*Int.J.Curr.Microbiol.App.Sci* (2024) *13*(09): 55-67

*International Journal of Current Microbiology and Applied Sciences*
*ISSN: 2319-7706* **Volume 13 Number 9 (2024)**
Journal homepage: http://www.ijcmas.com

**Original Research Article** https://doi.org/10.20546/ijcmas.2024.1309.005

# Changes in Protein and Amino Acid Content of Saffron Flowers Depending on Fertilization

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**Keywords**

Saffron, *Crocus sativus* L., protein, amino acid, saffron flowers, stigma saffron flowers

**Article Info**

***Received:***
18 July 2024
***Accepted:***
23 August 2024
***Available Online:***
10 September 2024

**A B S T R A C T**

Saffron is renowned for its high pharmaceutical value and steep price. The primary value of saffron lies in its flowers and stigmas. Cultivation of saffron in a plantation format is receiving significant attention globally. However, a key challenge faced in plantation cultivation of saffron is maintaining sufficient levels of the desired substances. A variety of biological and mineral fertilizers are commonly employed to enhance saffron production. Biological fertilizers show significant promise in saffron cultivation compared to chemical mineral fertilizers. However, the cultivation of saffron using zoohumus, an unconventional biological fertilizer, and its impact on the preservation of key substances have not been thoroughly investigated. Hence, this article examines the total protein and amino acid storage characteristics of saffron flower stigmas cultivated in conventional biohumus and non-traditional zoohumus. The results indicate that saffron flower stigmas grown in zoohumus exhibit higher levels of total protein and amino acid content compared to those grown in traditional biohumus.

## Introduction

Statistically, out of over 4,300 plants indigenous to Uzbekistan, 750 species are recognized for their medicinal properties. Among these, 112 species are officially approved for scientific medical use, with 70 species actively utilized in the pharmaceutical sector. One of the primary challenges in cultivating medicinal plants naturally and through cultural reproduction worldwide is the need to preserve the essential substances of the plant during cultivation. The second significant issue revolves around the economic and social aspects. When medicinal plants grown naturally are transitioned to plantation cultivation, there is often a struggle to retain the crucial chemical components efficiently. This results in substantial economic costs and excessive physical labor demands on human resources.In the context of Uzbekistan, significant challenges are encountered in the cultivation of chili peppers on extensive acreage, particularly in achieving the desired level of spiciness. Similar difficulties are also observed in the cultivation of red bell peppers (Eshkobilov *et al.,* 2023).

In the cultivation of saffron, renowned for its exceptional value, the storage of its key chemical components - crocin, picrocrotic, and safranal - has been observed to fluctuate based on the soil type (Cardone *et al.,* 2019). Scientific literature also highlights that when saffron is cultivated in moderately alkaline soil with low organic matter content, there is a decrease in color and taste properties, while the aroma intensity tends to increase. (Cardone *et al.,* 2019). Moreover, the main chemical components of saffron are influenced by factors such as the irrigation system, soil chemical composition, soil types, weather and climate conditions, as well as the agricultural and technological expertise related to the storage, drying, and processing of saffron flowers. These indicators can also be adversely affected by these variables (Rezaee *et al.,* 2013).

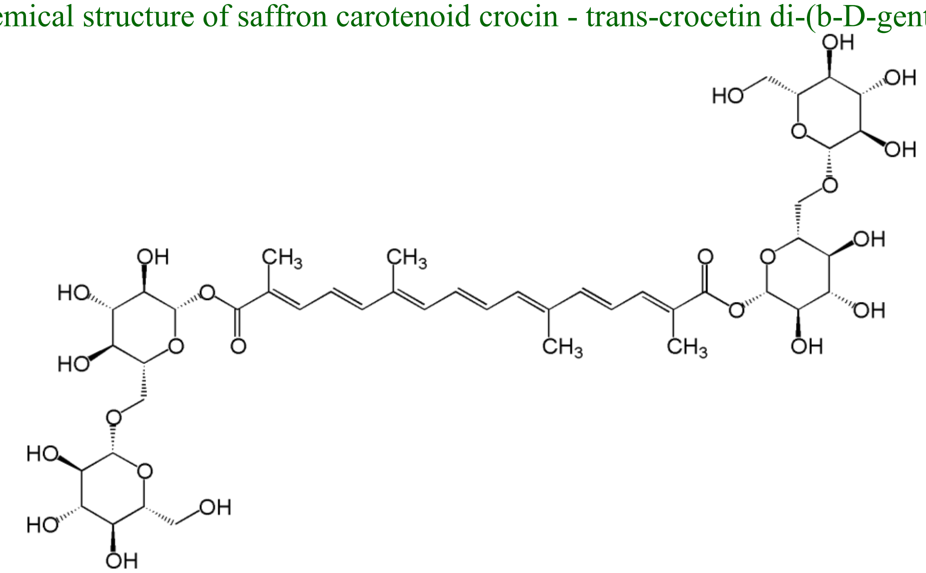
Hence, when cultivating medicinal plants and transitioning from natural growth to cultivated plantations, the primary focus should be on preserving the key chemical compounds inherent to the plant. Additionally, in the cultivation of medicinal plants within cultivated plantations, significant emphasis should be placed on minimizing the reliance on chemical fertilizers, particularly mineral-based ones.

