decrease in the percentage of normal seedlings (Mohammadi et al., 2011).

Moreover, the degree and rate of decline in seed viability largely depends on the plant species, storage conditions and the quality of the seeds (Balešević-Tubić et al., 2005). Another reason for the decline in seed quality of soybean seeds is that the root of the soybean embryonic axis is susceptible to deterioration due to its proximity to the follicular tip. Therefore, the seed is in direct contact with both water and oxygen, which enter through the hilum and cause lipid peroxidation (McDonald, 2004), leading to a decrease in seed viability and quality. The findings of this study are in agreement with the results obtained by many researchers (Hou et al. 1998; Alencar et al. 2010; Khaliliaqdam et al. 2012).

Seedling traits (shoot length, root length and dry matter content), which are indicators of seed performance in the field, decreased with increasing storage time. The severity of this decrease was observed more especially in the 10th and 12th months. Among the packaging materials, airless bag and glass jar materials were more effective than other packaging materials (cloth bag, paper bag) in preserving sprout length, root length and dry matter content of soybean seeds.

plastic bag and plastic container) were found to give better results. In the study, it was determined that the seedling characteristics of soybeans decreased during storage and airless bag and glass jar, which are packaging materials, can be the packaging materials that can minimize the decrease in seedling characteristics (Table 1; Figure 1).

Manonmani, (2002) reported that the decrease in root length, shoot length, seedling dry and fresh weight and seedling vigor index with increasing storage duration of seeds may be due to genetic differences, age-related deterioration and natural differences in seed structure and composition. In addition, Mohammadi et al. (2011) reported that seed deterioration caused a decrease in the percentage of normal seedlings. Seedling growth and mobilization rate of seed reserve showed a significant decrease with the progression of deterioration. The decline in seed quality is linked to biochemical changes in the seeds of oil crops (Kandil et al., 2013). Kandil et al. (2013) also found that root length, shoot length, root/shoot ratio, seedling fresh and dry weight and seedling vigor index decreased with prolonged storage period of soybean seeds. In addition, similar to the results in this study, they obtained the lowest root length, shoot length, root/shoot ratio, seedling fresh and dry weight and seedling vigor index values 12 months after storage. Chuansin et al. (2006) stated that seeds with high oil content, such as soybean, undergo rapid deterioration due to auto-oxidation of lipids and increased free fatty acid content during storage. These results support the findings of Maddah Arefi and Abdi (2003), Keshavulu and Krishnasamy (2005) and Tatić et al. (2012).

## CONCLUSIONS

In agricultural production, seeds should be stored without losing their viability and germination power or with minimum loss. As a matter of fact, significant differences in germination and seedling characteristics of soybean seeds stored in different periods and packaging materials were observed in the study. In the study, it was determined that germination and seedling characteristics decreased with the prolongation of soybean storage time. It was determined that airless bag and glass jar, which are packaging materials, can be the packaging materials that can minimize the decrease in germination and seedling characteristics values. As a result, it was determined that the storage period is very important to keep soybean seeds with long life, high germination and seedling characteristics after harvest and that soybean seeds stored in glass jars and airless bags can maintain germination and seedling characteristics.

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# THE EFFECT OF COCOA BUTTER REFINING ON THE PROBIOTIC MILK CHOCOLATE MANUFACTURING

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## ABSTRACT

Cocoa butter (CB) gives the continuous phase of chocolate that has a major influence on the final chocolate product quality, due to the CB has an optimal quality for chocolate shelf life. In this research, Bifido bacterium was fortificate into the milk chocolate for the preparation of probiotic milk chocolate and a crude cocoa butter with or without a silica pretreatment, was subjected to a steam refining in a packed column at four temperatures: 150, 200 and 250 °C. Then the impact of the refining treatments on the physicochemical quality of probiotic milk chocolate was evaluated; in the another step, the refined CBs were utilized to manufacture the probiotic milk chocolate. Various quality properties as particle size distribution (PSD), rheological behavior and texture were then determined to assess the cocoa butter refining treatment effect.

**Keywords:** Cocoa butter, refining, probiotic, Bifidobacteria, milk chocolate.

## INTRODUCTION

Chocolate is a delight and makes us happy; it is an indulgence everyone should have the opportunity to enjoy. While most people are aware of chocolate`s enjoyment, most people are not aware of the nutritional and health benefits the minor components in chocolate provide. Only a very few consumers, confectioners and food scientists know much about the cocoa butter, which is a key component in chocolate and responsible for many of the desirable sensort attributes unique to chocolate (Tokusoglu, 2015; Beckett,2009).

