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Chemical composition and biological activity of novel types of kombucha beverages with yarrow

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ABSTRACT

Kombucha beverages were produced by fermentation of new types of substrates – yarrow infusions and yarrow subcritical water extracts (SWE). Fermentation process parameters (pH, total acidity and yield of biomass), chemical composition (organic acids, total phenols and flavonoids and vitamin C content) and sensory analysis indicated that SWE were more suitable substrates for successful fermentation. Products obtained on infusions had more pronounced anticancer and antimicrobial properties whereas beverages produced on SWE had higher antioxidant potential.

1. Introduction

Kombucha is a fermented beverage which is globally consumed because of the health benefits reported by the users. This product has slightly acidic, carbonated and sweet taste, and is mostly prepared at home. Fermentation process is a result of the metabolic activity of kombucha culture (symbiosis of bacteria and yeasts) on sweetened black or green tea, as the most common substrates. During fermentation the cellulosic pelicle layer is also produced by acetic acid bacteria, and this biofilm has numerous applications (Jayabalan, Malbaša, & Sathishkumar, 2016a). Successful kombucha fermentation is conducted in glass vessels under static conditions, on substrates that contain source of carbon (mostly sucrose) and nitrogen (different tea components) atom, protected from direct sunlight at room temperature. Although black and green tea are the most common substrates for its prepatration, there are reports that infusions prepared from different medicinal plants can be used as well. Yarrow is a plant with a variety of applications, including medical, and is also used as a livestock feed. It is used as a food, for preparing infusions and as a spice. This herb has sweet and bitter taste. According to the scientific evidence, there are over a hundred active biological compounds in yarrow. Among the most notable are achilleine, apigenin, luteolin, azulene, camphor, coumarin, inulin, menthol, quercetin, rutin, succinic, salicylic and caffeic acids, thujone, etc. (Dervengji, 1977). This plant is widely used to treat various diseases including malaria, hepatitis and jaundice as well as for the treatment of wounds, hemorrhages, headaches, inflammation, pain, spasmodic diseases, flatulence, and dyspepsia (Akram, 2013; Chandler et al., 1982; Benedek & Kopp, 2007; Lehane & Saliba, 2008). Yarrow is commonly consumed in a form of infusion and there is no scientific evidence about its use for kombucha preparation. Thus, this study is a first attempt to make kombucha beverage based on yarrow infusion as well as on its extracts obtained by subcritical water.

Subcritical water extraction is advanced extraction technique that is gaining increasing attention nowadays in the extraction and recovery of bioactive compounds from different natural matrices. The technique is based on the extraction with hot water at temperatures below its critical point while maintaining high pressures in order to keep the water in a liquid state during the whole extraction process. These conditions alter physico-chemical properties of water, influencing its solvating properties. The increase of the temperature of liquid water produces a series of effects, including improved mass transfer as a result of the drop in water surface tension that allows better penetration into sample matrix. Moreover, the mass transfer kinetics is favoured by the disruption of intermolecular forces (i.e., van der Waals forces, hydrogen bonds and dipole attractions) in the sample matrix. However, the most important effects of the increase in water temperature are the weakening of hydrogen bonds, resulting in a lower dielectric constant. Thus, the use of SWE could be an alternative to the use of non-polar organic solvents in some applications. From a green chemistry perspective, the avoidance of organic solvents provides additional advantages in the extraction of highly to medium polarity compounds. Due to numerous benefits that it offers, SWE are a great candidate for production of functional

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ingredients (Švarc-Gajić, 2012, chap. 11).

The aim of this paper was to investigate the possibility of production of a new variety of kombucha beverages on yarrow infusions, which are prepared as usual substrates, and subcritical water extracts, as unique and innovative starting medium. The goal is to establish the basis for novel functional food production and therefore widen the food market by offering products with competitive quality characteristics and antioxidant, antimicrobial and antiproliferative activities.

2. Material and methods

2.1. Plant material

Yarrow (*Achillea millefolium* L.) was collected in the area of Vlasina Lake (Southeast region of Serbia) at an altitude of 1220 m, in August 2015. Yarrow flowers were stacked in a crate with perforated bottom, in order to ensure air flow. Drying was performed naturally in the draft and dark until moisture content of 10%. Dry plant material was packed in paper bags and stored in the dark until use.

2.2. Infusions

Infusions were obtained by steeping 1.13 (Y1.13), i. e. 2.26 g (Y2.26) of yarrow flowers in 500 mL of boiling tap water during 15 min.

2.3. Subcritical water extracts

Subcritical water extraction was performed in a homemade subcritical water extractor/reactor previously described by Cvetanović et al. (2017). The extraction procedure was as follows: the operating pressure of 45 bar, process temperatures of 115 and 140 °C, agitation rate of 3 Hz, extraction time15 min. Extracts were prepared with different sample-to-solvent ratio: YI (1.13 g yarrow flowers/500 mL, 115 °C), YII (1.13 g yarrow flowers/500 mL, 140 °C) and YIII (2.26 g yarrow flowers/500 mL, 115 °C).