Saffron *(Crocus sativus L.)* stands out as one of the most valuable plants globally, prized for its medicinal properties and distinctive flavor. The cultivation process of saffron, serving both medicinal and culinary purposes, demands significant labor (Milajerdi *et al.,* 2018). The flower is the focal point of saffron, a rare plant from the Iridaceae family, captivating individuals with its vibrant color, enticing aroma, and unique texture. Its prized attributes stem from its fragrant and vividly hued flowers. While saffron is cultivated in numerous countries worldwide, saffron grown in Iran holds exceptional value (Sadat Rafiei *et al.,* 2023).

The primary consumable part of saffron is its dried stigmas, which, according to scientific sources, contain the rare compounds crocin ($C_{44}H_70O_{28}$), picrocrocin ($C_{16}H_{26}O_7$, $C_{20}H_{24}O_4$), and safranal ($C_{10}H_{14}O$). The original value of saffron is determined by the storage conditions of these components. The distinctive aroma and flavor of saffron are attributed to the presence and quantity of these compounds (Han *et al.,* 2024; Mzabri *et al.,* 2019).

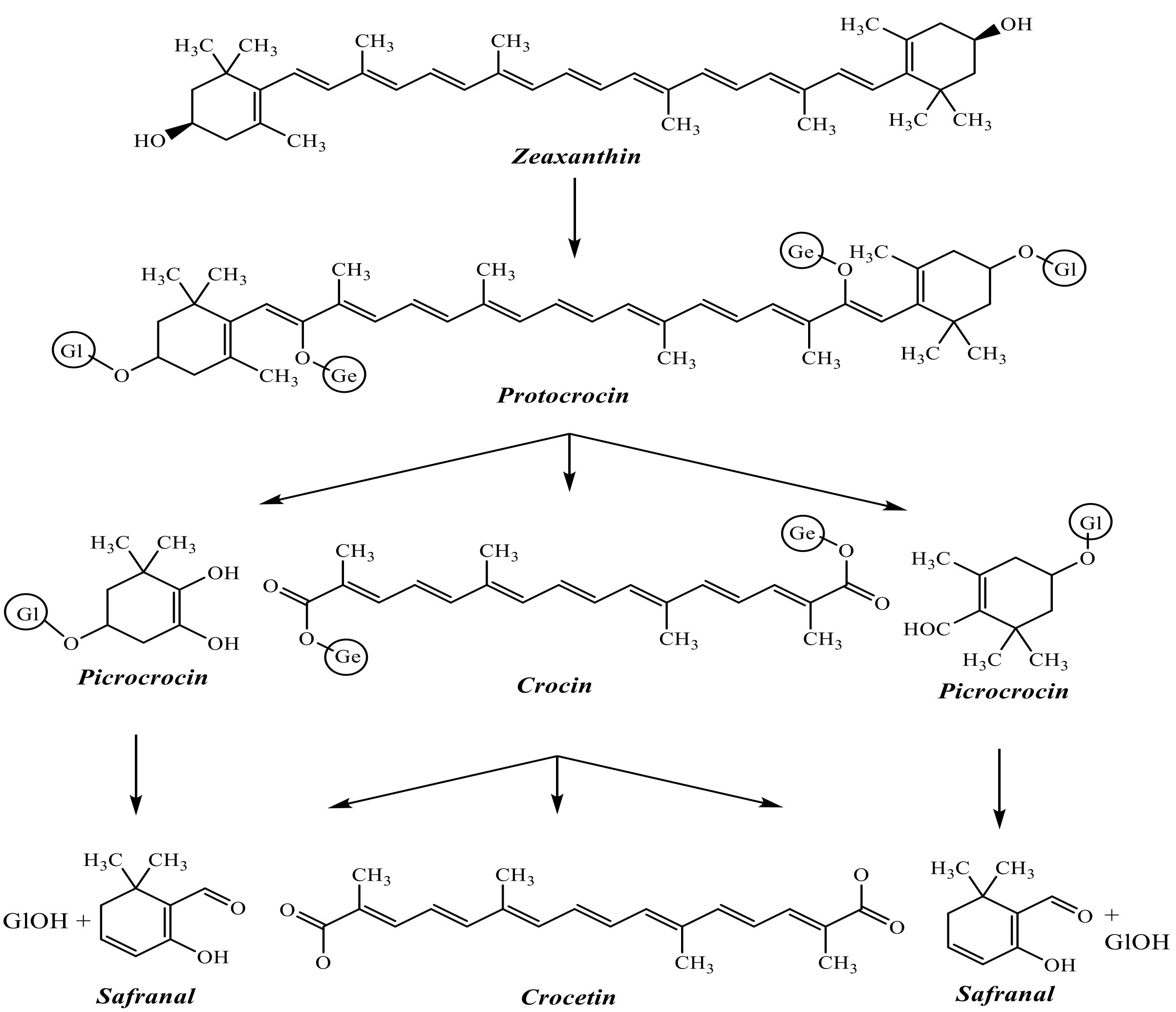
In this case,picrocrocin contributes to the distinct aroma and bitter flavor of saffron, while crocin imparts the yellow-brown hue. Safranal, as a volatile compound, lends the unique aroma and taste to saffron. Thesaffron flower is rich in various chemical components that are highly sought after by the global pharmaceutical industry, with carotenoids and flavonoids being among the most significant. Thecomposition of the saffron flower holds great importance in the realms of pharmaceuticals and cosmetics.

