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(RESEARCH ARTICLE)

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Biological effects of *Triticum sativum* (wheat) on some probiotic bacteria

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Abstract

In this study analyzed the effects of *Triticum sativum* (T) on *Lactobacillus bulgaricus*, (Lb) *Lactobacillus plantarum* (Lp), *Lactobacillus lactis* (Ll), *Enterococcus faecium* (Ef). In the study, fatty acid, flavonoid, resveratrol content, vitamin, phytosterol levels and the antimicrobial activities of T the fatty acid, flavonoid, resveratrol content, vitamin, phytosterol levels and antimicrobial activities of Lb, Lp, Ll and Ef extracts treated with T were determined and compared. For this purpose, the control (T), Lb, Lp, Ll and Ef treated with T (T+Lb, T+Lp, T+Ll and T+Ef) and Lb, Lp, Ll and Ef only cultures were used. According to the experimental results; It was determined that the total fatty acid level of T extracts increased in Lb, Ll and Ef groups and decreased in Ll group compared to the control, vitamin levels decreased significantly in Ef groups, and were at varying rates in Lb, Lp and Ll groups. It is thought that there was a general decrease in flavonoid and resveratrol contents of probiotic bacteria samples extracted with wheat compared to the control and that these contents were consumed by bacteria. According to these results, it was determined that the wheat used in the study activated the development of probiotic bacteria and they consumed these compounds in the environment. This is an indication that an advantage such as nutritional opportunity was provided to probiotic bacteria that are beneficial for human health. It is very important to consume these foods to help maintain the vitality of probiotic bacteria needed for a healthy life.

Keywords: *Lactobacillus bulgaricus*; *L. plantarum*; *L. lactis*; *E. faecium*; *Triticum sativum*; Probiotic bacteria; Prebiotic

1. Introduction

Probiotics are living microorganisms that have beneficial effects on health [1]. They affect human health directly and indirectly. They have important benefits in combating pathogens, protecting the immune system and intestinal epithelium [2]. Probiotics are competitive creatures, they prevent the development of pathogenic microorganisms by lowering the pH with the antimicrobial substances they produce, they do not allow pathogens to colonize by binding to intestinal epithelial cells [3,4]. In recent years, interest in probiotics has increased all over the world [5]. The presence of some microorganisms with probiotic properties is known in traditional foods such as yogurt, cheese, kefir, boza, etc. and has been consumed by people with these fermented foods for thousands of years [6-8]. Understanding the importance of probiotics led to the development of prebiotics. Prebiotics are non-digestible food substances that selectively increase the activity of a limited number of colonic bacteria and thus improve the health of the host and have a positive effect on the host. Prebiotics are naturally found in vegetables, fruits and grains such as asparagus, bananas, chicory, garlic, onions, wheat and tomatoes. Prebiotics promote the development of beneficial microflora in the intestine, have a positive effect on regular and healthy digestion, increase mineral absorption, strengthen the immune system, [8] inhibit the proliferation of pathogenic bacteria, reduce the risk of diarrhea and colon cancer, have beneficial effects on colon microflora, immune functions, mineral bioavailability, lipid metabolism and prevent colon carcinogenesis. Prebiotics bind pathogenic microorganisms to themselves and ensure their excretion in the feces [9]. Prebiotics promote the development of beneficial microorganisms (natural probiotics) in the digestive system, reducing

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the colonization of pathogenic microorganisms such as *E. coli* and *Salmonella* sp. In addition, these microorganisms synthesize vitamins, especially vitamin B, help digestion and absorption and stimulate the immune system [10]. Prebiotics, unlike probiotics, are non-living food additives and must be taken in minimum doses to be effective. The prebiotic *T. sativum* used in the study is an annual herbaceous plant species belonging to Poaceae that can grow almost everywhere in the world. It is the second most cultivated grain in the world, along with corn. It ensures regular bowel function and prevents constipation. It relieves nervous disorders and relaxes the mind. Wheat grain contains an average of 13% water, 51% starch, 9% protein, 2% fat, and 1.8% minerals. Wheat has been the most important foodstuff for humans in almost every era. It ranks first in agriculture due to its very important place in nutrition and nutritional content. Determining the phytochemical content (flavonoid, phytosterol, resveratrol) of plants used as prebiotics in foods is in favor of vitality. Because it has been stated in many studies that nutritional components have positive effects on health. This study, it was investigated how wheat, which is known to be beneficial for health and facilitates digestion, affects the development of probiotic bacteria. Some phytochemical contents of *T. sativum* extracts treated with probiotic bacteria were determined.

In this study, attention was drawn to the plant-probiotic relationship. As a result, the importance of the effect of plant nutrition on probiotic bacteria was emphasized.

2. Material and methods

The plant samples used in this study were taken from Fırat University campus, Elazig city center, placed in sterile bags and stored in a deep freezer at -20ºC until analysis The plant samples used in his study were taken from Fırat University campus, Elazig city center, placed in sterile bags and stored in a deep freezer at -20ºC until analysis

2.1. Extraction of lipids

Cell pellets whose wet weights were determined were homogenized with 3/2 (v v^{-1}) hexaneisopropanol mixture. The homogenate was centrifuged at 5000 rpm for 5 min at 4 °C and cell pellet remnant was precipitated. The supernatant was used in the vitamin and fatty acid analysis [11].