It`s cocoa butter`s unique mixture of triglycerides that impart the desirable melting properties of chocolate that set the standart of quality for indulgent confections (Timms and Stewart. 1999). However, this unique mixture of triglycerides has its unique problems: If not cooled properly or if it is stored for long periods of time and/or under fluctuating temperatures, the physical structure of the cocoa butter will alter and impart an unppealing``bloom`` on the chocolate`s surface,

As cocoa butter (CB) forms the continuous phase of chocolate, it has a major effects on the the final product quality; so it is crucial that the CB has an optimal quality. That is, CB has an optimal quality for chocolate shelf life. Various processing steps precede the final CB pressing but it may still include unwanted constituents sometimes making it necessary to refine the product (Tokusoglu, 2015 ; Vila Ayala et.al.,2007; Foubert et.al.,2002)

It is stated that CB should contain less than 1.75% free fatty acids (FFA, based on oleic acid) to be in compliance with the European Union Directive and needs to be free from off-flavors, molds and rancidity. Suitable flexible refining technologies are needed because as first step, a global decrease in CB quality is noticed (high level of FFA, more phosphorus and more alkalinity); as second step, there is also an increased industry's demand for different types of CBs (like color, neutral flavors degree, melting profiles). The conventional CB refining processing exists of a filtration followed by a batch or continuous deodorization.

## PROBIOTIC BACTERIA IN CHOCOLATE PROCESSING

Probiotic bacteria are healthfull for human by improving the gutmicro biota balance and the defenses against pathogenic flora and beneficial for immune system, blood cholesterol reduction, vitamin synthesis. The fortification of lactobacillus to planned chocolates creates a tastier option for transferring these Lactobacillus and Bifido bacterium to the stomach for the betterment of the microbial environment of the gut.


**Figure 1.** Probiotic Chocolate Manufacturing (As Low Sugar or Sugar-Free Chocolate) (Tokusoglu,2020)

Probiotic bacteria are healthfull for human by improving the gutmicro biota balance and the defenses against pathogenic flora and beneficial for immune system, blood cholesterol reduction, vitamin synthesis. The fortification of lactobacillus to planned chocolates creates a tastier option for transferring these Lactobacillus and Bifido bacterium to the stomach for the betterment of the microbial environment of the gut. (Figure 1.).

## METHODOLOLGY

In this research, Bifido bacterium was fortificate into the milk chocolate for the preparation of probiotic milk chocolate and a crude cocoa butter with or without a silica pretreatment, was subjected to a steam refining in a packed column at four temperatures: 150, 200 and 250 °C. Then the impact of the refining treatments on the physicochemical quality of probiotic milk chocolate was evaluated; in the another step, the refined CBs were utilized to manufacture the probiotic milk chocolate. The quality properties as particle size distribution (PSD), rheological behavior and texture were then determined to assess the cocoa butter refining treatment effect.

The silica pretreatment was carried out in a batch reactor. The crude CB was first heated to 83 °C and 0.5% (v/w) of a 13% (w/w) citric acid solution was added and thoroughly mixed with the CB. After 10 min, 0.5% (w/w) silica powder (Trysil, Germany) was added and agitation continued for another 20 min. A vacuum was applied to remove the water. The silica was separated from the CB in a plate-and-frame filter mounted with a standard filter cloth by pressurizing the reactor with compressed air.

## PRELIMINARY RESULTS ON THE RESEARCH

The refining situation and the the silica pretreatment had no effect on the fatty acid profile and triacylglycerol (TAG) distribution of the cocoa butters (CBs). Moreover, the distribution level of monoacylglycerol (MAG) and diacylglycerol (DAG) not altered. The main influence of the steam refining step was the FFA diminishing based on the increased temperatures ($T \geqslant 200$ °C). The alterations in physical properties were mainly related to the FFA removaling as they retarded and slowed down the crystallization situation in probiotic milk chocolate.
The cacao butter (CB) refining affected the probiotic milk chocolate physical properties due to the FFA affected the crystallization kinetics but no effects gave on the chemical quality of probiotic milk chocolate.