**Figure.1** Chemical structure of saffron carotenoid crocin - trans-crocetin di-(b-D-gentiobiosyl) ester




bis[(2S,3R,4S,5S,6R)-3,4,5-trigidroksi-6-({[(2R,3R,4S,5S,6R)-3,4,5-trigidroksi-6-(gidroksimetil)tetragidro-2H-piran-2-il]oksi}metil)tetragidro-2H-piran-2-il] (2E,4E,6E,8E,10E,12E,14E)-2,6,11,15-tetrametil-2,4,6,8,10,12,14-geksadekageptayenoat

**Figure.2** Chemical relationships between major compounds in saffron stigmas based on biosynthetic pathways




[Zeaxanthin→Picrocrocin→Safranal+D-Glucose (GlOH)]va [Zeaxanthin→Crocetin→Crocin (Crocetin+Gentiobii))] [Shahi *et al.*, 2016].

The renowned saffron suppliers globally include Safrante Global Company S.L.U., Evolva, Rowhani Saffron Co., Sara Nuts, Tarvand Saffron Co., Hijos de Eustaquio, Abad&Co.S.L., USMS Saffron Co.Inc., Royal Saffron Company, Saharkhiz International Group Companies, Gohar Saffron, Novin Saffron Co., and Azafranes Manchegos SL. These companies are highlighted in Table 1-2.According to statistics, as reported by Future Market Insights, the value of Saffronvalue in 2023 was $525.7 million (Saffron market trends, 2024).

The market share of saffron worldwide is projected to grow at an average rate of 7.3% from 2024 to 2030. (Saffron-market, 2024). The saffron market grew at a CAGR of 2.23% from 2018 to 2022, and is expected to reach a CAGR of 3% by 2033. This shows that the saffron market is growing at an average rate compared to other markets.

Scientific sources affirm the utilization of several biological fertilizers in the cultivation of saffron. Including cattle manure (Gholami *et al.*, 2017; Mollafilabi, 2002), sheep manure (Jahan and Jahani, 2007), poultry manure (Rezaie *et al.*, 2019; Koocheki, 2003; Koocheki *et al.*, 2007), biochar (Qasim *et al.*, 2024), vermicompost (Seyyedi *et al.*, 2018; Gholami *et al.*, 2017), microbiological agents (Chamkhi *et al.*, 2023; Javid *et al.*, 2013; Díez-Méndez *et al.*, 2017), and extracts from microalgae and macrophytes (Chaudhary *et al.,* 2023) are widely used in saffron cultivation.

From traditional agricultural practices, a range of fertilizers are commonly employed in saffron cultivation, including NPK (Alhasan, 2023), potassium-based mineral fertilizers such as K-silicate and K-sulphate (Seliem *et al.,* 2024), phosphorus (Rezaie *et al.,* 2019), urea humic acid (Rezaie *et al.,* 2019), and mineral fertilizers like urea (Rezaie *et al.,* 2019).

In contrast to saffron cultivated with mineral fertilizers, saffron grown using organic fertilizers displayed a robust correlation with factors such as the number of flowers, flower weight, and weight of flower stigmas (g/m2) (Jahan and Jahani, 2007). Specifically, when compared to N, NK, and PK mineral fertilizers, PK mineral fertilizer yielded 0.035 g/m2 of dry stigma, while cattle manure resulted in 0.093 g/m2 of dry stigma. Regarding the weight of dry flowers, NPK mineral fertilizer produced 0.92 g/m2 of dry flowers, whereas a minimal 0.33 g/m2 of dry flowers were obtained when N and PK were utilized.

