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The biological activity of fermented dairy products obtained by kombucha and conventional starter cultures during storage

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ABSTRACT

The effects of kombucha inoculum as a new starter culture for milk fermentation were investigated, during 14 days of storage. The antioxidant capacity, angiotensin converting enzyme (ACE) inhibitory activity, degree of proteolysis (DP), content of vitamin C as well as sensory properties of kombucha fermented milk, were analysed and compared with fermented milk products obtained by commercial probiotic (ABT-10) and yoghurt (YF-L812) starter cultures. The kombucha fermented milk product (K) showed similar trend of changes in pH, DP and sensory properties as products obtained by probiotic (P) and yoghurt (Y) starters. Significant ACE inhibitory was determined in all fermented products, which increased during storage. Kombucha product had the highest ACE activity (63.43%) at the end of storage compared with probiotic and yoghurt products. In all products, higher 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) than 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was determined, while both activities slightly decreased during storage. Based on estimated biological activity the kombucha product can be considered as an innovative fermented dairy product suitable for human nutrition.

1. Introduction

The consumption of yoghurt has a long tradition in many countries, with its general acceptance as a healthy and nutritious food. Nowadays, production of yoghurt with elevated benefits on human health has become one of the major focuses in dairy industry, as current trends on the market are towards development of functional foodstuffs, especially fermented milk products. Over the past years, yoghurt has been proved as an ideal food to be fortified with diverse functional ingredients, such as vitamins, probiotics, prebiotics (Sadeq et al., 2013; Tamime, Hassan, Farnworth, & Toba, 2007; Tripathi & Giri, 2014). On the other hand, the use of non-conventional, functional starter cultures holds great promises to manufacture various yoghurts with desirable technological, nutritional and beneficial health advantages (Tripathi & Giri, 2014). Recent research studies have focus on naturally occurring bioactive components in fermented milk products, such as bioactive peptides (Korhonen, 2009; Urista, Fernandez, Rodriguez, Cuenca, & Jurado, 2011). Nowadays, bioactive peptides have gained increasing attention for human health promotion and deferment of age-related diseases (Kittiphattanabawona, Benjakul, Visessanguan, & Shahidi, 2013).

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Milk proteins are well-known precursors of peptides exerting antioxidant, antihypertensive, antimicrobial, immunomodulatory, anticancer, mineral-binding and opioid activity (Chibuike & Aishwarya, 2014; Korhonen, 2009; Samaranayaka & Li-Chan, 2011). These peptides can be released during fermentation of milk by proteolytic starter cultures or by addition of proteolytic enzymes. A number of these peptides may be available in the final products, primarily depending on the proteolytic capacity of microorganisms taking part in milk fermentation. So far, in fermented milk products, such as cheese and yoghurt, the potential antihypertensive (namely, angiotensin I converting enzyme, ACE inhibitory) peptides have been mostly studied (Gonzalez-Gonzalez, Gibson, & Jauregi, 2013; Papadimitriou et al., 2007).

For centuries, kombucha has been known as a consortium of yeasts (Pichia, Zygosaccharomyces, Saccharomyces, Schizosaccharomyces, Saccharomycodes, Brettanomyces, Torulaspora and Candida) and acetic acid bacteria (Acetobacter and Gluconobacter) (Chen & Liu, 2000; Trovatti, Serafim, Freire, Silvestre, & Neto, 2011; Zhang, Zhang, & Xin, 2011). Otherwise, novel research has indicated the significant presence of lactic acid bacteria (LAB) (Marsh, O Sullivan, Hill, Ross, & Cotter, 2014; Wu, Gai, & Ji, 2004; Zhang et al., 2011). Marsh et al., (2014) suggested that Lactobacilli are more prevalent in kombucha than was previously understood, particularly at the later stages of fermentation. The same authors found Lactobacillus kefiranofaciens subsp. kefirgranum as the more abundant genera of LAB in kombucha culture. Petrušić, Radulović, Paunović, and Obradović (2011) also showed presence of Lactobacillus plantarum, Streptococcus thermophilus, Streptococcus bovis, Streptococcus lutetiensis, Brevibacterium sp. as a constituents in kombucha inoculums on black tea.