2.2. Preparation of fatty acid methyl esters

An aliquot was taken from the supernatant part of the cell pellet, 5 ml of 2% methanolic sulphuric acid was added. The mixture was vortexed and then kept at 50 ºC for 12 hours. Then it was cooled to room temperature, 5 ml of 5% sodium chloride was added and vortexed. Fatty acid methyl esters were extracted with 2x5 ml hexane. Fatty acid methyl esters were treated with 5 ml 2% KHCO3 solution and then the hexane phase was evaporated by the nitrogen flow, and then by dissolving in 1 ml fresh hexane, they were taken to auto sampler vials [12].

2.3. Gas chromatographic analysis of fatty acid methyl esters

Methyl esters were analyzed with the SHIMADZU GC 17 Ver. 3 gas chromatography (Kyoto, Japan). For this analysis, 25m of long Machery-Nagel (Germany) capillary colon with an inner diameter of 0,25µm and a thickness of 25 micron film was used. During the analysis, the colon temperature was kept at 120-220 ºC, injection temperature was kept at 240 ºC and the detector temperature was kept at 280 ºC. The colon temperature program was adjusted from 120 ºC to 220 ºC and the temperature increase was determined to be 5 ºC/min until 200 ºC and 4 ºC/min from 200 ºC to 220 ºC. It was kept at 220 ºC for 8 minutes and the total duration was set as 35 min and nitrogen gas was used as the carrier gas. During the analysis, before the analysis of fatty acid methyl esters, mixtures of standard fatty acid methyl esters were injected and the residence time of each fatty acid was determined. After this process, the necessary programming was made and the fatty acid methyl esters mixtures of the samples were analyzed [11].

2.4. HPLC analysis of adek vitamins and sterol amount

The 5 ml supernatant was taken to 25 mL tubes with caps, 5% KOH solution was added. After it was vortexed, it was kept at 85°C for 15 min. The tubes were then taken and cooled to room temperature and 5 mL of pure water was added and mixed. Lypophlic molecules that did not saponify were extracted with 2x5 mL hexane. The hexane phase was evaporated with nitrogen flow. İt was dissolved in 1 mL (50+50%, v v^{-1}) acetonitril/methanol mixture and then was taken to auto sampler vials and was analyzed. The analysis was made with the Shimadzu brand HPLC device. In the device as the pump LC-10 ADVP UV-visible, as the detector SPD-10AVP, as column oven CTO-10ASVP, as auto sampler SIL-10ADVP, as degasser unit DGU-14A and Class VP software (Shimadzu, Kyoton Japan) was used and during the mobile phase the acetonitril/methanol (60+40% v v^{-1}) mixture was used. The mobile phase flow rate was determined to be 1 mL A Uv detector was used for the analysis and as a column the Supelcosil LC 18 (15x4.6 cm, 5 µm; Sigma, USA)

column was used. For vitamin A and beta-charoten, detection of wave length 326 nm, for vitamin D and K, 265 nm, for vitamin E, 202 nm was used [12].

2.5. Statistical Analysis

The SPSS 10.0 software program was used. The comparison between experimental groups and the control group was made using ANOVA and LSD tests [11].

2.6. DPPH Radical Scavenging Activity

A methanolic solution of 25 mg/L free radical DPPH was prepared. During the tests, plant samples of 25, 50, 100 and 250 µL concentrations were added to the methanolic solution of 3.9 ml DPPH radical. The mixture was vortexed and incubated for 30 minutes at room temperature in a dark medium. The absorbance values were measured using a spectrophotometer at 517 nm against a blank [13,14]. Radical scavenging activity was calculated as a %. The DPPH radical scavenging activity was calculated using the following formula: (%)= [(Controlλ–Samplingλ)/(Controlλ)]x100

2.7. Identification of Resveratrol and Flavonoid Content

The flavonoid and resveratrol analysis was performed in an HPLC device and all procedures were performed at 25° [15].

2.8. Extraction and Analysis of Phytosterols

To the plant sample which was homogenized with hexane/isopropyl alcohol mixture (in proportion of 3/2 v/v) 5% KOH was added and it was hydrolyzed at 85ºC. The extraction was treated with n-heptane and analyzed in an HPLC device [16].

2.9. Sugar Analysis

10 g of plant was homogenized with distilled water, the pellet and supernatant sections were separated. After the volume of the total filtrate was determined, it was analyzed on the HPLC, a Shim-Pack HRC NH2 (150x4.6 mm, 5 µ) column was used. Acetonitrile+water (v/v) (75%/25%) mixture was used as mobile phase.[16].

2.10. Antimicrobial Activity

2.10.1. Test Microorganisms

In this study, 4 bacterial strains (*Bacillus megaterium* DSM 32 (Bm), *Staphylococcus aureus* COWAN 1 (Sa), *Escherichia coli* ATCC 25922 (Ec), *Klebsiella pneumoniae* FMC 5 (Kp)), 2 yeast *Candida albicans* FMC 17 (Ca) and *Candida glabrata* ATCC. 66032 (Cg)) and 2 Dermatophytes *Trichophyton* spp. (Tr) and *Epidermophyton* spp. (E) were used. The microorganisms were supplied by Microbiology Laboratory Department of Biology, Faculty of Science, Fırat University. Lb, Lp, Ll and Ef, probiotic bacterial cultures were provided from the culture collection of Anadolu University, Faculty of Arts and Sciences, Department of Biology, Microbiology Laboratory.