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# ANTIOXIDANT TRITERPENOIDS IN TABLE OLIVES: MASLINIC ACID AND OLEANOLIC ACID

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## ABSTRACT

In our study, the determination of antioxidant triterpenic maslinic and oleanolic acid levels were performed. The maslinic acid content in raw olive samples was 1.123 mg/g DM (average) in Akhisar Domat olives and Kuyucak Yamalak Kabası olives while oleanolic acid levels were determined as 0.402 mg/g DM (mean) and 0.272 mg/g DM, respectively for those samples. It was found that approximately 0.99-1.23 mg of maslinic acid can be consumed by consuming 5 table olives (approximately 15 g) per day.

**Key Words:** Olive, Maslinic Acid, Oleanolic Acid, Quality

## INTRODUCTION

For high value olive oil, the country of origin and type of olive cultivar has a dramatic effect on the price. Falsifying the provenance of such oils is an attractive proposition to fraudulent suppliers. Polar components such as phenolic acids and lignans are present in olive oils.(Zhou et.al.,2017; Tokusoglu,2016; Romero-García et.al.,2014; Bendini et.al.2007)

Secoiridoids; phenolics which include an elenolic acid moiety, are unique to the Olearacee family. These compounds are known to have beneficial health effects due to their antioxidant properties and are associated with the organoleptic properties. Measuring the levels of these polar components in oils from different cultivars and geographic origins is of interest for nutritional and authenticity reasons. In this study a statistical analysis of the polar chemical components of olive oils was used to determine potential markers for olive cultivars. These markers could be used to confirm the provenance of unknown samples (Tokusoglu,2016) .

The epicarp part of the olive fruit (O. europaea L.) contains triterpenes including oleanolic acid, maslinic acid, uvaol and erythrodiol. It is stated that maslinic acid and oleanilic acid are triterpenoids that are predominantly found as the main triterpenes. Various biological activities of triterpenoids, such as antioxidant, anti-inflammatory and antitumoral are reported. It is reported that the average amount of triterpenes consumed per person is 400 mg/kg/day, owing to the fact that the diet in Mediterranean countries is dominated by olive oil (Tokusoglu,2016).

## MATERIALS AND METHODS

### Turkish Olive Oil Samples

A number of authentic branded Extra Virgin Olive oils (EVOOs) were analyzed. Each of the oils were cold pressed from either a single cultivar olive or from a known blend of cultivars grown in a distinct geographic region in Turkey Izmir and Akhisar.

In our study, which was carried out to shed light on the determination of triterpenic acid levels in olives, a frequently consumed breakfast food product and special food in our country, we examined the raw and medium-sized olives of homogeneous caliber (201-230 grains/kg) obtained from three olive gardens in Manisa-Akhisar and Aydın-Kuyucak regions. After processing, maslinic acid and oleanolic acid levels were determined.

### LC/MS Analysis of Polar Components in Olive Oils

Polar Compound Extraction Liquid-liquid extraction was performed to enrich the polar components of the oils for improved sensitivity. Extraction also reduced the high levels of hydrophobic triacylglycerides and diacylglycerides, which would be retained on reversed-phase columns during the HPLC separation of the polar components. Olive oil samples were extracted three times with N,N- dimethylformamide (DMF). Extracts were pooled and washed twice with hexane to remove hydrophobic components. Residual hexane was removed from the DMF extracts by centrifugal evaporation. The DMF extracts were diluted 1:20 with water and 2 uL of each extract were injected for LC/MS analysis.

### LC/MS Method

Extracts were analyzed in triplicate with a single chromatographic method, with separate analyzes for the detection of components either with positive or negative mode electrospray ionization.

Components were separated by reversed-phase gradients on a Brownlee SPP C18 column with a Flexar*™ FX-10 UHPLC pump, using water/methanol eluents at 0.4 mL/min, with a gradient from 10% methanol to 50% methanol over 10 mins, then detected with an AxION® 2 TOF MS fitted with an Ultraspray'™ 2 ion source.

Many compounds were selectively detected in either positive or negative modes, although some compounds were detected in both modes. In negative mode, long chain fatty acids and a number of phenolics are detected. In positive mode (Figure 2) a number of phenolics, terpenes and fatty acids are observed as [M + H]+ or [M + Nal+ ions. For components of interest, the accurate mass and isotope patterns of the molecular ions in the original datasets were used to obtain elemental formulas, which were correlated to known compounds in olive oil (Figure 1- two chromatograms and Figure 2).

