# Determination of Amino Acids and Some Biochemical Properties in Biscuits Fortified with Spirulina

Rana Saleh Hussein1\* , Sabra Saad Yassin2 1Tikrit University/College of Agriculture/Department of Food Sciences. 2Tikrit University/College of Agriculture/Department of Food Sciences. \*Corresponding author's email: rana.s.h0220@st.tu.edu.iq.

### Abstract

The study was conducted in the laboratories of the College of Veterinary Medicine / Tikrit University, in the laboratories of the College of Science / Department of Biology / Tikrit University and in the laboratories of the Ministry of Science and Technology / Environment and Water Department / Baghdad. Thirty adult male albino mice weighing 210-220 g were used in this study. They were randomly distributed into six groups, one group fed on the animal house diet, and the other groups fed on the control biscuits and biscuits manufactured with spirulina fortification ratios. The results of estimating the amino acid content in the manufactured biscuits showed a significant increase in the 7% and 9% treatments, as the percentages increased with increasing the fortification ratio compared to the control treatment. The results of estimating the serotonin hormone showed a significant increase in the percentages of the manufactured biscuit treatments. The highest significant increase was recorded in the 9% treatment, reaching 44.384 ng/ml, while the lowest significant increase was recorded in the control treatment and the animal house feed, reaching 16.5114 and 14.7936 ng/ml, respectively. This indicates that spirulina works to increase this important hormone in rats, which plays a role in regulating many complex physiological systems within the central nervous system. The study aimed to manufacture functional biscuits by fortifying wheat flour with different proportions of spirulina powder 3, 5, 7, 9% and estimating the percentage of amino acids in biscuits fortified with spirulina powder, and knowing the effect of feeding on manufactured biscuits on the hormone serotonin in rats fed on biscuits.

Keywords: Spirulina, Biscuits, Amino Acids, Serotonin.

# Introduction

Functional foods are foods that contain other components that are beneficial and have a positive effect on health in addition to nutrients because they can be a source of essential nutrients. The concept of functional foods emphasizes that food is not only important for living but also plays a role in preventing and reducing risk factors for many diseases as it has a major role in promoting health and immunity [1]. Spirulina microalgae is considered to have high nutritional value. When fortified or enhanced with it, foods can be enriched with a wide range of foods that contain an ingredient with many health benefits beyond basic nutrition. Biscuits are a ready-to-eat baked snack that is widely consumed due to their low prices, variety of shapes, flavor, and long shelf life. Traditional biscuits are low in protein, fiber, and minerals, have few functional properties, and it is high in sugar and fat, making them unhealthy for daily use [2, 3]. Therefore, it is necessary to fortify it and add different functional components, which has led to an increase in the demand for nutritionally enhanced biscuits with the addition of nutritional and healthy components [4]. The blue-green microalgae Spirulina platensis is a common food all over the world since ancient times, spirulina is an excellent and exceptional food because it is rich in nutrients that have health-promoting benefits, as spirulina has many therapeutic properties [5]. Spirulina contains highly concentrated amino acids, fatty acids and other active compounds such biologically as carotenoids, phycocyanin, chlorophyll, vitamins and others [6]. The digestibility and absorption of spirulina is high due to the cell wall that includes soft mucous polysaccharides, so it is used by people who suffer from Dyspepsia [7]. Spirulina is a good source of most of the nutrients needed by our body and is a good source of many amino acids, including tryptophan, which is the basic unit in the synthesis of the hormone serotonin, which is important for brain health and is also important in regulating sleep, cognitive functions and appetite because it is a monoamine neurotransmitter in the central nervous system, the presence of which depends on the availability of the essential amino acid tryptophan, which cannot be synthesized within the body but is obtained from food sources [8.]

