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Production of functional basil drink enhanced with bifidobacterium

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Abstract
The study was conducted in the laboratories of the Department of Food Sciences / College of Agriculture / Tikrit University, for the purpose of producing a natural basil drink enhanced with the use of Bifidobacterium. It included fermenting the product by cooling at a temperature of 5°C for four weeks T_2, T_3, T_4 and T_5, and comparing it with the standard drink T_1. Several tests were conducted after each week of refrigerated storage, and several sensory tests were also conducted to determine the validity of the product. It was noted through the results of the study that there was a continuous decrease in the concentration of total soluble solids (TSS) of the therapeutic basil drink during the fermentation stages as a result of the consumption of the components of the functional drink produced by Bifidobacterium bacteria. In addition to the increased acidity of the medium due to the production of lactic acid by the probiotic bacteria, the results also showed an increase in the concentrations of total phenols, eugenol, flavonoids, carotenoids, and vitamins, including vitamin C and vitamin B3. Several sensory tests were also conducted, including taste, color, smell, texture, and general acceptability, and the results were acceptable after four weeks of fermentation.

Keywords: Basil drink, functional drinks, total phenolics, eugenol, carotenoids

Introduction
Basil, also known as basil, royal herb, or St. Joseph's plant as it is called in Europe, is one of the most important aromatic medicinal plants in the world. Its scientific name is *Ocimum basilicum*, and there are many species of it, reaching 160 species [1], including cinnamon basil, dark basil, lettuce basil, purple basil, red basil, dwarf basil, African basil, lemon basil, and others [2]. It is widely produced in tropical regions, especially Africa, Asia and South America. It is a perennial herbaceous plant that reaches a height of about a meter, and belongs to the Lamiaceae family [3]. The colors of its leaves range from green to purple [4]. It has a distinctive fragrant smell because it contains many aromatic compounds similar to the smell of cloves, especially in the type used in Italy because of the high concentrations of eugenol it contains. The lemon type has a citrusy smell because it contains high concentrations of limonene and many active ingredients, vitamins, phenolic compounds and pigments, which in turn work to enhance the health of the digestive system, cleanse the intestines, relieve pain, and raise the capacity of the immune system and cardiovascular health [5]. It has the ability to treat headaches, middle ear pain, coughs, colds, respiratory infections, and is an antimicrobial [6]. It has a calming effect that reduces anxiety and psychological stress, as it works to reduce the cortisol hormone in the blood, the secretion of which increases in cases of stress and psychological pressure [7]. The active compounds in basil are used as flavoring agents, as they are used as spices added to many types of commercially produced foods, including meat sausages, tomato paste, pickles, and various beverages [8]. The bio-enhanced basil drink is considered an ideal health food, as it contains many benefits that combine the components of the raw material used in the production process and the benefits of fermentation products [9]. The fermentation process depends on the nature of the food, the types of probiotics used, and the environmental conditions affecting the growth of organisms and metabolic patterns [10]. Fermentation is a desirable change due to the microbial activity of microorganisms that analyze carbohydrates, fats, proteins, and other food components, thus improving food digestion in the digestive system [11]. It increases nutrient absorption and contributes significantly to enhancing the taste and flavor of the produced food [12].

In addition to extending its storage life by controlling the growth and reproduction of many types of spoilage microorganisms [13]. Because of the importance of this product from a health and nutritional perspective, and the lack of studies on this topic, the study aimed to produce a therapeutic, bio-enhanced basil drink, study some of its biochemical properties, monitor it during the fermentation stages, and determine its shelf life.

Materials and Methods

Production of natural bio-enhanced basil drink: Basil leaves sourced from the local markets of Tikrit city were used, cleaned from dust and suspended materials, then 100 grams of basil leaves were taken and added to 900 ml of filtered and sterilized water with specific specifications, then blended in a GOSONIC blender, then left the mixture for 24 hours at a temperature of 25°C, then filtered and adjusted the concentration of total soluble solids of the product by adding sucrose according to the Iraqi standard specifications to 12% Then it was filled into pre-sterilized, hermetically sealed glass bottles and pasteurized at 80°C for 20 seconds, then rapidly cooled, and refrigerated at 5°C. Then it was inoculated with Bifidobacterium bacteria at a concentration of 1.5*108 cells/ml according to McFarland test under controlled sterile conditions. Then the products were kept in the refrigerator until the study was conducted.