## RESULTS

The dry matter content was determined as 35.56% DM in Akhisar Domat olives and 38.83% DM in Kuyucak Yamalak Kabası olives, and the maslinic acid content in raw olive samples was 1.123 mg/g DM (average) in Akhisar Domat olives and Kuyucak Yamalak Kabası olives, respectively. It was determined as 0.678 mg/g DM ($p < 0.05$). In olives sweetened after

Spanish processing, it was determined as 0.082 mg/g DM (average) and 0.066 mg/g DM, respectively. While oleanolic acid levels were determined as 0.402 mg/g DM (mean) and 0.272 mg/g DM in raw Akhisar Domat olives and Kuyucak Yamalak Kabası olives, respectively, it was not detected in processed Akhisar and Kuyucak samples ($p < 0.05$). In this context, approximately 0.99-1.23 mg of maslinic acid can be consumed by consuming 5 table olives (approximately 15 g) per day.

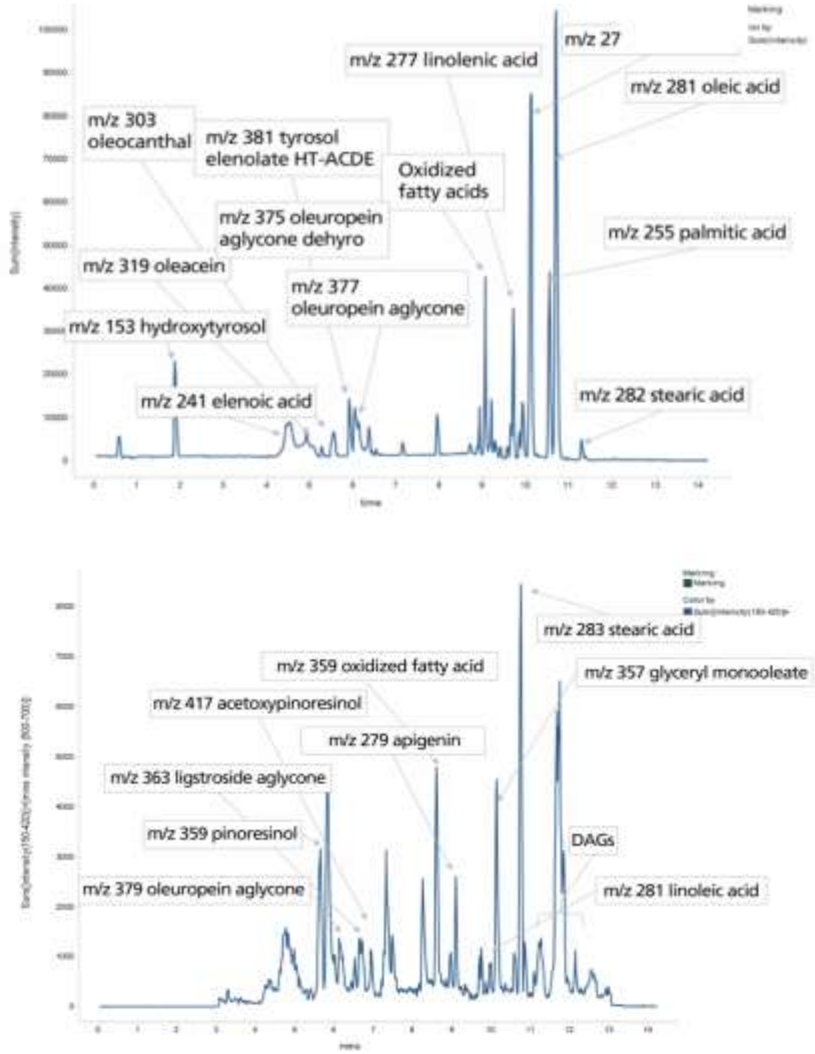
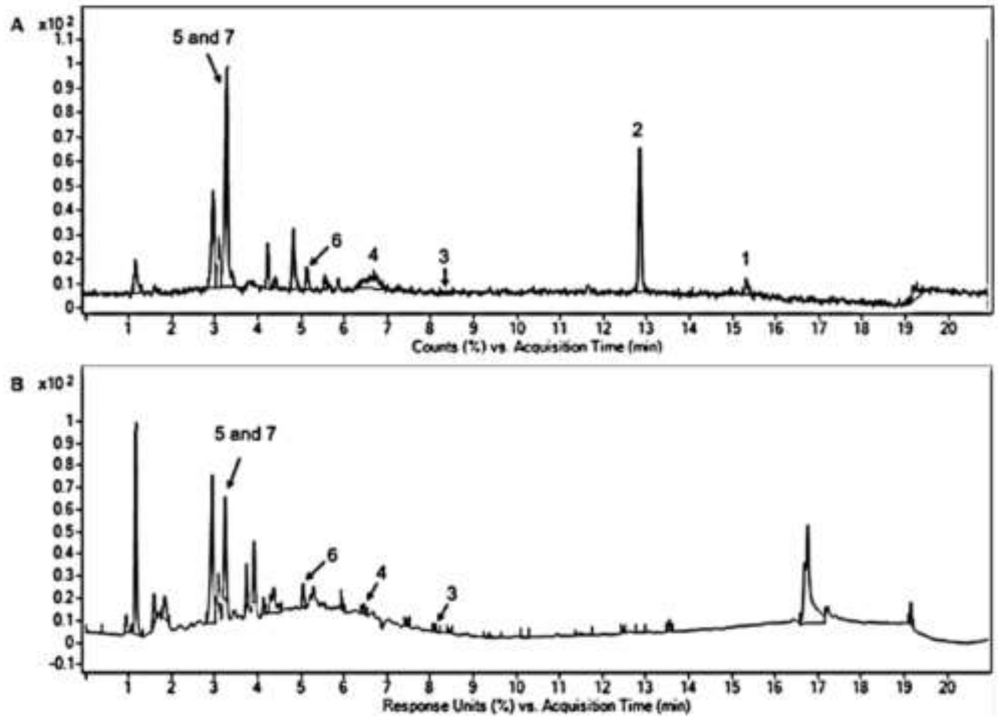