2.10.2. Preparation of Microorganisms Cultures and Cultivation

Bacteria strains were inoculated with a nutrient broth and were incubated at 35±1°C for 24 h. Yeast strains were incubated in Malt Extract Broth-Agar, while the dermatophyte fungi were incubated in Sabouraud Glucose Broth at 25±1°C for 48 h. The culture growing in the liquid medium was transferred to the broth tubes after performing turbidity adjustment according to a McFarland (0.5) standard tube. Müller Hinton Agar, Sabouraud Dextrose Agar and Potato Dextrose Agar which were sterilized in an erlenmayer flask and cooled to 45-50°C were inoculated with the cultures of bacteria, yeast and fungi in the broth at 1% proportion (10^6 bacteria/ml, 10^4 yeast/ml, 10^4 fungus/ml). After shaking well, a homogenous medium was obtained and 15 ml of the mixture was placed in petri dishes of 9 cm diameter. The soaked discs were placed on solidified agar and lightly pressed. The petri dishes prepared in this manner were maintained at 4°C for 1.5-2 h. The plates inoculated with bacteria were incubated at 37±1°C for 24 h; the plates inoculated with yeast and dermatophyte were incubated at 25±1°C for 3 days. At the end of these periods, the inhibition zones that formed on the medium were evaluated as mm. [17].

2.10.3. Growth of Lp and Treatment with Plant Extraction

After growing Lb, Lp, Ll and Ef in the MRS broth, and measuring it at spectrophotometer against a blank at 517 nm, it was cultivated in the environment containing the prepared minimal medium (0.019 M NaCl, 0.022 M KH_2PO_4, 0.049 M Na_2HPO_4, 0.019 M NH_4Cl, 0.002 M $MgSO_4$, 0.011 M glucose) [18] and the plant extract (the plant sample was treated

with a solvent and evaporated in evaporator, favorable growth media was prepared for the bacteria) at a level of 10^6 bacteria/ml under sterile conditions and an appropriate value was achieved. It was measured at spectrophotometer against blank at 517 nm. After incubation, the extracts which had been grown in the minimal medium were collected and measured in spectrophotometer against a blank at 517 nm for 6 h, 12 h, 24 h, 36 h, 48 h, 60 h and 72 h. After the measurements, the extracts were cultivated and incubated in MRS agar for the cell and plate count. The samples were centrifuged and pellets were collected at the point where growth was about to end. The fatty acid, vitamin, flavonoid, resveratrol and antimicrobial activities of these pellets were analyzed. The same procedures were applied to the Lb, Lp, Ll, Ef and plant (T) control group grown only in the minimal medium. In conclusion, the control (only plant) and Lb, Lp, Ll and Ef extract which was treated with plant were compared in terms of fatty acid, vitamin, flavonoid and antimicrobial activity.

3. Results and discussion

3.1. Fatty Acid Levels and Comparison of Some Probiotic Bacteria Treated with *T. sativum*

When the effect of wheat extracts on fatty acid profile was examined; it was found that the amount of palmitic acid (16:0) decreased significantly in all groups compared to the control group ($p<0.0001$). While the amount of stearic acid (18:0) was observed to increase significantly in the T+Ef group ($p<0.0001$), this increase was determined to be less in the T+Lb and T+Ll groups ($p<0.01$). It was determined that the increase in the T+Lp group compared to the control was not significant ($p<0.05$). It was found that oleic acid (18:1) level increased in T+Lb ($p<0.0001$) and T+Ef groups ($p<0.001$) compared to control, while this increase was found to be at a lesser level in T+Lp and T+Ll groups ($p<0.05$).

Table 1 Fatty acid (f.a) levels of some probiotic bacteria treated with *T. sativum* and comparison (µg/1g)

Sam.	16:00	18:00	18:1	18:2	18:3	Total µg /1g
T+Lb	78.00±7.40^{cd}	28.70±4.40^{c}	174.20±24.20^{cd}	45.65±4.95^{cd}	60.05±1.95^{d}	386.60±39^{d}
T+Lp	67.85±2.85^{cd}	19.85±4.85^{b}	34.10±21.20^{b}	11.62±5.62^{cd}	53.10±0.10^{d}	186.52±23.37^{cd}
T+Ll	151.50±1.50^{cd}	30.50±5.50^{c}	34.80±0.80^{b}	156.00±1^{cd}	83.70±1.70^{cd}	456.50±8.50^{cd}
T+Ef	104.10±4.10^{cd}	64,65±1,65^{cd}	60.87±18.52^{d}	36.60±3.60^{b}	121.05±18.05^{cd}	387.27±45.92^{d}
T	215.79±0	13,90±0	26.00±0	30.50±0	36.10±0	322.30±0
Lb	33.76±3.26^{cd}	14.20±1.90	51.10±5.10	49.43±0.31^{cd}	15.66±1.56	164.46±1.16^{cd}
Lp	20.21±0.68^{cd}	26.76±8.13	32.53±0.23	59,43±3.26	38.33±0.33	177.28±11.51^{cd}
Ll	36.30±2.10^{cd}	12.91±0.31	31.11±17.26	36.30±5.50	18.91±0.08	135.55±20.90^{cd}
Ef	42.55±1.55^{cd}	28.86±2.06	68.15±8.85^{b}	85.55±0.95^{cd}	11.66±0.33^{d}	236.78±6.51

(T+Lb: wheat+*L.bulgaricus*, T+Lp: wheat+*L.plantarum*, T+Ll: wheat+*L.lactis*, T+Ef: wheat+*E.faecium*, T: *T. sativum* (wheat): control, Lb: *L .bulgaricus*, Lp: *L.plantarum*, Ll: *L.lactis*, Ef: *E.faecium*, palmitic acid (16:0), palmitoleic acid (16:01), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), **cd: p<0.0001**, b: p<0.05, d: p<0.001 c: p<0.01 b: p<0.05).