Research findings have demonstrated a notable correlation among the weight of dry flowers, stigmas in dry flowers, and the proportions of phosphorus, mineral nitrogen, exchangeable potassium, and C/N in organic fertilizers. This balance is crucial for absorption and significantly influences the yield of saffron. In the saffron production of the five most productive saffron-growing regions in South Khorasan Province, Iran, 28 farms utilize cattle manure, representing 19% of the total fertilizer used (Koocheki, 2003).

According to scientific sources, saffron is not highly demanding in terms of soil nutrients. Hence, it can be cultivated even in soil with limited nutritional elements by supplementing it with fertilizers containing a light absorbable organic composition (Rahmati, 2004; Vatanpur-Azghadi *et al.,* 2003). Scientific sources attribute the soil's degradation to saffron cultivation, with each kilogram of dry saffron biomass absorbing and depleting approximately 10.2 kg of nitrogen, 3.2 kg of phosphorus, and 22.8 kg of potassium from the soil (Shahandeh, 1990). The state of Iran is considered one of the countries in the world that is effectively using various forms of biological fertilizers in relation to saffron-growing states, using on average up to 20-80 tons of cattle dung per hectare (Mollafilabi, 2002; Mollafilabi, 2003). Nevertheless, the trend of widespread use of mineral fertilizers has been maintained in the regions of Iran where saffron is most commonly planted, for instance, (Vatanpur-e-Azghadi and Mojtahedi, 2003) discovered that maintaining low levels of nitrogen and phosphorus could significantly enhance saffron yield. In contrast, Mollafilabi's research indicated that the application of 100 kg of ammonium phosphate per hectare before the initial irrigation post-saffron planting, along with 100 kg of mochevina per hectare during the first growing season, yielded favorable results (Mollafilabi, 2004).

But, recent evidence indicates that biological fertilizers, including vermicompost, are equally effective as mineral fertilizers, such as the application of 225/130 kg/ha of nitrogen and phosphorus in saffron production. Moreover, the utilization of 10 tons of vermicompost per year has been shown to enhance nitrogen and phosphorus retention, as well as increase the quantity and weight of medium and large saffron bulbs (Seyyedi *et al.,* 2018). In saffron production, the application of 10 tonnes of vermicompost was observed to be more beneficial compared to using 10 tonnes of cattle manure and 10 liters of Humaster Saffron oregano. This approach not only promotes the growth of saffron bulbs but also minimizes losses during harvesting, ultimately resulting in increased productivity (Gholami *et al.,* 2017). In addition, when chicken manure (15t/ha) is used for saffron cultivation, humic acid (2 kg/ha), urea 200 kg/ha, phosphorus-potassium (140 kg/ha) from mineral fertilizers. It was shown that the number of flowers is higher and the productivity of stigmas is increased compared to the used variants (Rezaie *et al.,* 2019).

Furthermore, the effect of saffron flowers on the formation of crocuses by the growth and development of plants by the rhizobacteria and their metabolic ingredients (epibrassinolides) has been studied, leading to a strong development of the root system of saffron by the metabolite 24 of epibrassinolides, resulting in a sharp increase in the amount of crocusin produced from the main ingredient of saffron, which has a positive effect on the productivity of its onions and flowers (Chamkhi *et al.,* 2023).

Furthermore, rhizobacteria such as Pseudomonas sp, Klebsiella sp, *Bacillus subtilis, Acinetobacter haemolyticus, Acinetobacter lwofii*, and Pantoea sp have been identified as key stimulators of the root system in saffron. These rhizosphere microorganisms play a crucial role in enhancing saffron plant growth (Díez-Méndez *et* *al.,* 2017; Javid *et al.,* 2023). The analysis of scientific sources indicates that biological fertilizers are of high importance in the production of saffron.

In this research, the main objective was to examine the alterations in the overall protein and amino acid composition of saffron in relation to the use of biological fertilizers.

## Materials and Methods

### Research object and its brief description

Underground corms of saffron crocus (*Crocus sativus* L.) were used as the research object. The professor A.K.Khamzayev provided corms of saffron crocus (*Crocus sativus* L.),which is grown in the Bobotog state forestry fields (since 2022, they have been cultivated in a natural conservation method). The vegetation of the plants has not completed, reaching its last phase. The research object is being grown at the experimental site of the Tashkent Botanical Garden (Botanical Garden) named after Academician F.N. Rusanov under the Botanical Institute of the Federal Republic of Uzbekistan. The diameter of the corm is 1-2 centimeters. The quantity of leaves is from 5 to 15, in pale yellow color; 2 mm wide, and has glabrous texture. The amount of flowers is from 1 to 4, the petals are in whitish yellow color, lower part (outer side) of which is purple, length is 2-4 cm. The stamens are shorter than a perigonium. The receptacle is long, 6-7 mm wide. It blooms in February-July, bears fruit in April-August. It has a very sharp and pleasant smell due to the presence of essential oil. From 90-100 thousand saffron flowers, 1 kg of dried flower buds are obtained.

