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Original Article

The role of probiotics in the regulation of microbial load in green detox smoothie to prevent foodborne and gastrointestinal infections

Gıda kaynaklı ve gastrointestinal infeksiyonları önlemek için, yeşil detoks içeceğinde mikrobiyal yükün düzenlenmesinde probiyotiklerin rolü

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ABSTRACT

Aim: The aim of this study was to reduce microbial load that can cause foodborne and gastrointestinal system infections in green detox smoothie by using probiotics instead of antimicrobials and heat treatments, and to develop green detox smoothie treated with probiotics for promoting gastrointestinal microflora.

Material and Methods: The microflora of green detox smoothie were determined by counting of total mesophilic aerobic bacteria (TMAB), yeasts and molds (YM), *Lactobacillus* spp., and *E. coli* on appropriate selective media. After 24 hours 37°C incubation of green detox smoothies with *L. acidophilus* and *L. reuteri* solely, all microorganisms were counted on appropriate media by streak plate method with respect to control.

Results: When treatments of *L. acidophilus* and *L. reuteri* were considered, significant reductions in the logarithmic counts of TMAB, YM and *E. coli* were seen. It was seen that inhibition of yeasts and molds were lower than inhibition of TMAB in green detox smoothie treated with probiotics. It was also determined that inhibitory effects of *L. acidophilus* and *L. reuteri* were the same against whole microorganisms ($P < 0.05$).

Conclusion: The applications of probiotics provide an alternative method for reducing microbial load of nutrients to the usage of antimicrobial substances and heat treatments. By the applications of probiotics, not only gastrointestinal microflora is promoted, but also microbial load of pathogens is eliminated in unprocessed nutrients such as green detox smoothie.

Keywords: Probiotic, antimicrobial, *Lactobacillus acidophilus*, *Lactobacillus reuteri*, foodborne infections, Gastrointestinal system infections, green detox smoothie

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ÖZ

Amaç: Bu çalışmanın amacı, antimikrobiyaller ve ısıl işlem yerine probiyotikler kullanarak, yeşil detoks içeceğinde gıda kaynaklı ve gastrointestinal sistem infeksiyonlarına neden olabilen mikrobiyal yükü azaltmak ve gastrointestinal mikroflorayı geliştirmek için probiyotik uygulanan yeşil detoks içeceği geliştirmektir.

Gereç ve Yöntemler: Yeşil detoks içeceğinin mikroflorası, uygun selektif besiyerlerde toplam mezofilik aerobik bakteri, maya ve küf, *Lactobacillus* spp. ve *E. coli* sayımları yapılarak belirlendi. Uygulama yapılmayan ve *L. acidophilus* ve *L. reuteri* ile tek olarak uygulama yapılan yeşil detoks içeceğinin 24 saat 37°C inkübasyonundan sonra, mikrobiyal yükü plak sayım besiyerinde (PCA) yayma plak yöntemi ile sayıldı.

Bulgular: *L. acidophilus* ve *L. reuteri* uygulamaları göz önüne alındığında TMAB, YM ve *E. coli* logaritmik sayımlarında anlamlı bir azalmalar görüldü. Probiyotik uygulanan yeşil detoks içeceğinde maya ve küf inhibisyonunun TMAB inhibisyonundan daha az olduğu görüldü. *L. acidophilus* ve *L. reuteri nin*, *E. coli* üzerindeki inhibitör etkisinin aynı olduğu da belirlendi ($P < 0.05$).

Tartışma: Probiyotik uygulamaları, besinlerin mikrobiyal yüklerini azaltmak için antimikrobiyal maddelerin ve ısıl uygulamaların kullanımına alternatif bir metod sağlamaktadır. Probiyotik uygulamaları ile sadece gastrointestinal mikroflora desteklenmekle kalmaz, aynı zamanda yeşil detoks içeceği gibi işlenmemiş besinlerde patojenlerin mikrobiyal yükleri yok edilir.

Anahtar Kelimeler: Probiyotik, antimikrobiyal, *Lactobacillus acidophilus*, *Lactobacillus reuteri*, gıda kaynaklı infeksiyonlar, gastrointestinal sistem infeksiyonları, yeşil detoks içeceği

Introduction

Usage of green detox smoothie have been emerged at health centers and gyms in worldwide due to acting as prebiotic, detoxicate xenobiotics. Especially, celery stalk and parsley added in green detox smoothie consisting of flavones that have antimicrobial and anti-inflammatory effects [1].