It was observed that linoleic acid level (18:2) increased significantly in T+Ll compared to control ($p<0.0001$), there was a partial increase in T+Lb ($p<0.001$), there was a significant decrease in T+Lp group ($p<0.001$), and the increase was maintained at a very low level in T+Ef group ($p<0.05$). It was determined that the amount of linolenic acid (18:3) increased in all groups compared to the control, and this increase was significant in T+Ef and T+Ll ($p<0.0001$), and partial in T+Lb and T+Lp groups ($p<0.001$). It was observed that the total fatty acid level was the highest in the T+Ll group ($p<0.0001$), this level increased partially in the T+Lb and T+Ef groups ($p<0.001$), and decreased in the T+Lp group ($p<0.0001$). It is thought that the partial increase in the fatty acid amount and levels of the probiotic bacterial groups extracted with wheat is due to probiotic bacteria. It is thought that the existing fatty acid decreases are also caused by bacteria and are used by them, thus affecting the fatty acid profile. This is an indication that an advantage such as nutritional opportunity is provided to probiotic bacteria, which are beneficial for human health. (Table 1). It has been determined that phytosterols reduce serum cholesterol levels in humans [19-22]. It has been determined that plant sterols and stanols reduce LDL cholesterol in all individuals and total cholesterol in many individuals, and that similar properties are generally observed on HDL and triglycerides [23]. A study reported that wheat has nutritious and valuable components [24]. In a study, when the composition of wheat was examined, it was stated that it consisted of 65-75% carbohydrates, 7-18% protein, 8-14% water, 1-3% lipids and 1-2% mineral substances, enzymes and vitamins [25,26]. In a study, it was determined that the level of unsaturated fatty acids increased in sprouted buckwheat seeds,

while the amount of saturated fatty acids decreased [27,28]. It was determined that the levels of vitamins, phenolic substances, aromatic amino acids, proteins and polyunsaturated fatty acids in wheat seeds increased with germination [29,30].

3.2. Lipophilic Vitamin and Phytosterol Levels and Comparison of Some Probiotic Bacteria Treated with *T.sativum*

When the effect of wheat extracts on lipophilic vitamin and phytosterol of probiotic bacteria was examined; It was observed that the amount of δ-tocopherol increased significantly in the T+Ef group compared to the control ($p<0.0001$), decreased in the T+Lb group ($p<0.001$), and this decrease was more pronounced in the T+Lp and T+Ll groups ($p<0.0001$). It was observed that the vitamin D level increased in the T+Ef group ($p<0.0001$), this increase was significant in the T+Ll group ($p<0.001$), and was partial in the T+Lb and T+Lp groups ($p<0.01$). It was found that the amount of α-tocopherol decreased significantly in the T+Lb and T+Lp groups compared to the control ($p<0.0001$), and this decrease was partial in the T+Ll and T+Ef groups ($p<0.001$). It was determined that the amount of vitamin K_1 decreased significantly in all groups ($p<0.0001$). It was observed that the amount of vitamin K_2 increased in the T+Lb, T+Lp and T+Ll groups ($p<0.01$), and this increase was significantly increased in T+Ef ($p<0.0001$). It was found that ergosterol increased significantly in T+Ef compared to the control ($p<0.0001$), and decreased significantly in the T+Lp and T+Ll groups ($p<0.0001$), and this decrease was partial in the T+Lb group ($p<0.001$). It was determined that stigmasterol level decreased the most in T+Lp ($p<0.0001$), followed by T+Lb and T+Ll ($p<0.001$) and T+Ef ($p<0.01$). While β-sitosterol level was found to decrease significantly in T+Lb and T+Lp groups ($p<0.0001$), a partial decrease was found in T+Ll and T+Ef groups ($p<0.01$). It was observed that the amount of retinol increased significantly in T+Ll group ($p<0.0001$), this increase was evident in T+Lp ($p<0.001$) and partial in T+Lb ($p<0.01$). It was found that retinol ast increased very slightly in all groups compared to the control ($p<0.05$). (Table 2). In a study it has been stated that wheat seeds contain many minerals, vitamins and proteins as well as magnesium, zinc, calcium, selenium, sodium, potassium, phosphorus, chromium, vitamins D, A, E, B_1, B_2, B_3, B_6 and B_{12} [31].