**Figure 1.** Chromatograms of Major Constituents for Studied Olive Oils of Akhisar.




A) Extracted ion chromatogram (EIC) scan and
(B) DAD chromatogram (λ = 280 nm) scan.
The compounds were identified as
**1** oleanolic acid , **2** maslinic acid ,
**3** 1-acetoxypinoresinol 3, **4** hydroxytyrosol acetate,
**5** 3,4-dihydroxyphenyl-2-methoxyethanol,
**6** luteolin-7-O-β-D-glucoside ,**7** and hydroxytyrosol

**Figure 2.** Ion Chromatograms of Studied Olive Oils of Akhisar

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# BIOMASS LIPID PROFILES OF SPIRULINA MICROALGAE AS FOOD SUPPLEMENT

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## ABSTRACT

Nutritional composition was determined for special cultivated Spirulina platensis and mentioned Thirteen strains of these microalgae were obtained as a percentage of total fat. Total PUFA, SFA contents, n3/n-6 ratios, and eicosapentaenoic acid (EPA)/docosahexaenoic acid (DHA) ratios were obtained. *Spirulina* samples contained 7.53% of average total lipid ($p < 0.01$). Special Spirulina was detected as a rich source of alpha linoleic acid (C18:2n-6) (ALA) and gamma-linolenic acid (C18:3n-6) (GLA) and were 15.03% 0.74%, respectively. Besides, the level of arachidonic acid (C20:4*n*-6) of *Spirulina* was 0.45% of the fatty acids whereas docosapentaenoic acid (DHA) (C22:5*n*-3) level was high and was in the range of 3.56%. This special cultivated microalgae could be an important food additive. It can be used in salads, soups, and dip foods in food sector and has been utilizated in infant formulas, popcorn, spice mixtures, fruit juice mix, potato chips, smoothies made with apples and blueberries, dilled beans, macaroni, and avocado dressing.

**Keywords:** Microalgae, Spirulina, proximate composition, fatty acids, GC, Gas Chromatography

## INTRODUCTION

The increasing worldwide population is projected to be around 9.7 billion in 2050 (UN DESA,2022) with massive demand for proteins from plant-based foods (fruits, vegetable, grains, nuts, and seeds) and animal-based foods (meat, poultry, fish, eggs, and dairy foods) and also there is a great interest on more sustainable food sources such as algal-based foods Sompura et.al.2024; Tokusoglu and Una, 2003).

Microalgaes represents the great interest for supplying the protein demand as well as a source of bioactive constituents as amino acids, polyunsaturated fatty acids (PUFAs), vitamins, and minerals (Tokusoglu and Unal,2003).

