Infusion parameters determining microbiological purity of tea brew for kombucha production

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INTRODUCTION. Herbal infusions have been used traditionally over many years as health-promoting beverages. Kombucha is a slightly sweet, acidic and alcoholic beverage produced from the sweetened tea infusion (*Camellia sinensis* L.), fermented by a symbiotic culture of bacteria and yeast (SCOBY). Parameters determining the preparation of tea infusion (temperature, time, type of water, type of tea and its form – whole leaves or powder) affect the extract content, chemical composition and antioxidant stability of the brew. Typically, tea infusion is conducted at 85 °C for up to 30 min. Alternatively, cold brewing, which does not decompose bioactive compounds, may be implemented. Herbs are known for possible heavy microbiological contamination. Preparation of tea infusions at low temperature in combination with sugar addition, may enhance the growth of undesirable microorganisms and impact the overall microbiological quality of kombucha, and consequently its sensory attributes. In addition, the presence of pathogens may be observed. Recognizing potential microbial hazards is also important in reference to the popularity of home-brewed kombucha.

The aim of the research was to determine the impact of the cold brewing parameters on the microbiological purity of tea infusions.

MATERIALS AND METHODS. The research involved the analysis of infusions (n=130) obtained from three types of tea - Black Kenia, Green Sencha and Black Oolong. Infusions (5 g/L) of a given type of tea were brewed with tap water or distilled water. Each type of tea was infused at 5 °C and 20 °C for 2, 6 and 12 hours as well as at 85 °C for 10 and 30 minutes.

Microbiological purity of tea infusions was determined using the standard pour plate method. The total viable count of mesophilic and psychrotrophic bacteria, the total number of fungi, the number of *Enterobacteriaceae* as well as the number of acidifying and spore-forming bacteria (Fig. 1A-C, Fig. 2A) were determined. Dominating bacteria (Fig. 2B-C) were identified by 16S rRNA gene sequencing.