### Cultivation schemes of the saffron crocus

In the research process, the land area was prepared by tilling and harrowing on a tractor at a depth of 40-45 cm. The row spacing was in the scheme of 60×70×90, and the depth planting corms was 12.0±1.0 cm.

### The soil composition of the saffron crocus

The initial chemical composition of the soil in the experimental field of the Botanical Garden is as follows: pH-6.5; electrical conductivity 3.4 $dS.m^{-1}$; the ratio of total organic is 0.68%; total nitrogen (N) 0.08%; absorbable potassium 230 mg/kg; absorbable phosphorus 40 mg/kg. In the dispersion analysis of physiological signs, the reliability of differences between samples was studied using generally accepted methods, including the methods of B.A.Dospekhov, on the basis of Fisher's criterion (f), standard error of deviation (SD), standard error of mean (SE) and least reliability differences ($P \leq 0.01$; $P \leq 0.001$) levels. All researches were performed in at least 3 repetitions. In all research methods, basic agrotechnic processings were carried out on a standard basis.

### Fertilization schemes of the saffron crocus

Zoohumus was used as a model biofertilizer, biohumus and untreated natural soil of the Botanic Garden were used as controls. Zoohumus prepared during breeding of *Tenebrio molitor* larvae in a standard nutrient medium in the scientific laboratory of the Tashkent Institute of Chemical Technology, Department of "Biotechnology" was used as a biological fertilizer. Zoohumus was presented by S. Salomova, independent researcher of Karshi State University (2021-2024). Furthermore, an independent researcher of the Department of "Biotechnology", Tashkent Institute of Chemical Technology, Sh.A.Eshqobilov presented biohumus, which was prepared on the basis of Californian earthworms grown in compost based on cattle manure and tree leaves (2021-2023).

### General proteins and identification of amino acids in proteins

For the determination of the total protein as one of the most optimal methods, the K'yel'dal ' method was used. The total protein content is calculated based on the nitrogen levels detected in the sample. The essence of the method is to produce ammonium sulfate salts by hydrolyzing the organic matter in the sample with concentrated sulfuric acid (amino groups in the protein structure).

Nitrogenous organic compounds $+H_2SO_4 \rightarrow (NH_4)_2SO_4 + CO_2 + CO_2\uparrow + H_2O$

Upon completion of hydrolysis, the resulting ammonium sulfate was processed with sodiumhydroxide to transform it into ammonia.

$$(NH_4)_2SO_4 + 2NaOH \longrightarrow Na_2SO_4 + 2NH_4OH \begin{cases} 2H_2O \\ 2NH_3 \end{cases}$$

Neutralization was absorbed into the resulting solution of ammonia or ammonium hydroxide sulfuric acid. the resulting acid is titrated with an alkaline solution. The nitrogen content is determined based on the calculated amount of ammonia. An accurate sample for analysis is drawn from the average ground homogeneous sample of the studied sample into a test tube, the error rate should not exceed 0.1%. The sample is quantitatively carried out in a K'yel'dal' flask.Subsequently, the experiment proceeded in accordance with the provided guidelines (Control methods. Chemical factors, 2004). Processing of the obtained results: the mass fraction of nitrogen (X) in the analyzed sample was calculated by the formula as a percentage of the mass of the sample by the volume after the titration of the amount of ammonia passed through dilute sulfuric acid:

$$X = \frac{(V_1 - V_0) * K * 0.0014}{m} * 100\%$$

$V_0$-In the experimental sample, the volume in milliliters of the 0.1 mol/l sodium hydroxide solution used to titrate the remaining 0.1 mol/l sulfuric acid solution.

During the course of the research, the method of storing amino acids in the general proteins of saffron stigmas was studied in relation to the cultivation of the plant (Table 3.5.2) (Steven *et al.,* 1988).

## Results and Discussion

### Effect of biological fertilizers on total protein content of saffron flower

In the course of the research, the preservation of total proteins in saffron flowers was investigated based on biological fertilization methods (Table 3). According to the obtained results, it was found that the total protein content of saffron flower stigmas grown on the basis of biohumus is 18.98%, and the nitrogen content is 3.037%. It was observed that the total protein content of saffron flowers cultivated with zoohumus is 19.39%, with a nitrogen content of 3.102%.

Upon comparing the results, it was observed that the total protein content of saffron flowers cultivated with traditional zoohumus is 0.41% higher than those grown with biohumus. Additionally, a 0.065% increase in nitrogen content was also noted.

These data show that biological fertilizers used in saffron cultivation are of great importance. Hence, the variable nature of the biological value and productivity of saffron cultivated with biological fertilizers emphasizes the importance of considering these factors in the plantation-based saffron cultivation process.

