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PHENOLIC CONTENT, ANTIOXIDANT ACTIVITIES AND STIMULATORY ROLES OF *CITRUS* FRUITS ON SOME LACTIC ACID BACTERIA

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Abstract: In this study, phenolic compounds and antioxidant activities in citrus fruits and their peels were determined, and their stimulatory roles on some lactic acid bacteria were investigated. Phenolic compounds in citrus fruits such as mandarin, lemon, orange and grapefruit were determined either in the juices or in the peel extracts. Total phenolic content was determined in a spectrophotometer at 685 nm using the adapted Folin-Ciocalteu method. Total flavonoid content was measured using LC/MS (liquid chromatography-mass spectrometry). The effects of the fruit juices and peel extracts on the selected lactic acid bacteria (*Lactobacillus delbrueckii* NRRL B5448, *Lb. casei* NRRL B1922, *Lb. acidophilus* NRRL B4495) were investigated. The tested lactic acid bacteria were significantly affected by chlorogenic acid, hesperidin, naringin and caffeic acid compared to the control samples ($P \leq 0.05$). Antioxidant properties of fruit samples were also measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The phenolics positively affected the metabolism of bacteria, with the stimulatory effects of the assayed samples being influenced by the phenolic profile.

Key words: Citrus fruits; flavonoid content; phenolic compounds; antioxidant; lactic acid bacteria

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INTRODUCTION

The significance and popularity of phenolic compounds in terms of health benefits and nutrition has increased in the last decade. The antioxidant properties of phenolic compounds, their abundance in diet and their probable role in the prevention of the various diseases associated with oxidative stress such as cancer, cardiovascular and degenerative diseases, are the main reasons for their popularity (Manach et al., 2004; Lau et al., 2006).

Phenolic compounds can be categorized into different groups as a function of the phenol rings. These are phenolic acids (benzoic or hydroxycinnamic acid derivatives), flavonoids, stilbenes and lignans. The flavonoids can be classified as flavonols, flavones, isoflavones, flavanones, anthocyanidins and flavanols (catechins and proanthocyanidins). Furthermore, polyphenols may be associated with various carbohydrates and organic acids (Lau et al., 2006; Rodriguez et al., 2009).

The presence of phenolic compounds in the diet is crucial in terms of health due to their chemopreventive effects on carcinogenesis and mutagenesis. On the other hand, the health effects of phenolic compounds depend on the amount consumed and on their bioavailability. In addition to their nutritional and antioxidant properties, phenolic compounds influence the organoleptic properties in foods, such as taste, flavor and color. Volatile phenols can also be produced by the metabolism of microorganisms such

as yeast and lactic acid bacteria (LAB) (Rodriguez et al., 2009; Shen et al., 2007).

The effects of phenolic compounds on the growth of Gram-negative and/or pathogenic bacteria have been widely investigated in the last few years (Puupponen-Pimia et al., 2005; Smith et al., 2005; Rodriguez et al., 2007; Vattem et al., 2005; Almajano et al., 2008). However, the impact of phenolic compounds on probiotic and lactic acid bacteria continues to be researched (Hervert-Hernandez et al., 2009).

The effect of phenolic compounds on bacterial growth depends on the microbial strain, polyphenol type and concentration tested (Almajano et al., 2008; Hervert-Hernandez et al., 2009). The bacteria belonging to *Lactobacillus* and *Bifidobacterium* genera are the most popular probiotic microorganisms used in food production. Although the antimicrobial activity of polyphenols is scientifically clear, the stimulating effects of phenolic compounds on probiotic and lactic acid bacteria needs to be further investigated (Vattem et al., 2005; Hervert-Hernandez et al., 2009).

In this study, the phenolic content in certain citrus fruits was determined. The flavonoid constituents of the fruit juices and peel extracts were identified by LC/MS. The effects of the extracts and the fruit juices on microbial growth of selected lactic acid and probiotic bacteria were tested and compared. The antioxidant properties of these fruit extracts and the juice samples were also measured to determine the potential health-benefits of the fruits.