Table 2 Lipophilic vitamin and phytosterol levels and comparison of some probiotic bacteria treated with *T. sativum* (μg/1g)

Sam.	**δ-t**	**D**	**α-t**	**K_1**	**K_2**	**E**	**S**	**β-s**	**R**	**Ra**
T+Lb	0.0233±0^{d}	0.0359±0^{c}	0.0082±0^{cd}	0.0035±0^{cd}	0.0014±0^{c}	0.0446±0^{cd}	0.0432±0^{d}	0.0988±0^{cd}	0.0299±0^{c}	0.0002±0^{b}
T+Lp	0.006±0^{cd}	0.0158±0^{c}	0.0065±0^{cd}	0.0040±0^{cd}	0.0006±0^{c}	0.0116±0^{d}	0.0305±0^{cd}	0.0876±0^{cd}	0.8775±0^{d}	0.0001±0^{b}
T+Ll	0.0074±0^{cd}	0.1555±0^{d}	0.0206±0^{d}	0.0025±0,002^{cd}	0.0012±0^{c}	0.0199±0^{cd}	0.0476±0^{d}	0.1848±0^{c}	2.9412±0^{cd}	0.0002±0^{b}
T+Ef	0.713±0^{cd}	0.3450±0^{cd}	0.0190±0^{d}	0.0061±0^{cd}	0.0290±0^{cd}	1.325±0^{cd}	1.0743±0^{c}	0.1650±0^{c}	0.0001±0	0.0002±0^{b}
T	0.0474±0	0.0034±0	1.36±0	0.46±0	-	0.56±0	6.505±0	9.16±0	-	-
Lb	0.0021±0cd	0.0021±0	0.0178±0	0.0055±0cd	0.0010±0	0.0024±0	0.0049±0cd	0.0124±0cd	0.0001±0	-
Lp	0.0018±0^{cd}	0.0144±0	0.0108±0	0.0047±0cd	0.0007±0	0.1418±0	0.0327±0cd	0.0386±0cd	0.0001±0	-
Ll	0.0002±0cd	0.0014±0	0.0331±0	0.0006±0cd	-	0.0109±0	0.0137±0cd	0.0120±0cd	-	-
Ef	0.0933±0cd	0.26±0	1.99±0	0.12±0cd	-	1.726±0	1.21±0cd	1.05±0cd	0.0733±0.0033cd	-

(T+Lb: Wheat+*L.bulgaricus*, T+Lp: Wheat+*L.plantarum*, T+Ll: Wheat+*L.lactis*, T+Ef: wheat+*E.faecium*, T: *T.sativum* (wheat): control, Lb: *L.bulgaricus*, Lp*: L .plantarum*, Ll: *L.lactis*, Ef: *E.faecium*), (cd: $p<0.0001$ d: $p<0.001$ c: $p<0.01$ b: $p<0.05$). (δ-t: δ-tocopherol, D: vitamin D, α-t: α-tocopherol, K_1: vitamin K_1, K_2: vitamin K_2, E: ergosterol, S: stigmasterol, β-s: β-sitosterol, R: retinol, Ra: retinol ast.).

According to a study, it has been stated that wheat contains vitamins A, B_1, B_2, B_{12}, E, K and D, and also that it gives strength to the body and nerves, facilitates digestion and is good for the heart [32]. It has been determined that the average phytosterol concentration in wheat is 6 mg. /100 g. They stated that the average phytosterol concentration in grains is 49 mg. /100 g. The highest of these is β-sitosterol with 62%, followed by campesterol with 21%, stigmasterol with 4%, beta-sitosterol with 4% and campestanol with 2% [23].

3.3. Comparison of Flavonoid and Resveratrol Contents of Some Probiotic Bacteria Extracts Treated with *T. sativum*

It was observed that there was no difference in the level of rutin between the groups ($p>0.05$), this level decreased compared to the control and the decrease was significant in the T+Lb, T+Lp, T+Ll and T+Ef groups ($p<0.0001$). It was determined that there was no significant difference in the amount of myricetin between the groups ($p>0.05$), this amount decreased very slightly in the T+Lb, T+Lp and T+Ll groups compared to the control ($p<0.05$). It was observed that the levels of morin, quercetin, catechin, naringin and resveratrol generally did not differ significantly among all groups ($p>0.05$). Kaempferol levels were found to decrease in the T+Lb, T+Lp, T+Ll and T+Ef groups ($p<0.01$). On average, it was found that naringenin levels were partially decreased in T+Lb, T+Lp, T+Ll and T+Ef groups compared to the control ($p<0.05$). In general, it is thought that there is a decrease in flavonoid and resveratrol contents of probiotic bacterial samples extracted with wheat compared to the control and that these contents are consumed by the bacteria. According to these results, it was determined that the wheat used in the study activated the development of probiotic bacteria and consumed these compounds in the environment (Figure 1). It was detect that soft wheat extracts had higher levels of total phenolic compounds and total antioxidant activities than hard extracts [32]. Wheat contains phytochemicals such as phenolics, carotenoids, lignans and vitamin E, which have beneficial effects on health. [33]. When buckwheat seeds are germinated, it has been observed that flavonols such as anthocyanins, rutin, quercetin, orientin, isoorientin, vitexin and isovitexin, which give these properties to them, increase significantly [28].

