Catechin-rich Tea Extracts Improve the Lactobacillus casei Growth During Lactic Fermentation

Dan C. VODNAR, Floricuța RANGA Oana L. POP, Carmen SOCACIU

Faculty of Agriculture, University of Agricultural Sciences and Veterinary Medicine, 3-5 Mănăștur str., 400372, Cluj-Napoca, România; dan.vodnar@usamvcluj.ro, carmen.socaciu@usamvcluj.ro

Abstract. Tea is rich in polyphenols and phenolics compounds that have been widely reported to have beneficial health effects. The aim of this study was to investigate the ability of catechins, major phenols in tea, to act as growth factors for Lactobacillus casei during lactic acid fermentation. Major catechins presented in tea extracts are gallocatechin, catechin, epigallocatechin, galocatechingallate, epicatechingallate, catechingallate. Different concentrations of green tea and black tea extracts have been added to MRS model media. Growth and viability of Lactobacillus casei was positively affected by the addition of green tea and black tea extracts. This indicates that green tea and black tea addition exert protective effect on Lactobacillus casei growth in MRS media, increasing the viability and biomass production, probably by acting as metabolic enhancer. This results indicated the possibility of using green tea and black tea as metabolic enhancers for the growth of Lactobacillus casei.

Keywords: Catechins, Lactobacillus casei, Fermentation, Probiotic, Tea.

INTRODUCTION

The human colon represents an ecologically complicated microflora, including more than 500 bacterial species (Macfarlane et al., 2003). The impact on host nutrition and health of the human microbiota is important and is due by its enzymatic and metabolic activity (Hooper et al., 2002; Isolauri et al., 2002). Habitual intestinal bacteria possess enzymatic activities different from those of probiotic bacteria (Brigidi et al., 2001), which exert beneficial effects on the host when consumed orally (Salminen et al., 2005).

Lactobacillus and Bifidobacteria are the most common types of probiotics and were extensively investigated for their beneficial effects (Susanna et al., 2010). Lactobacillus casei is a versatile lactic acid bacteria (LAB) that has been isolated from raw and fermented dairy and plant materials but also from humans and animal gastrointestinal tracts (Randazzo et al., 2004). L. casei strains are recognized as probiotics and have a large application into food industry (Minellia et al., 2004).

Prebiotics are non-digestible carbohydrates, which stimulate the growth of probiotic bacteria, affecting positively the host after ingestion (Ziemer et al., 1998). Commonly known prebiotics are carbohydrates but their effect may not always be beneficial, as they can also encourage the growth of non-probiotic bacteria as Bacteroides and Clostridium ssp., which were previously noted in colorectal cancer patients (Onoue et al., 1997). Therefore, the utilization of non-carbohydrate sources intended to protect and facilitate the growth of probiotic bacteria is needed.

Tea is the second most consumed beverage in the world and has been shown that contain high concentrations of epicatechin, catechin, epigallocatechin, galocatechin, epicatechin gallate and epigallocatechin gallate (Sheila et al., 1999). In vitro experiments demonstrate that several flavonoids are metabolized by human bacteria (Justesen et al., 2001).
However, only a few species of intestinal bacteria responsible for phenolic metabolism have been identified (Lee et al., 2006).

The aim of this study was to investigate the ability of catechin compounds to act as simulative factors for the growth of L. casei during lactic acid fermentation.

MATERIALS AND METHODS

Preparation of tea extracts

Green tea (CV) and Black tea (CN) were purchased from Romania market. The aqueous extracts were made by adding 10 ml water (100°C) to 0.1 g or 0.2 g tea leaves and brewing for 10 min with stirring and removing solid matter by filtration.

Inoculum preparation and fermentation conditions

Lactobacillus casei ATCC 393 was purchased from Microbiologics, Bioaqua, Romania. Bacterium was routinely grown in MRS broth. Inoculum culture were grown for 24 h in a 400 mL Erlenmeyer flask containing 180 mL sterile MRS broth medium on a rotary shaker at temperature of 37°C and a shaker speed of 150 rpm. Inoculum was added into flask containing 100 ml of MRS media with 1% and 2% (w/w) from 1% and 2% of green and black tea leaves extracts addition and for the control was used MRS broth.

Chemical Analysis

A Shimadzu HPLC system equipped with a LC20AT binary pump, a degaser, a SPD-M20A diode array detector (Shimadzu Corp., Kyoto, Japan) and a Supercosil ™ LC-18 column (Sigma-Aldrich Co), 5µm, 25 cm x 4.6 mm was used in order to quantify the catechins from green tea and black tea extracts. Gradient elution was performed with mobile phase A, composed of methanol: acetic acid: double distilled water (10:2:88 v/v/v) and mobile phase B, comprising methanol: acetic acid: double distilled water (90:3:7 v/v/v), at a flow rate of 1.0 ml/min. All solvents were HPLC grade solvents, filtered through a 0.45-µM membrane (Millipore, U.S.A.) and degassed in an ultrasonic bath before use. The chromatograms were monitored at 280 and 360 nm.

Biomass determination

The biomass concentration in fermented samples was determined gravimetrically after filtration; a drying step was performed at 80°C for 24 hours until a constant weight was reached.