MATERIALS AND METHODS

Chemical analysis

Preparation of juice sample

Samples of mandarin (*Citrus reticulata*), orange (*Citrus sinensis*), grapefruit (*Citrus grandis*) and lemon (*Citrus limonum*) were bought from a supermarket in the Balikesir region. The juice from the citrus fruits was extracted by cutting the fruit in half and hand-squeezing with a kitchen juicer. The juices were passed through a filter to remove the pulp and seeds and then centrifuged at 4000 rpm for 20 min in a laboratory centrifuge (Sigma 3K30, UK). After that, they were filtered through 0.45-μm pore-size membrane filters (Merck, Millex, syringe driven filter unit) and kept at -18˚C until analysis (Kelebek et al., 2008; Kelebek, 2010).

Preparation of peel samples and extraction

Extracts of citrus fruit peel samples were prepared according to the methods used by Diken (2009), Ramful et al. (2010) and Ramful et al. (2011). The citrus fruits were washed with tap water, and the peel removed with a manual peeler and cut into small pieces. The samples were then dried at 45˚C in a dry-heat oven (Nuve FN300, TR), ground into a fine powder in a shredder and stored at -18°C. The dried and frozen powders were vortexed and extracted in 80% aqueous methanol (v/v) at 4˚C for 1 night. They were then centrifuged at 4500 rpm for 15 min (Sigma 3K30, UK); supernatants of all extractions were pooled and stored at -20˚C. In order to determine total phenol, total flavonoids and for the purpose of antioxidant assays, a 5-mL 80% (v/v) concentration of methanol was added to the residues; this process was repeated twice and finally 2 mL of 80% (v/v) methanol was added to the extracts and 12 mL of total supernatants were analyzed. The pH of the citrus fruit extracts were determined with a Sartorious PT15 device (Goettingen, Germany).

Total phenolic determination

The total amount of phenolics in the extracts was determined according to the Folin-Ciocalteu modified procedure (Ramful et al., 2011). Briefly, 0.25 mL of diluted extract and 3.5 mL water were mixed with 0.25 mL of Folin-Ciocalteu's phenol reagent; after 3 min, 1 mL of 20% (w/v) sodium carbonate was added and the mixture was vortexed. A blank reagent was prepared using 0.25 mL 80% (v/v) methanol. After 40 min at 40˚C, absorbance was measured at 685 nm with a Perkin Elmer Lambda 35 spectrophotometer (US). Phenol content was estimated from a standard

curve of gallic acid and results were expressed as mg gallic acid equivalents (GAE) 100 g^{-1} dry weight (dw) of citrus materials.

Total flavonoid content

Total flavonoids were measured using a colorimetric assay according to Ramful et al. (2011): 150 µL of 5% aqueous $NaNO_2$ (w/v) was added to an aliquot (2.5 mL) of each citrus extract and the mixtures were vortexed. A reagent blank using 80% aqueous methanol (v/v) instead of sample was prepared. After 5 min, 150 µL of 10% aqueous $AlCl_3$ was added. One mL of 1 M NaOH was added 1 min after the addition of aluminum chloride. The solution was mixed and absorbance was measured against the blank at 510 nm with a Perkin Elmer Lambda 35 spectrophotometer (US). Total flavonoids were calculated with respect to the quercetin standard curve (concentration range: 10-600 µg/mL). Results are expressed in µg of quercetin/g of citrus material.

Antioxidant activity determination

The antioxidant capacity of the samples was determined spectrophotometrically by measuring the neutralization level of DPPH (2,2-diphenyl-1-picrylhydrazyl), which is a strong free radical. The extracts, which had previously been prepared according to defined instructions, were used to determine the DPPH radical scavenging activity of the citrus fruit samples (Benavente-Garcia et al., 2000; Sevim et al., 2013). To 250 µL of extract, 2500 mL DPPH and 2500 mL methanol (100 %) solutions were added and this was stored in darkness for 1 h before reading at 515 nm absorbance value. Only methanol and DPPH were used to measure the control solution (Ramful and Tarnus, 2011). The antioxidant or DPPH radical scavenging activity of the samples was determined using the absorbance values measured and the formulae below (Al-Saikhan et al., 1995).

$$AA\% = \frac{R_{control} - R_{sample}}{R_{control}} \times 100$$

where $R_{control}$ and R_{sample} refer to different citrus samples.

Chromatographic analysis
Sample preparation for chromatographic analysis

Twelve mL of 80% methanol (v/v) was added to 1 g of citrus fruit peel extracts and citrus juice extracts filtered through a 0.45-µm pore-size membrane filter before injection.