Green detox smoothie that is prepared fresh may cause foodborne infections due to having microbial load of which origin is soil generally. New alternative strategies for processing of nutrients to control pathogens have gained an attention due to antimicrobial chemicals and heat treatments used for control lead to spread resistant strains and disrupt heat sensitive health promoting molecules such as proteins and vitamins, respectively [2].

New applications of probiotics have been emerged in health and food industries. Probiotics that have health-promoting effects when are used in adequate level are used as a therapeutics or a supplement in nutrients [3]. Probiotics can have antimicrobial activities, regulate immune system, strength gastrointestinal system (GIS) by promoting microflora, acting as biocatalysts, consequently, facilitating digestion of polymers via fermentation [4-7]. While *Lactobacillus, Lactococcus* and *Bifidobacterium* are the main probiotics, *Streptococcus, Saccharomyces, Escherichia coli* are the other examples of probiotics [4]. *Lactobacillus acidophilus (L. acidophilus)* and *Lactobacillus reuteri* (L. reuteri) are Gram-positive lactic acid bacteria (LAB). While *L. acidophilus* is used in fermented dairy products generally. *L. reuteri* are inhabitants of GIS [8]. Many isolates of *L. acidophilus* produce bacteriocins [9], whereas *L. reuteri* produce and secrete reuterin (β-hydroxypropionaldehyde) and reutericyclin that are tolerant antimicrobial chemicals to proteolytic and lipolytic enzymes, when substrates of *Lactobacillus reuteri* are glucose and glycerol [10].

Stabilizing and supporting of intestinal microflora by probiotics plays a vital role through the lifetime [11]. Usage of probiotics such as *Lactobacillus* spp. can help to treat constipation and abdominal pain [12], and can avoid foodborne and GIS infections that are caused by *Helicobacter pylori* and enteric pathogens such as *Salmonella spp., Shigella spp., Clostridium difficile* and *Escherichia coli* [13].

Material and Methods

Determination of microflora of green detox smoothie

The microflora of green detox smoothie was determined by counting total mesophilic aerobic bacteria (TMAB), yeasts and molds (YM), *Lactobacillus spp*., and *E. coli* on plate count agar (PCA, Merck), yeast extract agar (YEA, Merck), de Man Rogosa and Sharpe agar (MRS, Merck) and eosin methylene blue agar (EMB, Merck) by standard spread plate technique, respectively.

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Preparation of green detox smoothie and inoculation of probiotics

10 spinach leaves, 5 rocket leaves, a pinch of parsley, one celery stalk and a half of cucumber were mixed by rondo after washing with tap water. 1.0 mL of bacterial suspension prepared in distilled water and adjusted to 0.5 McFarland (1.10^8 cfu/ml) was added into 9.0 mL of green detox smoothie. Inoculum was incubated at 37°C for 24 hours.

Determination of microbial counts

After incubation at 37°C for 24 hours, double fold dilutions of green detox smoothies untreated (control) and treated with *L. acidophilus* and *L. reuteri* were carried out with sterile distilled water. Then, 100 µL samples of each dilution were inoculated in PCA (Merck), yeast extract agar (YEA, Merck), de Man Rogosa and Sharpe agar (MRS, Merck) and eosin methylene blue agar (EMB, Merck) by streak plate method. Agars inoculated with 100 µL samples were incubated at 37°C for 24 hours. After incubation, plates were counted and converted to logarithmic values [14]. This process was repeated in triplicates.

Determination of pH

After incubation of green detox smoothie samples at 37°C for 24 hours, the pHs of green detox smoothie samples of which groups were untreated (control), treated with sole *L. acidophilus and L. reuteri* were measured by using a pH meter (WTW Inolab pH 720, Germany).

Statistical Analysis

The data were analyzed by the SPSS 21 software. Pearson $\chi 2$ test was used to detect existence of significance between treatments. All data were considered statistically significant, when p-value was equal to or less than 0.05 ($P < 0.05$).

Results

The microflora of green detox smoothie was found to be consisted of TMBA, YM, and *E. coli* in PCA, YEA, EMB agar, respectively, whereas LAB were not found in microflora of green detox smoothie (Table 1). The pHs of green detox smoothies of which groups were untreated (control), treated with sole *L. acidophilus* and *L. reuteri* were 6.57 5.21 and 5.5. There was an positive relationship between reduction of pH caused by *L. acidophilus* and *L. reuteri* and antimicrobial effects of *L. acidophilus* and *L. reuteri against* TMAB and yeast and molds grown in green detox smoothie. When treatments of *L. acidophilus* and *L. reuteri* were considered, significant reductions in the logarithmic counts of TMAB, YM and *E. coli* were seen ($P < 0.05$). TMAB, YM and *E. coli* were reduced approximately 2.5, 1 and 1.35 log cycle by treatment of *L. acidophilus*, respectively, whereas these reductions of TMAB, YM and *E. coli* were 2.5, 1 and 1 log cycle by treatment of *L. reuteri* (Table 1).