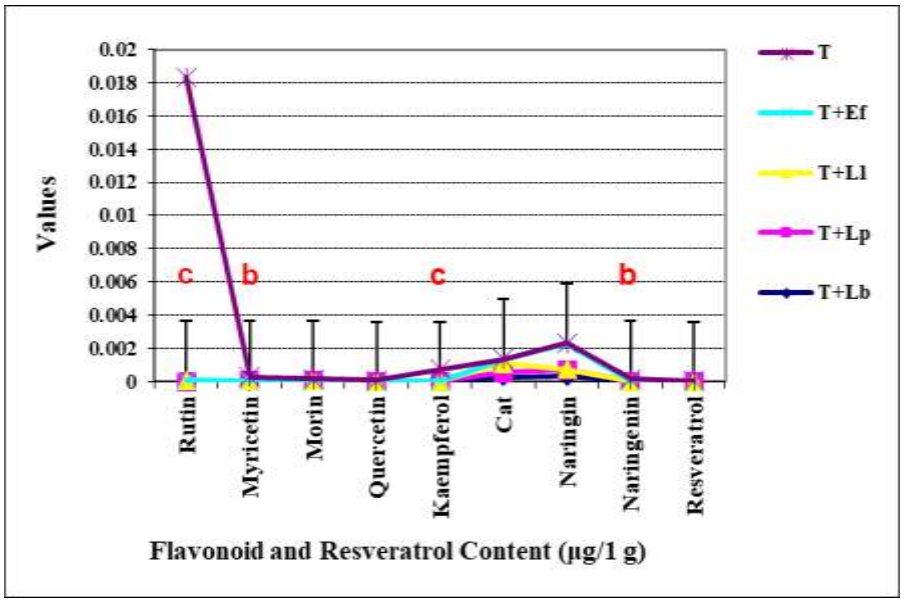

Figure 1 Comparison of flavonoid and resveratrol contents of some probiotic bacterial extracts treated with T (wheat). (T+Lb: wheat+*L.bulgaricus*, T+Lp: wheat+*L.plantarum*, T+Ll: wheat+*L.lactis*, T+Ef: wheat+*E.faecium*, T: *T.sativum* (wheat): control, (b:$p<0.05$, c: $p<0.01$, cd: $p<0.0001$)

3.4. Antimicrobial Activities and Comparison of Flavonoid Contents of Some Probiotic Bacteria Extracts Treated with *T. sativum*

When the antimicrobial activities of probiotic bacteria extracts treated with plants prepared for flavonoid analysis were examined; it was determined that the T+Lb group had an effect on Ca and E with a 10 mm inhibition diameter, the least effect was on the Kp and Cg group (8 mm), T+Lp had the most effect on Ca (16 mm), T+Ll had an effect on Ca (12 mm), E (11 mm) and Tr (8 mm), but did not have any effect on other groups, T+Ef showed activity against Ec and E groups (10 mm), and T from the control group had the most effect on Ca (10 mm) (Figure 2).

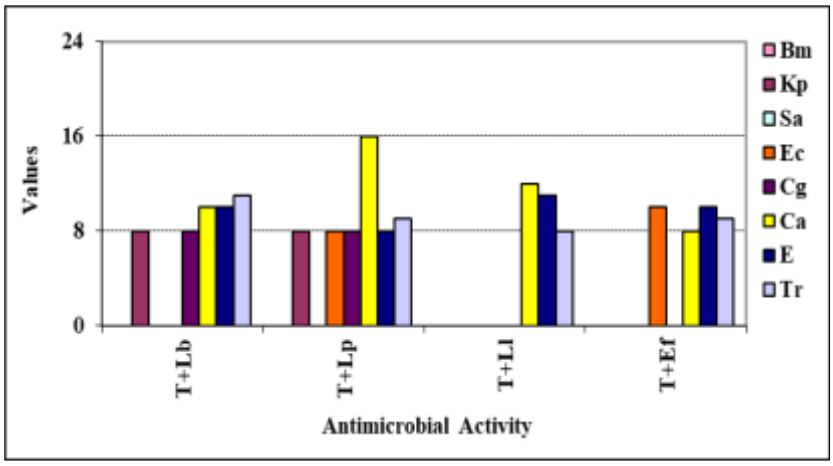

Figure 2 Antimicrobial activities of flavonoid contents of some probiotic bacterial extracts treated with plant groups used in the study (mm).

When the results were evaluated, it was thought that the effects of probiotic bacteria extracted with plants on some pathogens had uncontrolled activity, and that the reason for this was that probiotic bacteria better released the compounds in the environment in samples extracted in methanol and prepared for flavonoid analysis, and that these compounds affected pathogenic bacteria, yeast and fungi. In other words, it was determined that probiotic bacteria acted as catalysts here. On the other hand, the antimicrobial activity present in the control was not observed in some probiotic bacteria samples extracted with plants, and that the reason for this was thought to be related to the consumption of flavonoids in the environment by these bacteria and the consumption of these contents that may have an effect on pathogenic bacteria. According to these results, it was seen that all studies and study groups related to flavonoid and resveratrol analyses in the experimental process of our study were consistent and supportive of each other as a result of comparisons.