Total soluble solids test: The total soluble solids of the samples were estimated using a hand refractometer as in [14].

Total acidity test: Estimated by titration method against NaOH as shown by [15].

Eugenol test: These tests were performed after extracting the sample and injecting it into the HPLC device as mentioned in [16].

Total phenols test: It was estimated using UV-ViS Spectrophotometer as mentioned in [17].

Flavonoids test: It was calculated by dissolving the sample in ethanol, then adding ammonium chloride reagent and then calculating the absorbance using a UV-ViS Spectrophotometer as indicated by [18].

Carotenoids test: They were estimated by dissolving the sample in acetone and hexane, then filtering the solution and measuring the absorbance using a UV-ViS Spectrophotometer as stated in [14].

Vitamin Test: Vitamins were determined using HPLC technique according to the water mentioned in [14].

Sensory tests: Sensory evaluation of the samples was conducted by 22 evaluators from postgraduate students at the College of Agriculture, Tikrit University, according to [19].

Statistical analysis

The experiment was conducted according to the CRD design to determine the significant differences between the treatments at level (0.05) using the statistical program Minitab version (2010).

Results and Discussion

Table 1: Results of chemical tests for the bio-enhanced functional basil drink

Characteristic / Treatments	Acidity %	TSS %
T_1	0.05±0.65e	0.00±12.00a
T_2	0.05±0.95d	0.05±11.65b
T_3	0.05±1.25c	0.05±11.15c
T_4	0.05±1.55b	0.05±10.95d
T_5	0.05±1.75a	0.05±10.75e

* Different lowercase letters within a column indicate significant differences ($p \leq 0.05$) between treatments.

T_1: represents the control sample (free of probiotic bacteria), T_2: the first week of probiotic fermentation, T_3: the second week of probiotic fermentation, T_4: the third week of probiotic fermentation, T_5: the fourth week of probiotic fermentation.

Table (1) shows the results of the statistical analysis of the data for total soluble solids and total acidity, as it is noted that there are significant differences between all treatments during the fermentation stages, as it was found that there are significant differences between the value of treatment T_1 and treatments T_2, T_3, T_4 and T_5, and between treatment T_2 and treatments T_3, T_4 and T_5, and between treatment T_3 and treatments T_4 and T_5, and between treatment T_4 and treatment T_5 for both characteristics.

The decline in TSS concentration is due to the biochemical activities of probiotics, as their enzymes metabolize carbohydrates [20]. Carbohydrates are the building blocks for growth, reproduction, and fermentation [12]. probiotics enzymes primarily break down carbohydrates, followed by other components for energy production [21]. As for the acidity characteristic, probiotics work on converting the remaining carbohydrates in the fermentation medium into lactic acid, thus raising the percentage of total acidity. This contributes significantly to enhancing the taste and flavor of the produced food, in addition to extending its storage life by controlling the growth and reproduction of many types of spoilage microorganisms and pathogenic bacteria, in addition to its high nutritional value [22].

Table 2: Results of the test of active compounds of the functional basil drink enhanced with bioactive compounds (g/ml)

Characteristic / Treatments	Eugenol	Total phenols
T_1	0.95±0.05e	2.35±0.05d
T_2	1.35±0.05d	2.45±0.05d
T_3	1.65±0.05c	2.65±0.05c
T_4	1.95±0.05b	2.85±0.05b
T_5	2.15±0.05a	3.05±0.05a

* Different lowercase letters within a column indicate significant differences ($p \leq 0.05$) between treatments.