It was seen that inhibition of yeasts and molds were lesser than inhibition of TMAB in green detox smoothie treated with probiotics. When *L. acidophilus* and *L. reuteri* treatments were considered reductions of YM were both 1 log cycle, whereas reductions of TMAB were 1.35 and 1 log cycle, respectively. Loads of TMAB and YM were found to be reduced to 5.14 and 4.28 log cycle, respectively, from approximately the same log cycle grown in smoothie treated with *L. acidophilus*. Loads of TMAB and YM were found to be reduced to 4.36 and 5.43 log cycle, respectively, from approximately the same log cycle grown in smoothie treated with *L. reuteri*. Inhibitory effects of *L. acidophilus* and *L. reuteri* were the same against whole microorganisms ($P < 0.05$) (Table 1).

Table 1. Microbial loads of green detox smoothie

	Microbial loads of groups (Mean ± Std.)		
Microorganisms	**Untreated group (Control)**	**Treated groups**	
		L. acidophilus	***L. reuteri***
TMAB	6.83a ± 0.073	4.28c ± 0.304	4.36c ± 0.095
YM	6.21a ± 0.264	5.14b ± 0.285	5.43b ± 0.330
LAB	ND	7.27a ± 0.214	7.68a ± 0.464
E. coli	5.37b ±0.106	4.02c ± 0.06	4.23c ± 0.084

Abbreviations: ND, not detectedmeans ± Std. are data of three replicates Values followed by different letters are significantly different ($p<0.05$)

Discussion

L. reuteri that present in GIS provides an unfavorable environment for pathogenic bacteria by reducing pH of intestine [15]. *L. reuteri* produces and secrets reuterin and reutericyclin that are tolerant antimicrobial chemicals to proteolytic and lipolytic enzymes, when substrates of *L. reuteri* are glucose and glycerol [16], whereas *L. acidophilus* produces bacteriocin that is one of antimicrobial substance [17]. Researchers showed that incidence of diarrhea was decreased by the usage of *L. reuteri*, and nosocomial diarrhea was avoided by the usage of *L. reuteri in* children [13]. GIS infections are treated more effectively, when *Lactobacillus rhamnosus* GG (LGG), *L. reuteri* and *Saccharomyces boulardii* are used in the begining of infection [11]. Arqués et al. (2008) considered that reuterin showed bactericidal effect against all Gram-negative pathogens studied such as *Salmonella enterica, Campylobacter jejuni, Aeromonas hydrophila, Yersinia enterocolitica*, and *Escherichia coli* O157:H7 in milk [18]. Muthukumarasamy et al. (2003) revealed that *L. reuteri* had bactericidal effect against *E. coli* O157:H7 of which

inoculums were 3 log cfu/mL and 6 log cfu/mL in two ground beef before 20 days of storage period [19].

Other researchers considered that reuterin produced by *L. reuteri* had broad spectrum inhibitory effect against Gram-positive and Gram-negative bacteria such as *E. coli* O157:H7 [20, 21] grown especially in ground beef and milk [22, 23] and Listeria monocytogenes grown in cheese and milk treated with UHT 8 10 [24, 25], and prolonged shelf lifes.

Sikorska et al. (2013) determined that probiotics such as *L. acidophilus, L. casei, L. plantarum* and *Bifidobacterium bifidum* had antibacterial effects against methicillin resistant *Staphylococcus aureus* (MRSA). *L. acidophilus* was more effective against MRSA isolates than *Bifidobacterium bifidum*. Sikorska et al. (2013) and Hütt et al. (2006) revealed that antibacterial effects of probiotics can be mediated by acid products formed in fermentation process or antibacterials produced such as bacteriocin [13, 26].

Optimum pHs of bacteria and both yeast and molds for growth were 6.5-7.5 and 5-6, respectively [27]. In this study, eliminations of yeast and molds were lesser than elimination of TMAB in green detox smoothie treated with probiotics. This results were due to yeast and molds can resist to acidic environments more than bacteria [28].

Conclusion

The applications of probiotics not only promote microflora of GIS, but also can prevent foodborne and GIS diseases by reducing microbial load of pathogens in unprocessed nutrients without usage of antimicrobial chemicals leading to spread resistant strains or heat treatments degrading heat sensitive molecules such as proteins and vitamins. New applications of probiotic usage such as supplementation of nutrients with probiotics can be disseminated in health and food industries.

Declaration of conflicting interests

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