3.5. Antimicrobial Activities and Comparison of Fatty Acid Contents of Some Probiotic Bacteria Extracts Treated with Plants

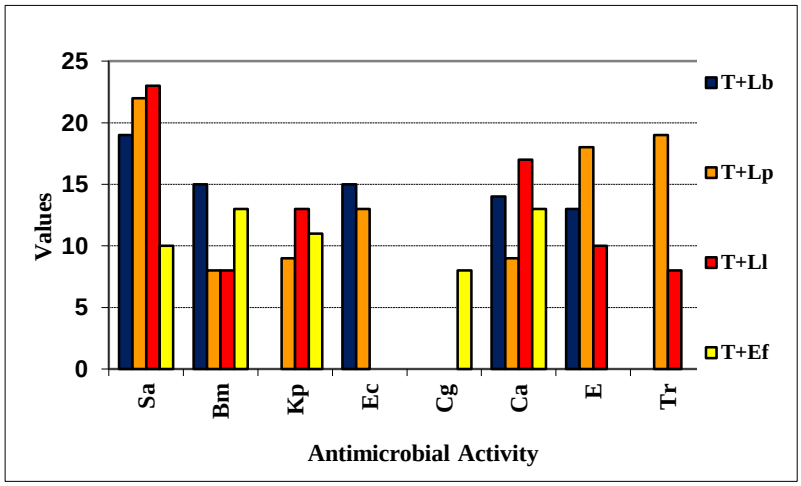

Figure 3 Antimicrobial activities (mm) of some probiotic bacteria fatty acid contents treated with the plants used in the study.

When the antimicrobial activities of probiotic bacteria extracts treated with plants prepared for fatty acid analysis were examined; It was determined that T+Lb group had an effect on Sa with an inhibition diameter of 19 mm, the least effect was on E group (13 mm), T+Lp had the most effect on Sa (22 mm), T+Ll had an effect on Sa (23 mm), Ca (17 mm), Kp (13 mm), E (10 mm), Bm and Tr (8 mm), and other than that, it did not have any effect on other groups, and T+Ef had the most activity against Ca group (13 mm). It was determined that T was generally effective on all plant groups except

Kp, and this effect was the most on E (25 mm). As a result, it was determined that the antimicrobial activities of the plant fatty acids in the control group had a high level of effect on pathogens, especially on dermophyte fungi and yeasts. It was observed that the probiotic bacterial samples extracted with plants were higher in some groups compared to the control, and had no effect in others. The reason for the high antimicrobial activity is thought to be that the plant fatty acid levels activate bacterial growth and increase the fatty acid levels in the environment, or that these compounds have a better effect on pathogens by better decomposing the fatty acid compounds in the environment. This study, it is thought that the probiotic bacteria are better decomposers than the chemicals used in the preparation of the control group, and thanks to this feature, they provide better release of the compounds in the environment. As a result of the study, it was proven that probiotic bacteria (probiotic bacteria) prepared for fatty acid analysis and not extracted with plants had an effect on pathogenic bacteria. However, it was generally seen that they had less activity than the samples extracted with plants. Thus, it was determined that the effect of the plants used in the study on the development of probiotic bacteria was significant. This effect is thought to be quite beneficial for health (Figure 3).

3.6. Antimicrobial Activities and Comparison of Vitamin Contents of Some Probiotic Bacteria Extracts Treated with Plants

When the antimicrobial activities of the probiotic bacteria extracts treated with plants prepared for vitamin analysis were examined; it was determined that the T+Lb group had an inhibition diameter of 22 mm on E, the least effect was on the Kp and Bm groups (8 mm), and T+Lp had the most effect on E (21 mm). It was determined that T+Ll had an effect against E (22 mm), T (20 mm), Cg (14 mm), Sa and Ca (12 mm), and that it had an effect on the other groups at the level of 8 mm. While it was observed that T+Ef showed the best activity against the E group (18 mm), it was determined that T showed activity on all groups except Sa, especially on dermophyte fungi and yeasts (Figure 4). As a result, it was determined that the antimicrobial activities of the plant vitamins in the control group were highly effective on pathogens, especially on dermophyte fungi and yeasts. It was observed that the probiotic bacteria samples extracted with plants were also high in some groups compared to the control, but decreased in some groups. The reason for the high antimicrobial activity in some groups is thought to be due to the fact that the plant vitamin levels activate bacterial growth and increase the vitamin levels in the environment, or that these compounds have a better effect on pathogens by better separating the vitamin and phytosterol compounds in the environment. As a result of the study, it was proven that the probiotic bacteria from the control group, which was prepared for vitamin analysis and not extracted with plants, also had an effect on pathogenic bacteria. However, it was generally seen that they had less activity than the samples extracted with plants. Thus, it was determined that the plants used in the study had significant effects on probiotic bacteria and had a positive effect on their development.

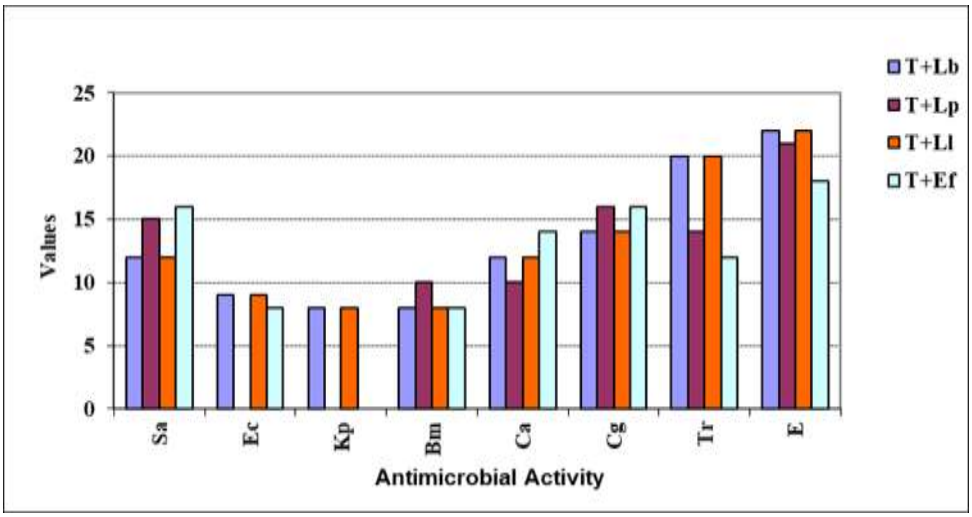

Figure 4 Antimicrobial activities of some probiotic bacteria vitamin contents treated with the plants used in the study (mm).