## MATERIALS AND METHODS

Three starter cultures of *Spirulina platensis*and were obtained from the Culture Collection of Algae and Protozoa, Dunstaffnage Marine Laboratory, OBAN, Argyll, Scotland, U.K. The isolation of mi- croalgae from cultures was done in the solid state via the agar method (Belcher and Swale 1982) at Pínar Deniz A.S ̧., Ízmir, Turkey.

The microalgae cultures were developed using the semicontinuous culture system in duplicate runs (Richmond 1986), then mas- sive productions were done in duplicate flasks.

Inoculation density of cultures in production were (1.5 to 1.8) × 106 cell/mL. These cultures were continuously aerated by using an air pump without additional carbon dioxide. The laboratory temperature was kept at 20 ± 2 °C, and 16 fluorescent lamps of 36 W each were provided for contin- ual light. The light reaching the surfaces of cultures was measured as E m–2 s–1. In all microalgae cultivations, Conwey culture medium (Pinar Deniz A.S., Izmir, Turkey) was used and the pH and salinity of cultures was routinely measured.

## RESULTS

The dry mass data were geneity test and variance was de- termined to be homogeneous ($p < 0.01$). Moisture content of *Spirulina* sample 1 was 3.11%, whereas *Spirulina* samples 2 and 3 were 4.08% and 3.76%, respectively, and was low, with a mean of 3.65%. This mean value is in the range of general recommendations for a quality less than 10% (Tokusoglu and Unal,2003; Becker 1994). There was no significant difference be- tween the 3 *Spirulina platensis* samples ($p < 0.01$). The biomass of *Chlorella* had 3.87% moisture, whereas *Isochrisis* had 6.48% moisture. There was no significant dif- ference between the *Spirulina* samples and *Chlorella* as dry weight ($p < 0.01$). The results of the biomass analysis are expressed on 100 g dry wt basis (Table 1). Table 2 indicates the fatty acid composition of three Spirulina microalgae samples (Table 2).

**Table 1—The proximate composition parameters of 3 microalgae samples†**

| Parameters | Spirulina 1 | Spirulina 2 | Spirulina 3 |
|---|---|---|---|
| Moisture(%) | 3.11 ± 0.05 | 4.08 ± 0.02 | 3.76 ± 0.04 |
| Total ash(%) | 7.43 ± 0.06 | 7.51 ± 0.05 | 10.38 ± 0.05 |
| Crude protein(%) | 63.26 ± 0.04 | 64.43 ± 0.03 | 61.32 ± 0.02 |
| Crude lipid(%) | 7.09 ± 0.03 | 7.14 ± 0.03 | 8.03 ± 0.06 |
| Available carbohydrate(%) | 15.17 ± 0.02 | 15.09 ± 0.04 | 15.81 ± 0.07 |
| Energy (kJ) | 1562.22 ± 2.11 | 1582.41 ± 2.26 | 1575.18 ± 3.68 |

†Data are based on dry wt(%), mean ± SD (n = 3)

**Table 2—Fatty acid (FA) composition of 3 microalgae as percent of total lipid**

| Fatty Acid | Spirulina Plantensis 1 | Spirulina Plantensis 2 | Spirulina Plantensis 3 | Chlorella Vulgaris | Isochrisis Galbana |
|---|---|---|---|---|---|
| C14:0 | 0.43 | 0.46 | 0.41 | 0.38 | 8.40 |
| C14:1 | 0.53 | 0.48 | 0.30 | tr | 1.29 |
| C16:0 | 27.86 | 26.61 | 27.19 | 15.41 | 28.37 |
| C16:1*n*-7 | 1.84 | 2.27 | 1.92 | 1.17 | 6.57 |
| C18:0 | 5.80 | 8.82 | 6.66 | 6.24 | 5.82 |
| C18:1*n*-9 | 32.86 | 34.71 | 35.74 | 33.14 | 19.73 |
| C18:1*n*-7 | 1.17 | 1.64 | 1.33 | 1.13 | 2.40 |
| C18:2*n*-6 | 10.37 | 14.45 | 11.25 | 9.73 | 1.14 |
| C18:3*n*-6 | 4.60 | 3.64 | 5.52 | tr | 0.54 |
| C18:3*n*-3 | 0.62 | 0.68 | 0.71 | 1.93 | 0.46 |
| C18:4*n*-3 | 0.71 | 0.57 | 0.81 | tr | 0.48 |
| C20:0 | - | - | - | 0.19 | 0.74 |
| C20:4*n*-6 | 0.34 | 0.35 | 0.41 | tr | 1.07 |
| C20:5*n*-3 | 2.33 | 2.21 | 2.91 | 3.23 | 1.93 |
| C22:5*n*-3 | - | - | - | 3.11 | tr |
| C22:6*n*-3 | 3.33 | 2.30 | 3.51 | 20.94 | 18.79 |