In order to further scientifically substantiate the results of this research, the amino acid composition of saffron flower stigmas grown on the basis of traditional biohumus and zoohumus was analyzed.Comparing the total amino acid composition with standard amino acids is depicted in (Figure 1).

According to the obtained results, the total amino acid content of saffron flowers grown in biohumus amounted to 38.9 mg/g, while it was determined to be 45.5 mg/g in saffron flowers grown in zoohumus (Figure 2).In conclusion, it was found that saffron flowers cultivated under biohumus conditions contained 1.339641 mg/g of glutamine, while saffron flowers grown under zoohumus conditions had a content of 6.472112 mg/g (Figure 4).

Also, it was found that threonine amino acid was stored in the amount of 0.394265 mg/g in the saffron flower grown in the conditions of biohumus fertilization, and in the amount of 1.60769 mg/g in the saffron flower grown in the conditions of zoohumus (Figure 3).

Upon analyzing the data, it was observed that saffron flowers cultivated with zoohumus fertilizer exhibited higher levels of glutamine amino acid at 5.132471 mg/g and tyrosine amino acid at 1.213425 mg/g.

Furthermore, it was discovered that arginine amino acid was stored at 0.561764 mg/g in saffron flowers cultivated with biohumus fertilization and at 0.844156 mg/g in saffron flowers cultivated with zoohumus.

When our analysis was conducted, it was noted that saffron flowers grown under zoohumus conditions preserved a higher amount of 0.844156 amino acid at a level of 0.282392 mg/g.

According to the results obtained, it was determined that saffron flowers cultivated under biohumus conditions retained a quantity of 2.72617 mg/g of alanine amino acid, while saffron flowers grown under zoohumus conditions exhibited a level of 3.030166 mg/g.

Similarly, it was found that saffron flowers cultivated under biohumus conditions retained a quantity of 2.14945 mg/g of tryptophan amino acid, while saffron flowers grown under zoohumus conditions exhibited a level of 3.634474 mg/g.

**Table.1** Proportion of major saffron supplier companies by world region (2024)

| North America | Europe | Asia-Pacific region | Middle East |
|---|---|---|---|
| **Great American Spice** | HEA&Co | Royal Saffron | Gohar Saffron |
| **Mehr Saffron** | Golden Saffron Co. | King Kesariya | Rowhani Saffron Co. |
| | Azafran de la Mancha | Royal Spices International | Linkage Internationals |
| | | Shahsaffron | Iran Saffron Company |
| | | Shiva Saffron | Ajfan store |
| | | | Tarvand Saffron Co. |
| | | | Grandor Co. |
| | | | Novin Saffron |
| | | | Saffronir |

**Table.2** The proportion of the countries that supply large quantities of saffron in the world by region (2024)

| Asia Pacific | Europe | Middle East and Africa | South America | North America |
|---|---|---|---|---|
| **China** | Great Britain | South Africa | Brazil | USA |
| **South Korea** | France | Egypt | Argentina | Canada |
| **Japan** | Germany | Nigeria | Other countries | Mexico |
| **India** | Italy | The Persian Gulf Cooperation State | | |
| **Australia** | Spain | Middle East and the rest of Africa | | |
| **Indonesia** | Sweden | | | |
| **Malaysia** | Austria | | | |
| **Vietnam** | Other countries | | | |
| **Taiwan** | | | | |
| **Bangladesh** | | | | |
| **Pakistan** | | | | |
| **Other countries** | | | | |

**Table.3** Protein content in saffron flowers cultivated using different fertilizers.

| № | Sample | Nitrogen quantity (%) | The quantity of total proteins % |
|---|---|---|---|
| 1 | Biohumus | 3,037 | 18,98 |
| 2 | Zooghumus | 3,102 | 19,39 |