In a study investigating the antibacterial activity of wheat seeds against *S. aureus* and *E. coli* bacteria that cause contamination in the cosmetics and food industry, it was stated that wheat has the potential to be used as a natural antimicrobial against *S. aureus* and *E. coli* species [34]. It was determined that chloroform extracts of wheat germ had no antibacterial effect against Staphylococcus aureus and the highest effect was on *E.coli*. [35]. Another study determined that wheat essential oils showed low antimicrobial activity against the tested microorganisms [36]. It is stated that buckwheat seeds have antioxidant, antimutagenic, anticarcinogenic and antimicrobial properties that are

important components for protection against diseases [28]. When buckwheat seeds are germinated, it has been observed that flavonols such as anthocyanins, rutin, quercetin, orientin, isoorientin, vitexin and isovitexin, which are thought to provide antimicrobial properties, increase significantly [28].

3.7. Free Radical (DPPH) Neutralization Activity

It was determined that T. showed DPPH scavenging activity at 100 µl maximum and 25 µl minimum concentrations. T. was found to be most effective at 100 µl maximum concentration. It was observed that the wheat used in the study had DPPH scavenging activity. Studies have shown that plant phenols have the ability to inhibit and neutralize superoxide, alkyl and hydroxyl free radicals that damage DNA and other cell components [37]. Studies have focused on foods containing phenolic substances and antioxidants that reduce the risk of contracting diseases and protect human health. It has been stated that wheat, among these foods, is a natural nutritional antioxidant source due to the phytochemicals it contains and prevents many chronic diseases in humans and animals [38]. In the analyses of antioxidant activities of bread wheat, it was determined that varieties showed significant differences between 11.89% and 26.33% in terms of antioxidant activity [39]. In a study, it was stated that wheat is a natural nutritional antioxidant source due to the phytochemicals it contains [38].

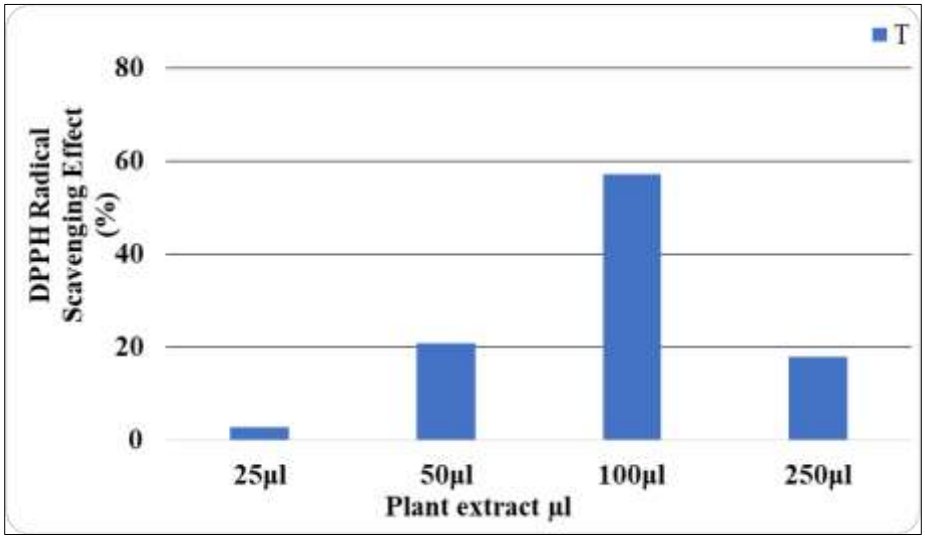

Figure 5 DPPH radical scavenging activity T (µl).

3.8. Sugar contents

When wheat was examined in terms of sugar content, it was found that fructose in T. were at significant levels, and sucrose and maltose were at partial levels (p<0,0001). In a study conducted in buckwheat seeds, it was observed that the rate of disaccharides (such as maltose and sucrose) decreased as a result of germination, while the level of fructose and glucose increased [27,28].

Table 3 Sugar contents of wheat (T) extracts used in the study (µg /1 g)

Sam.	Glucose	Fructose	Arabinose	Sucrose	Maltose
T	0,015±0,00	0,0115±0,00	0,001±0,00	0,076±0,00	0,0599±0,00

(cd: p<0,0001)

4. Conclusion

As a result, it was determined that wheat was effective on Lb, Lp, Ll and Ef bacteria development as a result of the parameters used in this study. Before moving on to the disease and treatment stage, using these plants used as prebiotics is very important in the disease protection stage. Consuming such plants for health will provide us with both material and spiritual gains. However, there is still a need for studies to be investigated, tested and answered in this field. We believe that this study will guide the research that can be done.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declared no conflict of interest.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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