RESULTS

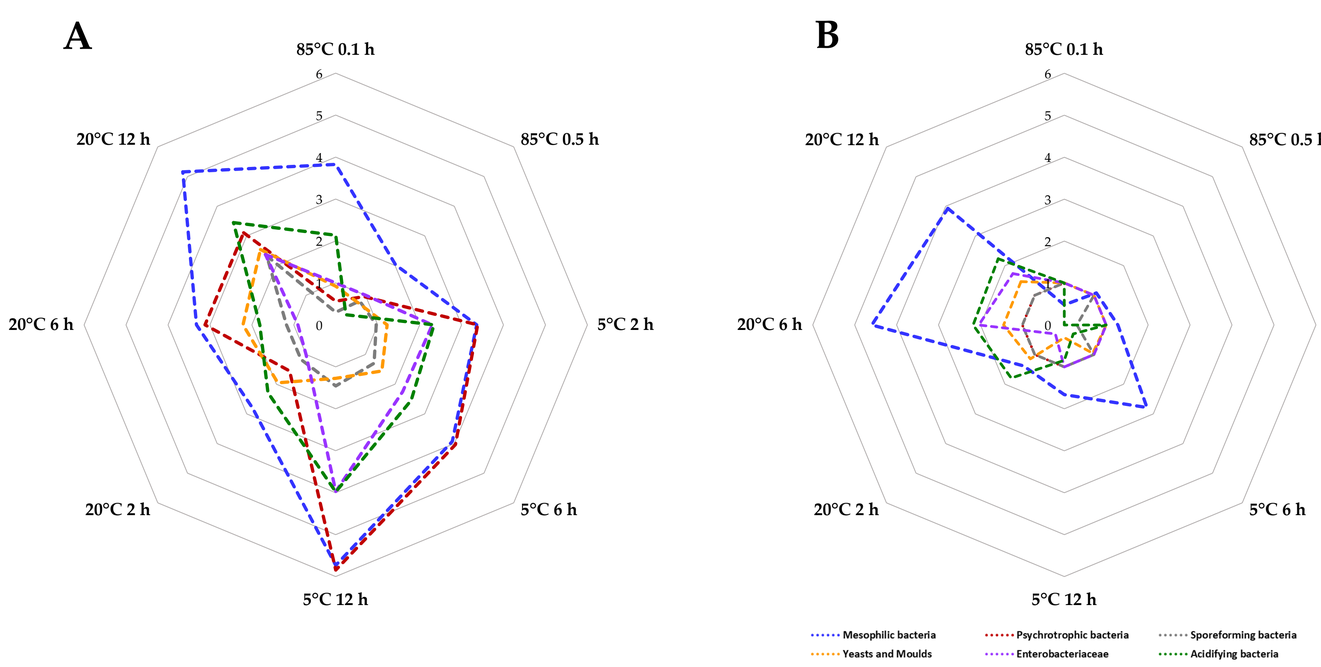

Figure 1. Impact of infusion parameters (time and temperature) on microbiological quality (expressed as log CFU/mL) of Black Oolong (A), Black Kenia Milma (B) and Green Sencha (C) tea.

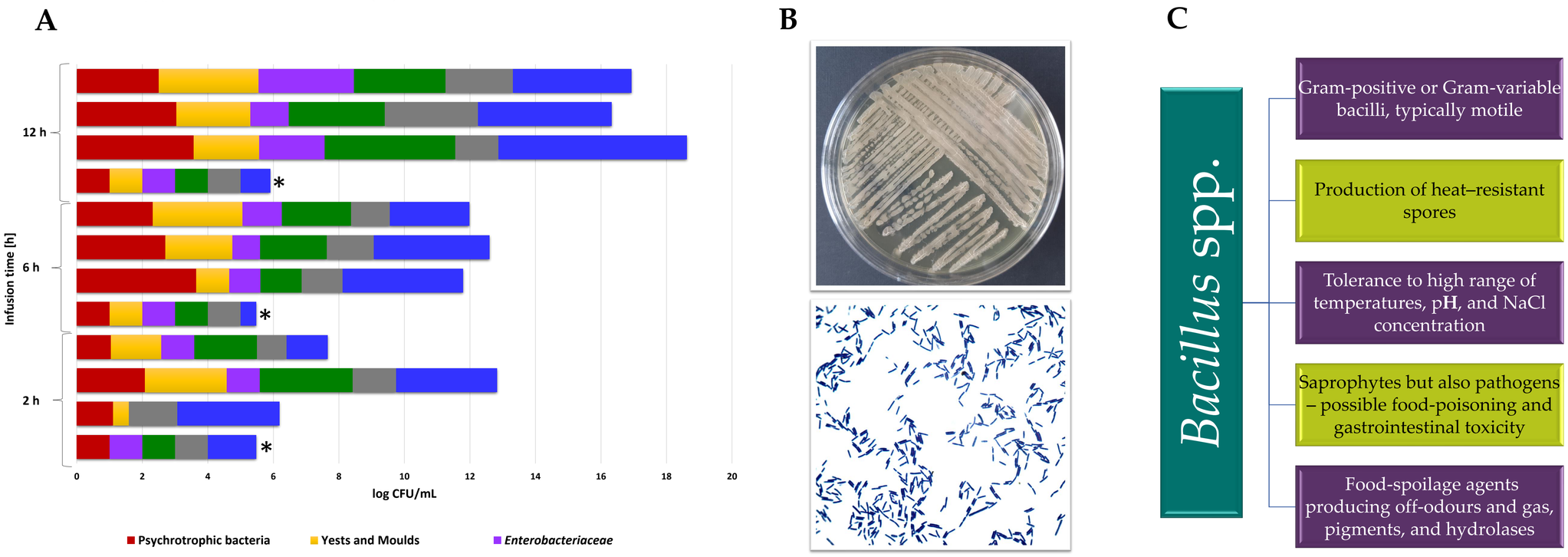

Figure 2. Microbial groups contaminating Black Oolong tea (infusion in 20°C, 12 h) (A), macro- and microscopic morphology of *Bacillus* spp. and main features of *Bacillus* spp. (C). *Tea infusions shortly pasteurized in 85 °C for 3 min.

CONCLUSIONS. The results indicated diversified level of microbiological purity of the analysed samples. Parameters of infusion process influenced the number of microorganisms in the tested samples. Amongst the detected isolates of microorganisms contaminating tea infusions (n=100), the most frequently occurring bacteria (as dominating microflora) were identified by 16S rRNA gene sequencing. Tea infusions were dominated by bacteria belonging to *Bacillus* spp.

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