Chromatographic analysis of citrus juices

For determining the phenolic compounds in citrus juices, Agilent Technology 6130 Quadrupole LC/MS equipment was used (Kelebek et al., 2008; Kelebek, 2010). Samples were filtered through a 0.45-µm membrane filter and passed through a C18 column. The mobile phase consisted of two solvents: solvent A – 10 mM ammonium acetate/0.2% formic acid (v/v), and solvent B – methanol/solvent A (% A/B; 90/10). Chlorogenic acid, gallic acid (Fluka, US), naringin (Sigma-Aldrich, US), caffeic acid (Sigma-Aldrich, US), hesperidin (Sigma-Aldrich, US), p-coumaric acid (Fluka Sigma Aldrich, US), rutin hydrate (Sigma Aldrich, US), neohesperidin (Sigma Aldrich, US) and quercetin (Sigma Aldrich, US) were used as standards in the study. The chromatographic peak corresponding to each phenolic compound was identified by comparing retention time with that of the standard. A calibration curve was prepared using standards to determine the relationships between peak areas and concentrations.

Chromatographic analysis of citrus peels

Samples were filtered through a 0.45-µm membrane filter and injected into the Agilent Technology 6130 Quadrupole LC/MS equipment under the above conditions. Water-acetonitrile (70-30% v/v) was used as the solvent. The chromatographic peak corresponding to each phenolic compound was identified by comparing retention time with that of the standard. A calibration curve was prepared using standards to determine the relationship between peak area and concentration.

Microbial analysis

Halkman (2005) and Hervert-Hernandez and Goñi (2011) assays were modified and used in the study.

Growth of lactic acid bacteria

Lyophilized lactic acid bacteria cultures *Lactobacillus acidophilus* NRRL B 4495, *Lb. casei* NRRL B 1922 and *Lb. delbrueckii* NRRL B 548 were obtained from the US Department of Agricultural Research Service. Microorganisms were activated in liver infusion broth at 28˚C (*Lb. casei* NRRL B 1922) and 37˚C (*Lactobacillus acidophilus* NRRL B 4495, *Lb. delbrueckii* NRRL B 548) for 2 days. The activated microorganism culture contained about 6.5-8.5 log CFU/mL.

Analysis of microbial growth

Citrus fruit juices and extracts of citrus peel were filtered through a 0.45-μm pore-size membrane and their effects on lactic acid bacteria were determined.

Agar diffusion test

Sterile empty 6 mm paper disks were used to screen microbial growth activity of citrus peel extracts and juices. For this purpose, activated LAB cultures were inoculated on de Man Rogosa and Sharp (MRS) agar (Merck) in Petri dishes. Sterile paper disks on inoculated MRS Petri dishes were soaked with sterilized citrus juice and citrus peel extracts. The Petri dishes were incubated at 37˚C for 48 h under anaerobic conditions. The zones of growth or inhibition surrounding the disks on each MRS plate were determined at the end of the incubation period. For the controls included in the assay, citrus juice and peel extracts were replaced by sterile water and methanol, respectively. Positive activity was defined as an intense growth zone of microorganism >10 mm surrounding a disk, and negative activity was defined as an inhibition zone >10 mm surrounding a disk (Irkin et al., 2010).

Growth in broth

One hundred μL of sterile citrus juice and peel extracts were put into the 9-mL MRS broth-containing tubes (Merck) and 1 mL of LAB culture was added and mixed. Then all the tubes were incubated at 37°C for 48 h. In control tubes, 1 mL of LAB culture was added to the sterilized 5 mL of MRS broth. All the tests were done in triplicate (Abbasoğlu, 1996). Microorganism intensity was determined using a densitometer (DEN-1, UK); then 1 mL of sample from the tubes was added to 9 mL of sterile saline peptone water (0.1% peptone and 0.85% NaCl) and mixed serial decimal dilutions were prepared; LAB counts were determined using MRS agar at 35°C for 48 h.

Statistical analysis of data

Three replicate trials were conducted for each duplicate experiment. Data were subjected to analysis of variance (ANOVA) and Duncan's multiple tests, as well as general multivariate analysis tests SPSS 16.0 (SPSS Inc., Chicago, IL, USA) to determine if there were significant differences ($p < 0.05$) in populations.

RESULTS AND DISCUSSION

According to the pH analysis of the citrus fruit juice and fruit peel extracts, the lowest pH values were obtained from the lemon and grapefruit juices, and the highest pH values from the peel extracts of orange and mandarin (Table 1).

The total concentrations of phenolic compounds obtained were, from highest to lowest, from grape-

Table 1. pH values of fruit juices and fruit peel extracts ±S.D (n=3).