RESULTS AND DISCUSSION

The identification and the quantitative evaluation of six catechins found in green and black tea extracts was done by HPLC analysis as shown in Table 1. The major catechin derivatives found in the tea extracts are gallicatechin, catechin, epigallocatechin, gallocatechingalat, epicatechingalat, catechingalat. It can be noted that in black tea and green tea extracts epigallocatechin was the major compound identified followed by catechin and epicatechingallat.
The catechin derivatives found in (1%) black and green tea leaves water extracts

<table>
<thead>
<tr>
<th>Catechin derivatives</th>
<th>Concentration (mg %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Green tea</td>
</tr>
<tr>
<td>Galocatechin</td>
<td>18</td>
</tr>
<tr>
<td>Catechin</td>
<td>44</td>
</tr>
<tr>
<td>Epigalocatechin</td>
<td>46</td>
</tr>
<tr>
<td>Galocatechingallat</td>
<td>3.5</td>
</tr>
<tr>
<td>Epicatechingallat</td>
<td>18.7</td>
</tr>
<tr>
<td>Catechingallat</td>
<td>1</td>
</tr>
</tbody>
</table>

The quantification of each identified catechin has been monitored during fermentation processes. Thus, the catechin concentration (Figure 1) after 8 hours of fermentation decreased with 0.3 mg% while at the end of the fermentation process registered a decrease level of 0.5 mg%.

Generally, galocatechin (Figure 2) concentration decrease after 6 hours of fermentation. Trial using 1% of green tea addition registered, at the final process, a concentration up to 0.60 mg%. Epigalocatechin (Figure 3) concentration remained unchanged in trial using green tea 2%.

In trials incorporating black tea extracts 1% and 2%, the concentration of galocatechingallat (Figure 4) decrease proportionally with the fermentation time reached up to 2.5 mg% and 4.6 mg%, respectively. The epicatechingallat (Figure 5) level during 36 hours of fermentation decrease in sample incorporating 2% of green tea from 3.6 to 3.1 mg%. Content of catechingallat (Figure 6) in trials with 2% addition of green tea and black tea extracts has showed no variation from the beginning to the end of fermentation.

Growth of *L. casei* was positively affected by the addition of green tea and black tea extracts, and the viability of the cells in inoculum was 75%, after 24h of fermentation the value was 210% then decreased up to 210% at the end of fermentation in control media (Figure 7). Viability of *Lactobacillus casei* in all variants was influenced by the concentration of teas extracts addition, trial CN 1% has 225% after 24h of fermentation, trial CN 2%: 342%, trial CV 2%: 236% and trial CV 1%: 228%.

![Fig.1. Catechin quantification during fermentation processes.](image1)

![Fig.2. Galocatechin quantification during fermentation processes.](image2)
The cells were well adapted to this medium and biomass formation began immediately after adding the inoculum. At the end of process (after 38 hours) biomass formulation was around 2.1 g l$^{-1}$ (Figure 8) with highest value 2.4 g l$^{-1}$ at 28h in trials with 2% of black tea addition.

Growth of pathogenic *E.coli* and *S. typhimurium* were strongly inhibited by the presence of tea catechins and particularly by their aromatic compounds (Lee *et al.*, 2006). Growth of bacteria belonging to the *Clostridium* and *Bacterioides* genera that comprise the human intestinal microflora was also generally inhibited by tea polyphenolics and aromatic compounds (Lee *et al.*, 2006).
Fig 7. Viability of *L. casei* during fermentation processes.

Fig 8. Biomass production of *L. casei* during fermentative processes.

Our results are in agreement with that reported by Lee *et al.* (2006) regarding the ability of *Lactobacillus* *ssp.* to grow in media containing polyphenols. Currently, it is estimated that 500-1000 different strains populate the gastro-intestinal tract, but only a few identified species were able to catalyze the metabolism of phenolics compounds (Griffiths *et al.*, 1972).

On the other hand, antimicrobial activities of tea extracts have been demonstrated by Aziz *et al.* (1998). The inhibition capacity varies depending on the bacterial species and the chemical compound (Lee *et al.*, 2006).

Ingested probiotics from *Lactobacillus* *ssp.* and *Bifidobacteria* *ssp.* exert beneficial effect to the host by improving the intestinal microbial balance. An increase of the probiotic bacteria level into colon decreases the formation of ammonia and amine into intestine and reduces acid production (Yamamoto *et al.*, 1997).

Our studies are in agreement with that reported by Lee *et al.* (2006) indicated that phenolics are likely to benefit the growth of probiotic bacteria, consisting on the ability of polyphenols in green tea, to act as antioxidant and antiradical agents, to modulate the oxidative stress in the medium generated by the metabolic activities and consequently provide a better environment for the growth and multiplication of strains.
This result may account for the ability of phenolics-rich foods to protect against gastric and possible cancer colon. Currently most prebiotics are non-digestible carbohydrates intended to stimulate growth of probiotic bacteria, but they could remain in the gastrointestinal tract and are not hydrolyzed or absorbed.

CONCLUSIONS

It is generally believed that the tea catechins could positively influence the human health due to their anti-oxidant activity. This study, suggest that tea extracts (green and black tea) may have beneficial effects on the growth of \( L. \text{casei} \) during lactic acid fermentation. Water infusion prepared from black tea have higher epigallocatechin content than water extract from green tea, while more effective at promoting the growth of \( L. \text{casei} \) has been green tea extract addition. Growth of \( L. \text{casei} \) was positively affected by the addition of green tea and black tea extracts, indicating the protective effect on \( L. \text{casei} \) growth in MRS media, increasing the viability and biomass production, probably by acting as metabolic enhancer.

ACKNOWLEDGEMENTS

This work has supported by a research grant of University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca.

REFERENCES