Kombucha has been traditionally used for fermentation of sweetened black or green tea (Dufresne & Farnworth, 2000; Teoh, Heard, & Cox, 2004). Kombucha fermented product contains ethanol, carbon dioxide, a high concentration of acid (gluconic, acetic and lactic) and a number of other health-promoting metabolites. Therefore, it is considered to be beneficial beverage in cases of: digestive ailments, diabetes, hypercholesterolaemia, high blood pressure, combating stress and cancer as well as body vitalisation, among others (Aloulou et al., 2012; Guttapadu, Yang, & Wieger, 2000; Houda, Amina, & Emna, 2012; Jayabalan et al., 2011; Semantee, Ratan, & Parames, 2013; Thummala, Ramachandran, Jagadeesan, & Uppala, 2013). Recent studies presented the technological and nutritional potential of kombucha as an innovative starter culture in dairy industry (Iličić et al., 2012; Malbaša, Lončar, Milanović, & Kolarov, 2009a; Vukic et al., 2014). As the result of milk fermentation by kombucha inoculums, products similar to yoghurt or kefir are produced (Iličić et al., 2013; Milanović et al., 2008, 2012).

The aim of this study was to investigate the effects of kombucha inoculum as a new starter culture for milk fermentation, during 14 days of storage. The antioxidant capacity, angiotensin converting enzyme (ACE) inhibitory activity, degree of proteolysis (DP), content of vitamin C as well as sensory properties of kombucha fermented milk, were analysed and compared with fermented dairy products obtained by commercial probiotic (ABT-10) and yoghurt (YF-L812) starter cultures.

2. Materials and methods

2.1. Materials

All the chemicals used for the experiments were of analytical grade. DPPH (2.2-diphenyl-1-picrylhydrazy), angiotensin-I converting enzyme (ACE) from rabbit lung, N-hippuryl-His-Leu hydrate were obtained from Sigma (St. Louis, MO, USA), m-phosphoric acid (Riedel-de Haën, Seelze, Germany), acetonitrile, acetic acid and vitamin C (J.T. Baker, Deventer, Holland) HPLC grade.

2.2. Methods

2.2.1. Sample production

Fermented milk products were manufactured in laboratory conditions, from pasteurised (72 °C (161 °F) for 15 seconds) and homogenised milk with 2.8% fat (Dairy Subotica, Subotica, Serbia).

The following starter cultures were used for milk fermentation:

1. Kombucha inoculum – Kombucha was cultivated on black tea (*Camellia sinensis* – oxidised, 1.5 g L^{-1}) with saccharose concentration of 70 g L^{-1}. The tea was cooled at the room temperature, after which inoculum from a previous fermentation was added in concentration of 10%. Incubation was performed at 25 ± 2 °C for 7 days (Malbaša et al., 2009b). Kombucha inoculum in concentration of 10% (30 mL) was applied for milk fermentation. Total number of viable cells was as follows: approximately 5×10^4 of yeast cells per cm^3 of the reaction mixture and approximately 2×10^5 of bacteria cells per cm^3 of the mentioned mixture (Lončar, Kanurić, Malbaša, Đurić, & Milanović, 2013).
2. Probiotic starter culture, ABT-7 – lyophilised probiotic culture – Probio-Tek® contains LA-5®, *Lactobacillus acidophilus*, BB-12®, *Bifidobacterium*, *Streptococcus thermophilus* (Chr. Hansen, Hørsholm, Denmark).
3. Yoghurt starter culture – lyophilised yoghurt culture YF-L812 contains *Streptococcus thermophilus and Lactobacillus delbrueckii* ssp. *bulgaricus* (Chr. Hansen).

Commercial starters were added according to manufacturer's specification, 0.005 g 100 g^{-1}. All samples were produced in triplets at 42 °C. Fermentation was continued until pH = 4.5 were reached. Then samples were cooled to 4 °C, homogenized by mixing, packed in polypropylene glasses and stored in refrigerator at 4 ± 1 °C. Depending on the used starter culture different samples were produced. The samples were labelled as K0, K7, K14, P0, P7, P14, Y0, Y7, and Y14. Letters K, Y, P indicate used starter culture (K – kombucha, P – probiotics, Y – yoghurt) and number 0, 7 and 14 indicate the day of storage.

Chemical quality was tested in fermented dairy products after production using the following methods (Carić, Milanović, & Vucelja, 2000): dry matter (DM) (IDF/ISO 21A:1982): milk fat (MF) according to Gerber (IDF 105:1981); total proteins (TP) (IDF 20:1962); ash (A) (IDF 90:1979);