Sample	pH
Orange juice	3.60±1.9
Mandarin juice	3.21±1.4
Grapefruit juice	3.00±2.5
Lemon juice	2.34±1.8
Orange peel extract	6.62±2.2
Mandarin peel extract	6.58±3.4
Grapefruit peel extract	6.45±3.2
Lemon peel extract	6.23±2.1

fruit, mandarin, lemon and orange juices, and from the grapefruit, orange, mandarin and lemon peels (Table 2). The concentration of total phenolic compounds measured in mandarin samples was 47.1-78.7 mg/g GAE in research conducted by Ye et al., (2011). However, in our research the total phenolic compound levels found in the mandarin samples was found to be higher than these values. This was probably the result of plant variety and planting conditions of the fruit used in our research. In other research carried out by Kelebek (2010) in Turkey, the concentration of total phenolic compounds was found to be similar to our research and ranged from 441-725 mg/l.

In our research, the total flavonoid content, from highest to lowest, was obtained from the grapefruit, mandarin, orange and lemon juices and from the lemon, grapefruit, orange and mandarin peels (Table 2).

The antioxidant activities of citrus samples, from highest to lowest, were obtained from the orange, lemon, grapefruit and mandarin juices and from the grapefruit, lemon, mandarin and orange peels (Table 2). We found no correlation between antioxidant activity and the levels of total phenolic compounds and flavonoids in the citrus samples. Gardner et al. (2000) found 66% and 81% antioxidant activity in orange and Jaffa orange juices, respectively. Similarly, they did not observe any correlation between antioxidant activity and the phenolic compounds identified in the extracts.

Zulueta et al. (2007) determined that orange juice is rich in phenolic compounds (naringin, hesperidin) and consequently has a high antioxidant capacity. They also showed that lemon juice had a greater antioxidant capacity than other citrus fruits. Tripoli et al. (2007) determined that the antioxidant capacities of orange juices seem to be widely influenced by anthocyanin concentrations.

Majo et al. (2005) showed that some phenolic compounds demonstrate antioxidant activity. They proved hesperidin has a higher antioxidant activity than neohesperidin. They suggested it could be caused by the lack of hydroxyl group in the 7th position. According to our research, orange juice, lemon juice and their peels contained the highest levels of hesperidin; this could be related to their high antioxidant capacities (Table 3).

Some examples of chromatographic diagrams related to the grapefruit juice and the orange peels are given in Figs 1 and 2. In Table 3, the phenolic compound concentrations in citrus fruits determined from the chromatographic peak values are given as ppm. Gallic acid, p-coumaric acid and quercetin were not found in either the citrus juices or citrus peels used in our research.

Chlorogenic acid, from highest to lowest, was determined as 2.145, 1.950, 0.698, 0.415 and 0.225 ppm in the peels of grapefruit, orange, lemon, mandarin and grapefruit juice, respectively. Chlorogenic acid content in the Dörtyol-Hatay variety was determined as 16.27 mg/ l in research carried out on phenolic constituents of orange by Kelebek and Selli (2011). The differences observed between the studies may be due to the variety used, and the planting and climatic conditions.

Table 2. Total phenolic (mg gallic acid eq. (GAE)/L±S.D.), total flavonoid compounds (mg quercetin/L±S.D.) and the antioxidant activities (AA%±S.D.) determined in the citrus fruit juices and their peel extracts (n=3).

Sample	Content of total phenolic compounds (mg GAE/L and mg GAE/ g)	Total Flavonoid Content (mg quercetin/L) and (mg quercetin/g)	AA%
Grapefruit juice	657.65± 69.20	2.30± 11.4	42.99± 12.1
Mandarin juice	636.73± 68.74	1.69± 4.60	30.57± 2.4
Lemon juice	579.41± 91.14	0.34± 3.37	58.31± 9.6
Orange juice	523.44± 87.20	1.50± 7.18	77.15± 5.2
Grapefruit peel	13.71± 9.70	6.43± 6.60	54.48± 7.2
Orange peel	11.08± 9.55	5.18± 2.85	24.99± 3.7
Mandarin peel	9.31± 12.43	4.27± 1.51	36.12± 1.8
Lemon peel	5.35± 5.81	8.63± 7.18	43.60± 2.5

Table 3. Concentrations of phenolic compounds in citrus samples determined by chromatographic analysis (ppm±S.D.) (n=3); - sign: not determined.