T_1: represents the control sample (free of probiotic bacteria), T_2: the first week of probiotic fermentation, T_3: the second week of probiotic fermentation, T_4: the third week of probiotic fermentation, T_5: the fourth week of probiotic fermentation.

Table (2) shows the results of the statistical analysis of the data for total phenols, as it is noted that there are significant differences between the value of treatment T_1 and treatments T_3, T_4 and T_5, and between treatment T_2 and treatments T_3, T_4 and T_5, and between treatment T_3 and treatments T_4 and T_5, and between treatment T_4 and treatment T_5, while no significant differences were noted

between treatments T_1 and T_2. Through the same table, the results of the statistical analysis of the Eugenol characteristic show significant differences between the value of treatment T_1 and treatments T_2, T_3, T_4 and T_5, between treatment T_2 and treatments T_3, T_4 and T_5, between treatment T_3 and treatments T_4 and T_5, and between treatment T_4 and treatment T_5. Fermentation processes represent the slow decomposition of organic components by microorganisms [9]. These changes are due to the work of its enzymes to digest food components (carbohydrates, fats, and proteins), improve the metabolism and absorption of nutrients within the human digestive system, and produce many active compounds such as total phenols, pigments, and vitamins, and raise their concentrations [23].

Table 3: Pigment content for the bio-enhanced functional basil drink g/ml

Characteristic / Treatments	Carotenoids	Flavonoids
T_1	0.18±0.01e	2.25±0.05d
T_2	0.23±0.01d	2.55±0.05c
T_3	0.28±0.01c	2.75±0.05b
T_4	0.32±0.01b	2.95±0.05a
T_5	0.36±0.01a	3.05±0.05a

* Different lowercase letters within a column indicate significant differences ($p \leq 0.05$) between treatments.
T_1: represents the control sample (free of probiotic bacteria), T_2: the first week of probiotic fermentation, T_3: the second week of probiotic fermentation, T_4: the third week of probiotic fermentation, T_5: the fourth week of probiotic fermentation.

Table (3) shows the results of the statistical analysis of the pigments in the functional basil drink. As for flavonoids, significant differences were observed between the value of treatment T_1 and treatments T_2, T_3, T_4 and T_5, and between treatment T_2 and treatments T_3, T_4 and T_5, and between treatment T_3 and treatments T_4 and T_5, and between treatment T_4 and treatment T_5, while no significant differences were observed between treatments T_4 and T_5. And through the same table, the results of the statistical analysis of the carotenoid pigment show that there are significant differences between the value of treatment T_1 and treatments T_2, T_3, T_4 and T_5, between treatment T_2 and treatments T_3, T_4 and T_5, between treatment T_3 and treatments T_4 and T_5, and between treatment T_4 and treatment T_5. The functional basil drink contains many natural pigments, the most important of which are flavonoids and carotenoids, which are considered natural antioxidants in the body [24]. Fermentation is one of the most important and widespread methods for protecting against spoilage and increasing storage life, in addition to enhancing taste, color, flavor, and texture, and producing biologically active compounds such as pigments, phenolic compounds, and vitamins [21]. Probiotic bacteria have the ability to produce a diverse mix of metabolic products and continuously increase their concentrations in the fermentation medium if appropriate conditions are available, such as flavonoids, carotenoids, anthocyanins, phenols, and other metabolic products [25].

Table 4: Vitamin content for the bio-enhanced functional basil drink g/ml

Characteristic / Treatments	Vitamin B_3	Vitamin C
T_1	0.07±0.01e	0.75±0.05e
T_2	0.10±0.01d	0.95±0.05d
T_3	0.13±0.01c	1.15±0.05c
T_4	0.17±0.01b	1.45±0.05b
T_5	0.20±0.01a	1.75±0.05a

* Different lowercase letters within a column indicate significant differences ($p \leq 0.05$) between treatments.
T_1: represents the control sample (free of probiotic bacteria), T_2: the first week of probiotic fermentation, T_3: the second week of probiotic fermentation, T_4: the third week of probiotic fermentation, T_5: the fourth week of probiotic fermentation.