**Figure.3** The standard chromatogram of the total amino acid content of saffron flowers

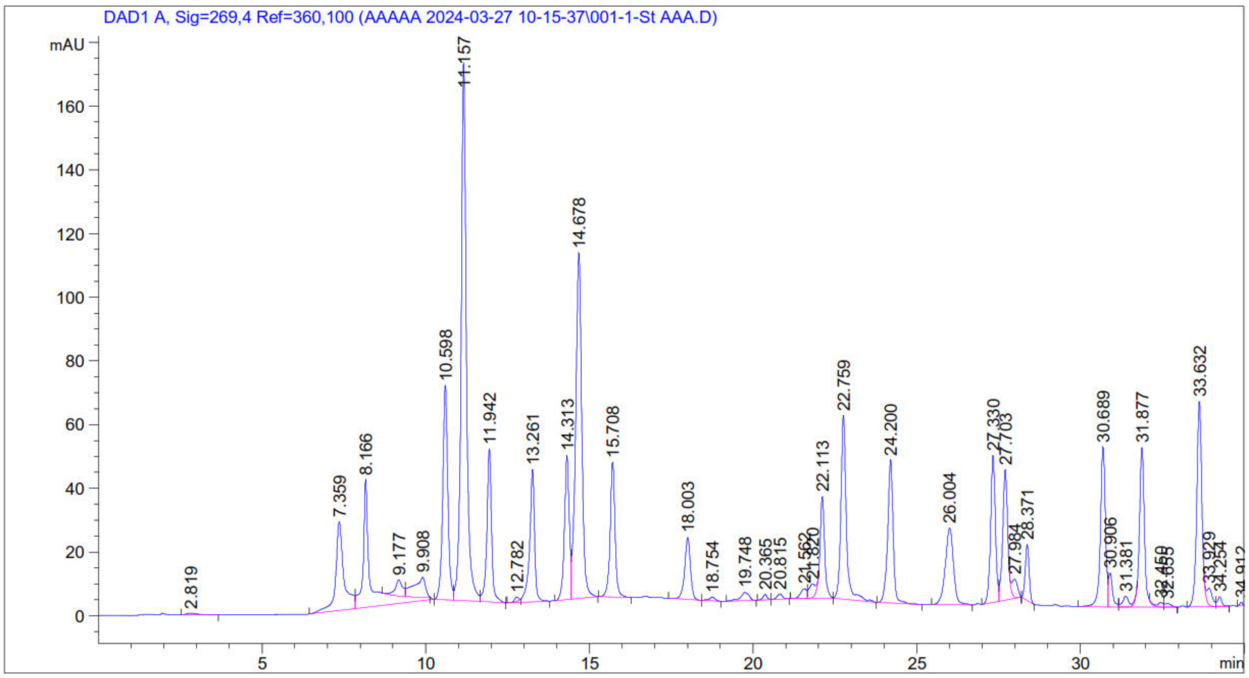


**Figure.4** The chromatogram of the amino acid content of saffron flowers grown in biohumus

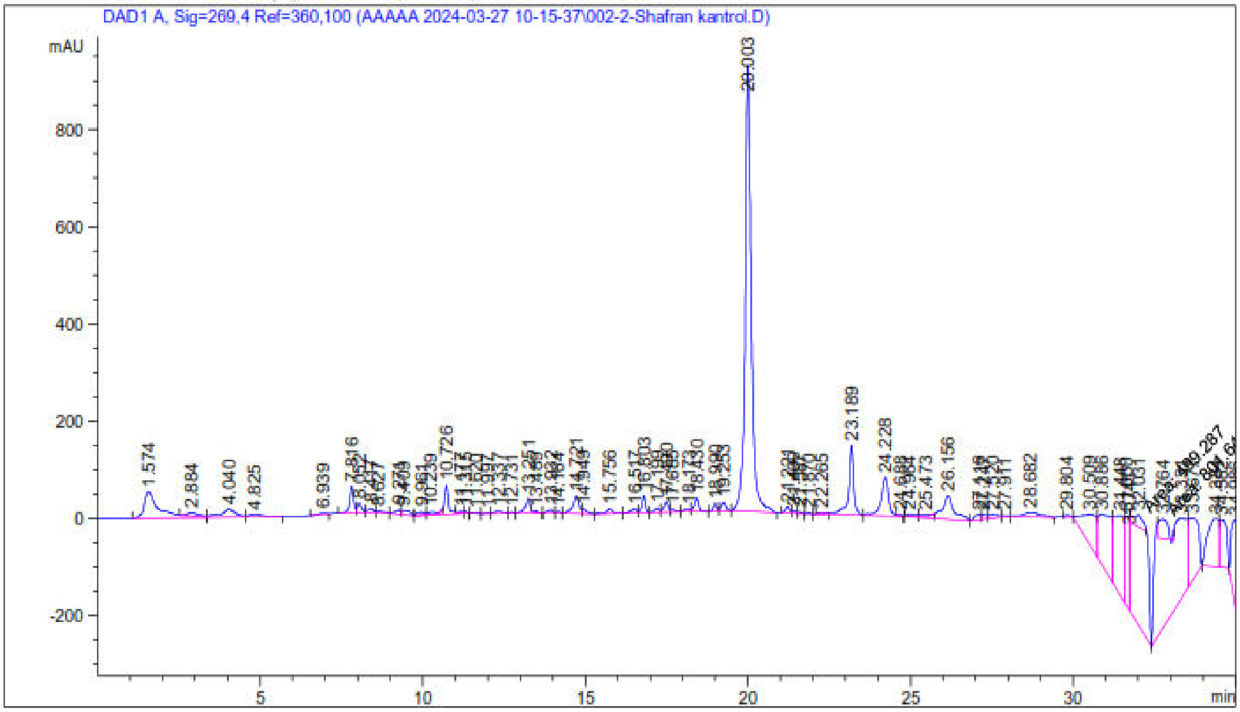
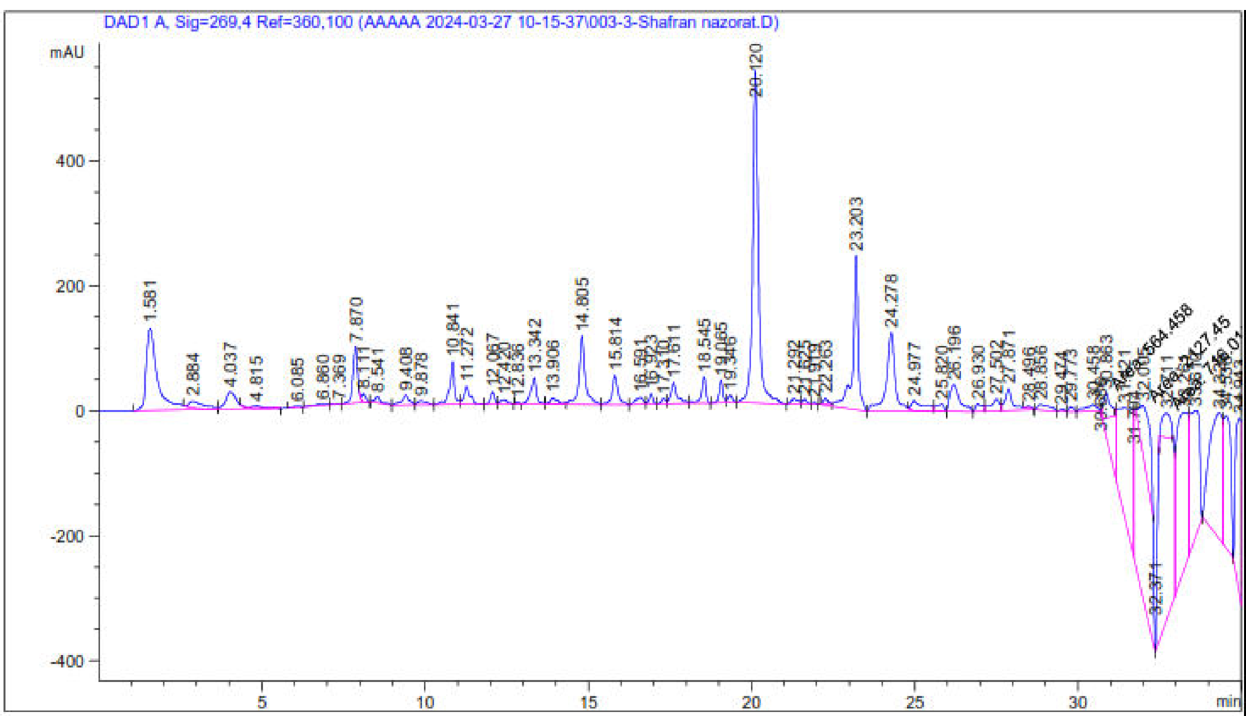