Samples	Gallic acid	Chlorogenic acid	Caffeic acid	Coumaric acid	Naringin	Rutin hydrate	Hesperidin	Neohesperidin	Quercetin
Grapefruit juice	-	0.225± 7.2	-	-	87.690± 6.1	-	3.809± 9.3	5.318± 2.3	-
Grapefruit peel	-	2.145± 4.6	0.166± 3.3	-	577.351± 7.3	-	-	-	-
Lemon juice	-	-	0.040± 0.6	-	-	3.72± 5.4	50.623± 5.4	-	-
Lemon peel	-	0.698± 1.2	-	-	-	-	79.003± 1.5	-	-
Mandarin juice	-	-	-	-	-	-	81.674± 1.8	-	-
Mandarin peel	-	0.415± 6.3	-	-	-	-	-	-	-
Orange juice	-	-	0.070± 2.3	-	-	-	133.180± 2.2	-	-
Orange peel	-	1.950± 2.8	0.190± 8.4	-	0.982± 1.7	-	170.044± 2.4	-	-

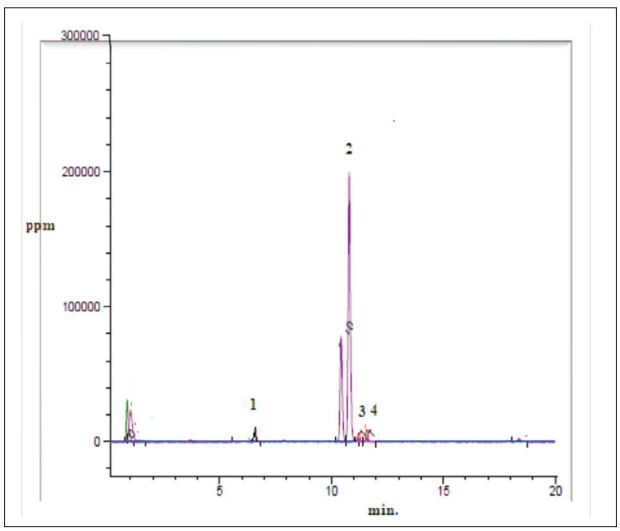

Fig.1. Concentrations (ppm) of phenolic compounds in grapefruit juice sample determined by LC/MS chromatographic analysis. (1: chlorogenic acid; 2: naringin; 3: hesperidin; 4: neohesperidin)

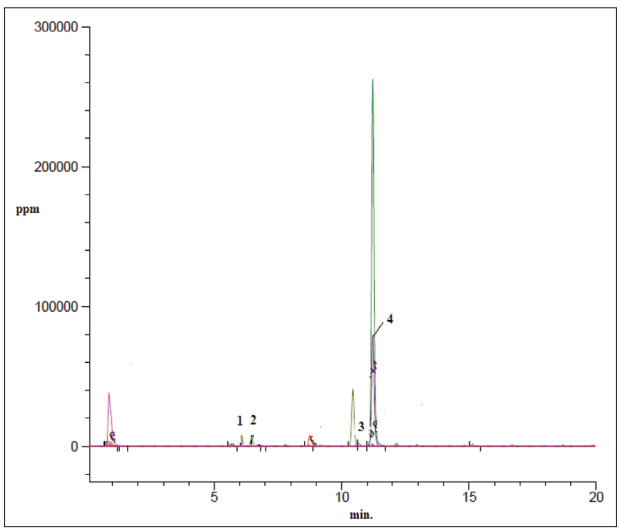

Fig. 2. Concentrations (ppm) of phenolic compounds in orange peel sample determined by LC/MS chromatographic analysis. (1: chlorogenic acid; 2: caffeic acid; 3: naringin; 4: hesperidin)

Caffeic acid contents were found to be 0.19, 0.166, 0.07 and 0.04 ppm in orange and grapefruit peels, and orange and lemon juice, respectively.

The caffeic acid content in the orange varieties Moro and Sanguinello was reported as 5.23-6.79 mg/l by Kelebek et al. (2008) and was much higher than the levels found in our research.

Naringin, which is one of the common compounds found in citrus fruits, was determined as 577.351, 87.69 and 0.982 ppm in grapefruit peel, its juice, and orange peel, respectively. In parallel to our research, the level of naringin was found to be 0.95 mg/l in the Dörtyol-Hatay variety of orange by Kelebek and Selli (2011).