Table (4) shows the results of the statistical analysis of vitamins (vitamin C and vitamin B3) in the functional basil drink, as it is noted that there are significant differences between all treatments during the fermentation stages for both characteristics, as it was shown that there are significant differences between the value of treatment T_1 and treatments T_2, T_3, T_4 and T_5 and between treatment T_2 and treatments T_3, T_4 and T_5 and between treatment T_3 and treatments T_4 and T_5 and between treatment T_4 and treatment T_5. Bio-enhancers have the ability to improve the vitamin content of functional products, especially vitamin C and the B-complex vitamin, at different concentrations. The variation in the high levels of these vitamins is due to the compositional difference between the active products and the ability of bio-enhancers to produce these components [26]. Several studies have shown the potential of probiotics to produce a complex mixture of metabolites and vitamins [25].

Table 5: Sensory test results for the bio-enhanced functional basil drink

Characteristic / Treatments	General Admission	Consistency	Smell	Color	Taste
T_1	9.00±0.00a	9.00±0.00a	9.00±0.00a	9.00±0.00a	9.00±0.00a
T_2	9.00±0.00a	9.00±0.00a	9.00±0.00a	9.00±0.00a	9.00±0.00a
T_3	9.00±0.00a	9.00±0.00a	9.00±0.00a	9.00±0.00a	9.00±0.00a
T_4	9.00±0.00a	9.00±0.00a	9.00±0.00a	9.00±0.00a	9.00±0.00a
T_5	9.00±0.00a	9.00±0.00a	8.00±0.00b	9.00±0.00a	8.00±0.00b

* Different lowercase letters within a column indicate significant differences ($p \leq 0.05$) between treatments.
T_1: represents the control sample (free of probiotic bacteria), T_2: the first week of probiotic fermentation, T_3: the second week of probiotic fermentation, T_4: the third week of probiotic fermentation, T_5: the fourth week of probiotic fermentation.

Table (5) shows the results of the statistical analysis of the sensory tests in the functional basil drink K, as it is noted that there are significant differences in the taste and smell characteristics between treatment T_1 and treatment T_5, between treatment T_2 and treatment T_5, between treatment T_3 and treatment T_5, and treatment T_4 and treatment T_5, while no significant differences were noted between treatments T_1 and T_2 and T_3 and T_4. Regarding the

characteristics (color, texture, and general acceptability), the results of the statistical analysis showed no significant differences between all treatments and for all characteristics during the fermentation stages. Lactic fermentation is an important technique for preserving food products and improving their quality, in addition to enhancing the flavor, texture, and nutritional value of fermented foods and products [27]. It is considered one of the most important and widespread methods for preserving food products and enhancing taste, color, and flavor [22], through the production of a group of biological products such as organic acids, like lactic acid, and some flavoring materials [13].

Conclusion

The study successfully demonstrated the potential of producing a functional, bio-enhanced basil drink fermented with *Bifidobacterium* spp. The results revealed that fermentation had a significant effect on enhancing the drink's biochemical and sensory properties over time. A progressive and statistically significant decrease in total soluble solids and an increase in acidity were observed, indicating active microbial metabolism. Concurrently, the concentrations of key bioactive compounds such as total phenols, eugenol, flavonoids, carotenoids, and essential vitamins (B_3 and C) significantly increased throughout the fermentation process, especially by the fourth week.

These enhancements reflect the probiotics' enzymatic action, contributing to the generation of health-promoting metabolites. Importantly, despite slight reductions in aroma and taste in later stages (T_5), the overall sensory acceptability remained high across treatments. This highlights the feasibility of developing a nutraceutical beverage combining the health benefits of basil with probiotic fermentation.

In conclusion, the bio-enhanced basil drink represents a promising functional food product with improved nutritional quality and health benefits. Such fermented beverages can serve as innovative dietary interventions aimed at boosting immunity, digestive health, and antioxidant intake. Future studies may further optimize the fermentation parameters and assess the product's effects in clinical settings.

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