2.4. Kombucha inoculum and fermentation process

The native kombucha culture contains five yeast strains (*Saccharomycodes ludwigii*, *Saccharomyces cerevisiae*, *Saccharomyces bisporus*, *Torulopsis* sp. and *Zygosaccharomyces* sp.) and key bacterium belongs to the strains of the genus *Acetobacter* (Malbaša, Lončar, Vitas, & Čanadanović-Brunet, 2011).

Kombucha inoculum, used in this investigation, was fermentation liquid of kombucha obtained after 7 days long fermentation on yarrow infusion and subcritical water extract, sweetened with 35 g/500 mL sucrose, at 25 °C. Kombucha starter was added in the amount of 10% (v/v). Kombucha was selected as widely used microbial culture that can produce a beverage with potential functional characteristics. Yarrow was selected to introduce a new substrate for kombucha fermentation and infusions and subcritical water extracts to demonstrate the influence of different production parameters on the quality of obtained beverages.

Following kombucha beverages were produced: K-Y1.13, K-Y2.26, K-YI, K-YII and K-YIII.

2.5. Chemicals and reagents

Folin-Ciocalteu reagent, chlorogenic acid and rutin were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Aluminium chloride hexahydrate, sodium nitrite, sodium hydroxide and sodium carbonate were purchased from Merck (Darmstadt, Germany). Potassium ferricyanide and ferric chloride, were obtained from Zorka (Šabac, Serbia). Vitamin C standard and acetic acid were purchased from J.T. Baker, The Netherlands. Ammonium-acetate was purchased from Centrohem (Serbia), *orto*-phosphoric acid from Kemika (Zagreb, Croatia), lactic and oxalic acid from Laborat. Laphoma Hemikali (Skopje) and succinic, citric and malic acid from SupelcoAnalytical (Bellefonte).

Chemicals and reagents were of analytical and HPLC grade.

2.6. pH

pH values were measured using a pH-meter (WTW series Inolab pH 720).

2.7. Total acidity

Total acidity was determined by volumetric analysis with a standard solution of sodium hydroxide and phenolphthalein as indicator (Malbaša, Lončar, Djurić, & Došenović, 2008). Results were expressed as grams of acetic acid per liter of sample.

2.8. Yield of biomass

The yield of the obtained biomass was determined by mass measurement. The cellulose floating pellicle layer was removed from the fermented liquid surface, rinsed with distilled water and dried with filter paper (Malbaša, et al., 2008). Results were expressed in grams.

2.9. Organic acids analysis

HPLC analysis of organic acids (oxalic, formic, acetic, lactic, succinic, malic and citric) was performed using reversed phase chromatography, with Agilent 1100 Series HPLC, USA, according to Kordiš-Krapež, Abram, Kač, & Ferjančić (2001), with minor modifications. System consisted of degasser, binary pump, ZORBAX® SB-C18 column (4.6x150 mm, 5-μm) and UV-DAD detector. Analysis was performed in isocratic mode with 6 mmol/L phosphoric acid (pH 2.1) as mobile phase. Liquid chromatography parameters were set at: column temperature 28 °C, detection wavelenght 220 nm and flow rate 1.0 mL/min. Organic acids standards were used for external standard method calibration. Results were expressed in g/L.

2.10. Total phenols analysis

The Folin-Ciocalteu method (Singleton & Rossi, 1965; Kähkönen, 1999) was used to determine the total phenolics content. The reaction mixture was prepared by mixing 0.1 mL of the sample, 7.9 mL of distilled water, 0.5 mL of Folin-Ciocalteu reagent and 1.5 mL of sodium carbonate (20%, w/w). After incubation at room temperature for 1 h, absorbance was measured at 750 nm. The blank was prepared by replacing the sample with distilled water. Chlorogenic acid was used as a calibration standard and the results were expressed as chlorogenic acid equivalents per mL of sample (mg CAE/mL).

2.11. Total flavonoids analysis

Flavonoids in obtained extracts and beverages were determined using colorimetric assay based on the procedure described by Markham (Markham, 1989). SCW extracts and infusions of yarrow as well as kombucha beverages (1 mL) were mixed with 5% $NaNO_2$ solution (0.3 mL). After 5 min aluminium choride hexahydrate (10%, 0.3 mL) was added and allowed to stand for 6 min. Sodium-hidroxide (1 mol/dm^3, 1 mL) was added to the mixture. Immediately, distilled water was added to bring the final volume to 10 mL. The blank was prepared by replacing the sample with distilled water. Immediately after mixing, absorbance was measured at 510 nm. Rutin was used as a calibration standard and results were expressed as rutin equivalents (RE) per mL of sample (mg RE/mL).

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