In research conducted by He et al. (2011), the naringin content was determined as 53.8-627.2 ppm in citrus pulp and 53.9-1588 ppm in citrus peel. The presence of naringin was not established in some samples.

Similarly, the naringin content in a Turkish orange variety was measured as 270.21-464.13 mg/l in research performed by Kelebek (2010). The naringin content of grapefruit peel used in our research was higher.

Rutin hydrate in lemon juice was determined as 3.72 ppm and not detected in the other citrus samples. While hesperidin, which is one of the most common phenolic constituents found in citrus fruits, was not found in grapefruit and mandarin peels, it was found at concentrations of 170.044, 133.18, 81.674, 79.003, 50.623 and 3.809 ppm in orange peel, orange juice, mandarin juice, lemon peel, lemon and grapefruit juice, respectively.

Hesperidin was measured as 108.9-978.7 ppm in the pulp and 837-7995 ppm in the peel by He et al. (2011), while Kelebek and Selli (2011) found the content of hesperidin to be 104.21 mg/l in the Dörtyol orange variety. In mandarin samples, Ye et al. (2011) determined the hesperidin content as 142-261 mg/g in their research. The hesperidin content was 137.2 mg/L in the freshly squeezed fruit juice and 955.6 mg/kg in the peel in the paper of Stuetz et al. (2010) who examined citrus fruits. Hesperidin content was much higher in their research than in ours. In the study performed by Kelebek et al. (2008), hesperidin levels ranged between 112.98 and 143.20 mg/l in the juices of the Moro and Sanguinello orange varieties, their results being very close to ours.

Neohesperidin in our research was only determined in grapefruit juice as 5.318 ppm. Neohesperidin levels were determined as 3.1-130.5 ppm by He et al. (2011) and 1.44 mg/l in Dörtyol orange by Kelebek and Selli (2011). Kelebek (2010) measured the neohesperidin content as 14.72-24.24 mg/l in the *Citrus paradisi* variety of grapefruit.

Table 4. Effects of citrus samples on the growth of lactic acid bacteria with agar diffusion test method.

Samples	*Lb. delbrueckii* NRRL B5448	*Lb. casei* NRRL B1922	*Lb. acidophilus* NRRL B4495
Grapefruit juice	-	-	+
Grapefruit peel	+	++	++
Lemon juice	-	-	+
Lemon peel	++	++	++
Mandarin juice	+	+	++
Mandarin peel	++	+	+
Orange juice	++	++	++
Orange peel	++	++	++

++: high stimulatory effect; +: medium stimulatory effect; -: inhibitory effect

The LAB grown on the Petri plates were counted. The stimulatory and inhibition effects of citrus samples observed on MRS agar with the agar diffusion method (Table 4) and the numbers of LAB are given in Table 5.

The microbiological results in Table 4 show that *Lb. delbrueckii* NRRL B5448 was inhibited by lemon and grapefruit juices, but its development was supported by lemon peel, orange juice and its peel, and mandarin peel.

Table 5. Growth of lactic acid bacteria in broth medium with/without the citrus samples (MacFarland Turbidity value (McF) and log CFU/mL±S.D.).

Samples	*Lb. delbrueckii* NRRL B5448			*Lb. casei NRRL* B1922			*Lb. acidophilus* NRRL B4495		
	Before the incubation McF	After the incubation McF	M.org numbers after the incubation log CFU/mL	Before the incubation McF	After the incubation McF	M.org numbers after the incubation log CFU/mL	Before the incubation McF	After the incubation McF	M.org numbers after the incubation log CFU/mL
Grapefruit juice	2.3±1.4	9.9±1.0	10.32±2.3^{a*}	2.3±1.2	10±1.4	10.04±2.3^{a}	2.2±1.0	10±1.0	10.32±3.1^{a}
Grapefruit peel	2.6±1.3	10±1.1	10.25±2.7^{a}	2.2±1.7	11±1.0	11.20±2.9^{c}	2.5±1.1	11±1.1	11.58±4.3^{b}
Lemon juice	2.7±1.2	9.8±1.1	9.07±2.5^{b}	2.4±1.9	9.9±1.1	9.11±1.8^{a}	2.3±1.4	10±1.0	10.20±2.1^{a}
Lemon peel	2.6±1.8	10±1.0	10.53±3.4^{c}	2.4±1.3	11±1.0	11.57±3.1^{c}	2.6±1.3	11±1.0	11.34±3.2^{c}
Mandarin juice	2.5±1.2	10±1.0	10.44±2.2^{a}	2.2±1.5	10±1.1	10.27±3.7^{b}	2.2±1.0	11±1.0	11.53±5.1^{b}
Mandarin peel	2.4±1.7	10±1.0	10.62±2.3^{c}	2.3±1.9	10±1.0	10.34±1.9^{b}	2.2±1.0	10±1.0	10.39±3.4^{a}
Orange juice	2.6±1.0	10±1.0	10.54±1.9^{c}	2.2±1.3	11±1.0	11.20±1.3^{c}	2.6±1.3	11±1.1	11.25±4.4^{c}
Orange peel	2.3±1.2	10±1.1	10.36±1.5^{a}	2.4±1.2	11±1.0	11.53±1.6^{c}	2.3±1.6	11±1.2	11.17±1.6^{c}
Control	2.7±1.9	10±1.0	10.23±1.7^{a}	2.4±1.7	10±1.4	10.07±1.1^{a}	2.4±1.2	10±1.0	10.11±1.2^{a}

