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
ÖZ

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

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

Bulgular: L. acidophilus ve L. reuteri uygulamaları göz önüne alındığında TMAB, YM ve E. coli logaritimsı sayım oranında anlamlı bir azalmalar görüldü. Probiyotik uygulanan yeşil detoks içeceğiında maya ve küf inhibisyonunun TMAB inhibisyonundan daha az olduğu görüldü. L. acidophilus ve L. reuteri nin, E. coli üzerindeki inhibitör etkinisinin aynı olduğu da belirlendi (P < 0.05).


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.
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 (1.10^10 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 χ2 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 smoothie of which groups were untreated (control), treated with sole L. acidophilus and L. reuteri were 6.57 5.21 and 5.5. There was a 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).

Discussion

L. reuteri that present in GIS provides an unfavorable environment for pathogenic bacteria by reducing pH of intestine [15]. L. reuteri produces and secretes 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 beginning 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

### Table 1. Microbial loads of green detox smoothie

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Untreated group (Control)</th>
<th>Treated groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. acidophilus</td>
<td>6.83a ± 0.073</td>
<td>4.28c ± 0.304</td>
</tr>
<tr>
<td>L. reuteri</td>
<td></td>
<td>4.36c ± 0.095</td>
</tr>
<tr>
<td>YM</td>
<td>6.21a ± 0.264</td>
<td>5.14b ± 0.285</td>
</tr>
<tr>
<td>LAB</td>
<td>ND</td>
<td>7.27a ± 0.214</td>
</tr>
<tr>
<td>E. coli</td>
<td>5.37b ± 0.106</td>
<td>4.02c ± 0.06</td>
</tr>
<tr>
<td>Abbreviations: ND, not detected</td>
<td>means ± Std. are data of three replicates Values followed by different letters are significantly different (p&lt;0.05)</td>
<td></td>
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</tbody>
</table>
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

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The authors received no financial support for the research and/or authorship of this article.

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