**Figure.5** Chromatogram illustrating the amino acid storage of saffron cultivated in zoohumus.




In the present study analysis of the results, it was determined that saffron flowers grown under zoohumus conditions retained a higher amount of 0.303996 mg/g of alanine amino acid, while tryptophan amino acid was found to be preserved at a higher level of 1.485024 mg/g.

Based on the obtained results, it was noted that under the influence of biological fertilizers used in saffron cultivation, strong changes occur in the chemical composition of saffron flowers. The obtained results can be important in obtaining BAS based on saffron flower.When analyzing the total protein content depending on fertilization, it was found that the content of total proteins and amino acids (glutamine, serine, alanine, tryptophan) is high in the stigmas of the saffron flower grown in biohumus. Saffron crocus grown on the basis of zoohumic fertilizer can be somewhat rich in non-exchangeable amino acids, which can serve to further increase its value in pharmaceuticals.

## Author Contributions

Nodira K. Ruzmetova: Investigation, formal analysis, writing—original draft. Xurshidbek O. Abdullayev: Validation, methodology, writing—reviewing. Sodikjon X. Abdinazarov:—Formal analysis, writing—review and editing. Gayrat N. Juraev: Investigation, writing—reviewing. Nortoji A. Khujamshukurov: Resources, investigation writing—reviewing.

## Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent to Publish** Not applicable.

**Conflict of Interest** The authors declare no competing interests.

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**How to cite this article:**

Nodira K. Ruzmetova, Xurshidbek O. Abdullayev, Sodikjon X. Abdinazarov, Gayrat N. Juraev and Nortoji A. Khujamshukurov. 2024. Changes in Protein and Amino Acid Content of Saffron Flowers Depending on Fertilization. *Int.J.Curr.Microbiol.App.Sci.* 13(9): 56-67. **doi:** https://doi.org/10.20546/ijcmas.2024.1309.005