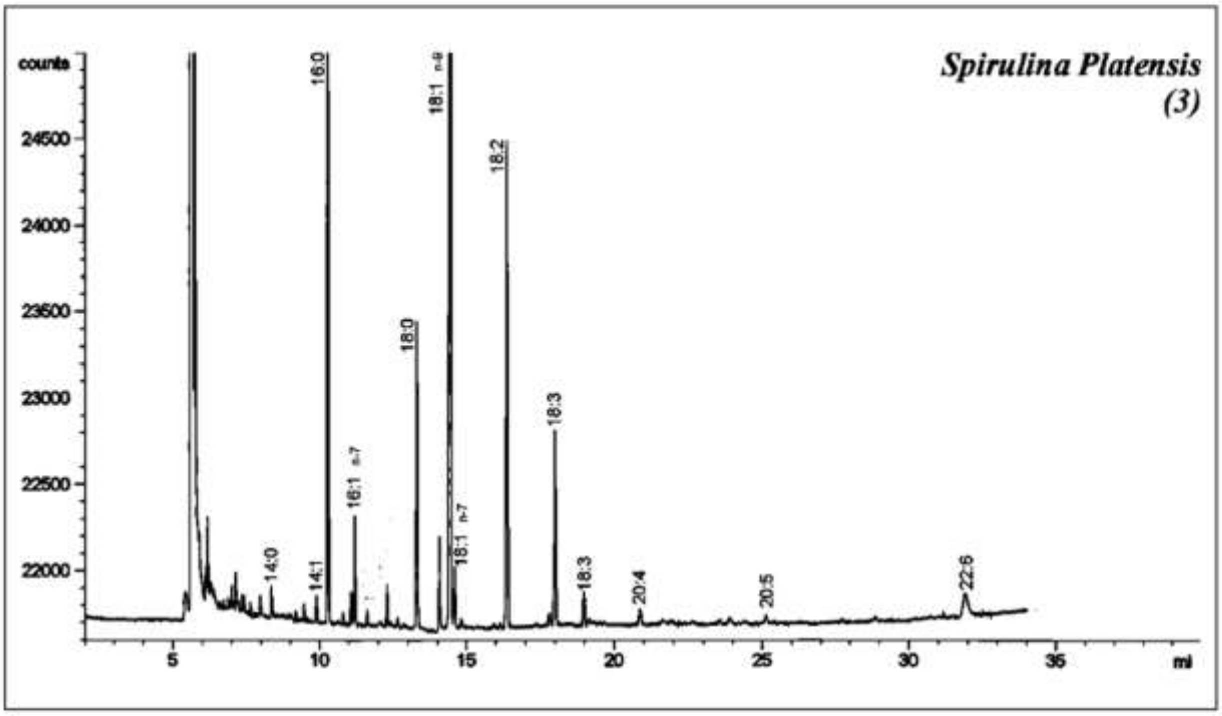


**Figure 1.** Gas Chromatogram of Fatty Acid Methyl Esters for Spirulina Platensis No 3

Nutritional composition was determined for special cultivated Spirulina platensis and mentioned thirteen strains of these microalgae were obtained as a percentage of total fat. Total PUFA, SFA contents, n3/n-6 ratios, and eicosapentaenoic acid (EPA)/docosahexaenoic acid (DHA) ratios were obtained. Spirulina samples contained 7.53% of average total lipid ($p < 0.01$). Special Spirulina was detected as a rich source of alpha linoleic acid (C18:2n-6) (ALA) and gamma-linolenic acid (C18:3n-6) (GLA) and were 15.03% and 0.74%, respectively. Besides, the level of arachidonic acid (C20:4n-6) of Spirulina was 0.45% of the fatty acids whereas docosapentaenoic acid (DHA) (C22:5n-3) level was high and was in the range of 3.56%. This special cultivated microalgae could be an important food additive. It can be used in salads, soups, and dip foods in food sector and has been utilizated in infant formulas, popcorn, spice mixtures, fruit juice mix, potato chips, smoothies made with apples and blueberries, dilled beans, macaroni, and avocado dressing.

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