# Material and Methods

Samples were prepared and collected, and American organic spirulina powder was purchased from the drug stores of Tikrit city. Biscuits were manufactured by adding five percentages of spirulina powder to the biscuit dough. The treatments were divided into a control treatment of 0% (without adding spirulina powder). The remaining four treatments included adding spirulina powder at a rate of 3, 5, 7, and 9% to the biscuit sample, and the biscuits were manufactured by adding ground sugar to margarine and were mixed by a hand mixer for 5 minutes until a good cream was formed, after which egg yolks were added then add wheat flour and baking powder and then powder Spirulina was kneaded according to the studied proportions and kneaded well until the ingredients homogenized and the dough was left to rest for 30-45 minutes and the dough was rolled out to a thickness of 3.5 mm and cut using a round biscuit mold and then placed in the oven tray coated with parchment paper at a temperature of 180 °m for 10-15 minutes after baking it is left to cool at room temperature until the biscuit temperature reaches the desired temperature. Packaging was carried out in polyethylene bags and left until the required analyses were performed or consumed [9]. and biscuits were manufactured. Healthy and disease-free laboratory animals were brought from the College of Veterinary Medicine / Tikrit University, 30 adult male Albino rats, whose weights ranged from 210-220 grams. The rats were randomly distributed into six groups with similar weights. The animals were fed regularly and the basic food was the manufactured biscuits and the animal house feed. After 28 days, the experiment period ended and the experimental animals were starved for 10 hours, then chloroform was used to anesthetize them. Then the rats were dissected from the chest area and blood was drawn directly from the heart for the purpose of conducting the necessary tests. The blood was drawn into a tube containing the anticoagulant EDTA containing approximately 6 ml of blood that was centrifuged using a centrifuge at a speed of 3000 rpm for 5 minutes to obtain the serum that was stored in Eppendorf tubes at a temperature of -20  $^{\circ}$  C until the analyses and tests were conducted. Estimation of Amino Acids Content:

The amino acids in the biscuit mixtures and

spirulina powder were estimated according to method [10], 2 g of the sample was taken, then hydrochloric 12 ml of acid (HCL)

concentration and 6 molar were added and the mixture was placed in the oven at a temperature of 110°C for 24 hours, after which the sample was filtered using a plastic filter (0.45um) and taken to the device for injection, 1 ml of the sample was taken and 200 microliters of 5% Orth phthalein aldehyde were added to it and the sample was shaken for 2 minutes and 100 microliters of the last mixture were taken and injected into the (Amino acid analyses) device. The eluting phase consisting of (methanol: acetonitrile: 5% formic acid) was used in ratios of (25: 75) volume/volume and at a flow rate of 1 ml/min, using a C18-NH2 (250/4.6 mm) separation column to separate amino acids, while a fluorescence detector was used to detect amino acids at wavelengths (EX=445nm, EM=465nm). The (clarity 2015) program was used to analyze amino acids.

Biochemical Tests:

Measuring the percentage of the hormone serotonin

The level of serotonin was estimated according to the method developed by [11], through the following steps:

.1Sample preparation: Plasma samples were collected, and then stored appropriately until use.

.2Plate preparation: (Plates) We prepare ELISA plates by adding a solution containing specific parts of the target serotonin to each area on the plate.

.3Binding: (Coating) The samples were added to the plates and left for half an hour until serotonin binds to the surface of the plate.

.4Preparation for reaction: The plates were washed to remove unbound samples and antiserotonin solution (antibodies) was added and left to react.

.5Detection: The plates were then washed again to remove unbound antibodies and a

stabilizing compound or alkaline phosphatase was added which will react with the serotonin bound antibodies.

.6Signal measurement: The amount of immobilized complex formed by the reaction between the immobilized complex and the antibodies was measured.

.7Data analysis: The results were analyzed using known amounts of serotonin as a reference to estimate the amount of serotonin in the samples.

Results and Discussion

Amino acid content in biscuits made from fortifying wheat flour with spirulina powder:

The results of the current study showed, as shown in Table (1), the amino acid content in biscuits made from fortifying wheat flour with spirulina powder at rates of 3, 5, 7, 9%. It was found that the amino acid aspartic acid increases with increasing the substitution rate and recorded the highest value at the substitution rate of 9%, reaching 14.12%, with significant difference from the other a treatments, which reached 6.54% for the control sample and 8.58, 11.48, 13.52%, respectively, for rates of 3, 5, 7%. As for the amino acid glutamic acid, it increased with increasing the substitution rates of spirulina powder, as the highest value for the substitution rate was recorded at 9%, reaching 25.68%, and then the substitution rate of 7%, 5%, and 3%, which reached 25.48, 24.85, and 24.25%, respectively, compared to the control treatment, which was 11.22%. Also, the amino acids lysine, serine, threonine, isoleucine, alanine, valine, tyrosine, arginine, methionine, histidine. leucine, proline, tryptophan, phenylalanine and glycine recorded the highest value for the substitution rate of 9%, with significant differences from the other treatments and substitution rates of 7, 5, 3% respectively. compared to the control treatment, which recorded the lowest value. These results are consistent with [12, 13] who indicated that all biscuit treatments made from fortifying wheat flour with spirulina powder contained the highest amount of essential amino acids compared to the control treatment which had the lowest value of amino acids