*: mean values within the same column with different superscript letters are statistically different ($p<0.05$).

The microbiological development observed on *Lb. delbrueckii* NRRL B5448 may be related to the chlorogenic acid and hesperidin (79.003 ppm) in lemon peel, naringin and hesperidin (170.044 ppm) in orange peel, chlorogenic acid in mandarin peel and hesperidin (133.18 ppm) in orange juice.

Lb. casei NRRL B1922 was inhibited by the lemon and grapefruit juices, while its growth was stimulated by mandarin juice, orange juices and the peels of lemon, grapefruit, orange and mandarin. The inhibition effects observed for grapefruit and lemon juices on *Lb. delbrueckii* NRRL B5448 and *Lb. casei* NRRL B1922 were probably caused by the very low pH of these fruits.

The growth-promoting effects seen on *Lb. casei* may be related to hesperidin in lemon peel, caffeic acid in grapefruit peel, caffeic acid with hesperidin in orange peel, caffeic acid with hesperidin in orange juice and hesperidin in mandarin juice determined as 79.003, 0.166, 0.19, 170.044, 0.07, 133.18 and 81.674 ppm, respectively.

The growth of *Lb. acidophilus* NRRL B4495 was stimulated by grapefruit, lemon, orange peels, mandarin juice and orange juice, and affected moderately by grapefruit and lemon juices and mandarin peel extract. Growth of *Lb. acidophilus* NRRL B4495 could be promoted by naringin with caffeic acid in grapefruit peel extract, hesperidin in lemon peel, hesperidin and caffeic acid in orange peel, hesperidin in mandarin juice and caffeic acid, naringin with hesperidin in orange juice with the following respective values in ppm: 577.351, 0.166, 79.003, 170.044, 0.19, 81.674, 0.19, 0.982 and 170.044 ppm.

Several authors studied the interactions among probiotic bacteria and citrus fibers in probiotic products. Cesur (2014) researched dried citrus peels (orange, mandarin and lemon) that were used in a kind of fermented dairy product called "kefir". It was determined that total *Lactobacillus* and *Lactococcus* microorganism numbers were above 8.66 and 11.41 log CFU/mL during the 27 days, respectively. Sendra et al. (2008) found that citrus fibers enhanced *L. acidophilus* CECT 903 and *Lb. casei* CECT 475 survival in MRS broth during refrigerated storage.

As mentioned in previous research, phenolics can be used as a nutrient by lactic acid bacteria. In addition, the consumption of nutrients can be increased by the phenolic constituents available and, therefore, stimulate bacterial growth (Hervert-Hernandez et al., 2009; Hervert-Hernandez and Goni, 2011; Parkar et al 2008; Limkhuansuwan and Chairpraset, 2010). Based on the presented data, citrus fruit juices and their peels can be used in the production of functional foods and probiotics, for sustaining and developing vitality in probiotic microorganisms and for the enrichment of products in terms of phenolic constituents. They can be combined with certain fermented foods. Thus, the viability of lactic acid bacteria can be extended to foods such as probiotic yoghurts, kefir, fermented sauces and pickles, etc. In addition, the consumption of citrus fruits and peels with foods containing high antioxidant activities and the stimulating effects of lactic acid bacteria will contribute to the regulation of metabolism by intestinal microflora.

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