Table (1) Amino acid content in biscuits made from fortifying wheat flour with spirulina powder

Biscuits with	Biscuits	Biscuits	Biscuits with		treatments
9% substitution rates	with 7% substitution rates	with 5% substitution rates	3% substitution rates	The control	Amino Acid
14.12 A	13.52 B	11.48 C	8.58 D	6.54 E	Aspartic acid
25.68 A	25.48 B	24.85 C	24.25 D	11.22 E	Glutamic acid
4.55 A	3.97 B	3.87 C	3.65 D	2.24 E	Lysine
6.15 A	5.98 B	5.93 C	5.88 D	5.56 E	Serine
5.94 A	5.88 B	4.48 C	4.25 D	3.12 E	Threonine
5.62 A	5.58 B	5.14 C	4.54 D	3.85 E	Isoleucine
5.67 A	5.65 B	4.65 C	4.25 D	3.54 E	Alanine
6.68 A	6.65 B	6.58 C	6.28 D	5.25 E	Valine
9.82 A	9.25 B	9.14 C	5.48 D	4.58 E	Tyrosine
5.82 A	5.66 B	5.52 C	5.48 D	5.44 E	Arginine
5.98 A	5.82 B	5.62 C	5.42 D	3.56 E	Cysteine
4.85 A	4.76 B	4.62 C	4.58 D	3.14 E	Methionine
10.05 A	9.58 B	8.47 C	8.25 D	4.68 E	Proline
5.14 A	5.09 B	3.65 C	3.25 D	3.02 E	Histidine
8.82 A	8.58 B	8.08 C	7.77 D	7.65 E	Leucine
10.50 A	7.00 B	4.85 C	3.50 D	3.01 E	Tryptophan
8.85 A	8.48 B	8.03 C	7.98 D	5.22 E	Phenylalanine
4.48 A	4.42 B	4.13 C	3.95 D	3.88 E	Glycine

#### Serotonin

Serotonin, or 5-hydroxytryptamine (5-HT), is a monoamine neurotransmitter in the central nervous system. Its synthesis depends on the availability of the essential amino acid tryptophan (L-tryptophan), which cannot be manufactured biologically and therefore must be obtained from dietary sources. In addition, the rate of serotonin synthesis depends on the availability of tryptophan. Serotonin has long been associated with many basic mental aspects of behavior, including sleep, appetite,

#### Hormone:

cognition, and social and emotional behaviors [14]. Figure (1) shows the effect of feeding biscuits made from fortifying wheat flour with spirulina powder at rates of 3, 5, 7, and 9% on serotonin hormone values in rats. The

results showed a significant increase (p<0.05) in serotonin hormone values with increasing fortification rate, as their values in 3% spirulina biscuits, 5% spirulina biscuits, 7% spirulina biscuits, and 9% spirulina biscuits were 17.8193, 26.8107, 30.9794, and 44.3848

ng/ml, respectively, compared to the control treatment, which was at 16.5114 ng/ml, while for the feed treatment, it was at 14.7936 ng/ml. This indicates that spirulina increased this important hormone in rats, which is known for its role as a neurotransmitter within the functions, specifically regulating the glomeruli [15.[ nervous system, but it is manufactured and has multiple functional roles in other organs, including the intestines, kidneys, and liver, as hepatic serotonin regulates blood flow and wound healing, while renal serotonin works to maintain kidney



Figure (1) Effect of feeding on feed and biscuits made from fortifying wheat flour with spirulina powder on the hormone serotonin in rats .

# Conclusion

The present study showed that spirulina is a good source of most of the nutrients necessary for our body, especially the brain. It is the best and richest source of protein and has recorded the highest values in it. It is also a good source of essential and non-essential amino acids and other nutrients important for the health of the

# [1] **References**

Chen, G., Li, Y., Li, X., Zhou, D., Wang, Y., Wen, X., ... & Li, N. (2021). Functional foods and intestinal homeostasis: The perspective of in vivo evidence. Trends in Food Science & Technology, 111, 475-482. body. Therefore, spirulina can be considered an interesting food for many reasons. It is very nutritious and rich in antioxidants, protein and iron. It can be given to infants, especially in the growth stage, safely without any risk.

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