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Effect of mixed culture inoculation on chemical and sensory properties of aronia (*Aronia melanocarpa*)

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ABSTRACT

The aim of this study was to investigate the effects of mixed inoculation on blocking astringency in aronia (*Aronia melanocarpa*) concentrates with respect to chemical and sensory properties. Mixed inoculation treatment included inoculation with four different ratios of mixed starter cultures (*Lactobacillus bulgaricus, Streptococcus thermophilus*, and *Saccharomyces cerevisiae*). The effect on astringency in aroina concentrates after mixed inoculation was demonstrated by three intimately reciprocal tests: bovine serum albumin precipitation, condensed tannin, and acetaldehyde analyses. After mixed inoculation, increased acetaldehyde components in aronia concentrates reacted with condensed tannin to form the modified tannin, resulting in decreased condensed tannin contents. This trend was supported by reduced aggregation of tannin-protein complex. Furthermore, sensory analyses (consumer testing and ranking descriptive analyses) showed moderated astringency perceptions after mixed inoculation. For consumer testing, the liking scores of all sensory attributes (overall liking, astringency, bitterness, sweetness, sourness, and berry taste) in mixed inoculated samples were slightly higher than those of non-inoculated aronia concentrate. The findings of ranking descriptive analysis indicated the significantly decreased scores at astringency attribute after mixed inoculation. In conclusion, these results suggested that mixed inoculation can be used to moderate astringency in aronia concentrates in both chemical and sensory aspects.

1. Introduction

Aronia (*Aronia melanocarpa*) is a member of the *Rosaceae*, native to North America and also cultivated in Europe (Strigl, Leitner, & Pfannhauser, 1995). In the food industry, aronia is extensively used for the production of jams, wines, preserves, juices, and many other food products (Brand, 2010). Aronia contains remarkable quantities of health-promoting compounds, such as anthocyanins and other polyphenols and is, thus, prescribed for various adult diseases such as hypertension, hyperglycemia, and diabetes (Kim et al., 2016; Yamane et al., 2017). However, aronia produces a conspicuous astringent sensation (tactile, drying and tightening sensation) in the mouth (Bajec & Pickering, 2008), and this property contributes to its aversion by consumers (Suomela et al., 2012).

Inoculation with microorganisms has been earlier proposed as a strategy to moderate the unpleasant astringency of berries (McRae et al., 2012). During the process, less astringency sensation can be usually perceived due to the decline of condensed tannin (proanthocyanidin) which is mainly responsible for the astringency perception (Es-Safi, Fulcrand, Cheynier, & Moutounet, 1999; Gawel, 1998). Factors contributing to the reduction of condensed tannin in berries are diverse and widely discussed in the previous studies, and the role of “acetaldehyde” produced after inoculation with yeast has been highlighted (Paiano et al., 2014). Generally, the inoculation with yeast moderated the astringency in berries (Sheridan & Elias, 2015). On the other hand, an inoculation with lactic acid bacteria improves the taste and aroma of berries (Lonvaud-Funel, 1999).

Although many researches have reported the effects of inoculation with yeast and lactic acid bacteria as mentioned above, there are no studies covering the impact of mixed culture inoculation on both the chemical and sensory characteristics of aronia, and therefore the present study was required for further information. In this study, yeast (*Saccharomyces cerevisiae*) was used for moderation of astringency of aronia owing to the production of acetaldehyde and was mixed with the lactic acid bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) for the improvement of taste. The objectives of this study were

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twofold. The first objective was to evaluate the changes in some primary parameters affecting the astringency of aronia after mixed culture inoculation, in respect of chemical analyses. The second objective was to determine if oral sensory profile and preference of aronia could be different between before and after mixed culture treatment. It was hypothesized that the mixed culture inoculation of aronia would degrade the condensed tannin contents and would, thus, improve the acceptability and preference for aronia.

2. Materials and methods

2.1. Materials

Aronia concentrates (30 °Brix) were obtained from Aronia farming association of Danyang (Danyang, Korea) and were stored at 4 °C. Gallic acid, tannic acid, sodium dodecyl sulfate, triethanolamine, sodium chloride, (+)-catechin hydrate, and bovine serum albumin were purchased from Sigma Aldrich (St. Louis, MO, USA). The other reagents used were of analytical grade.

2.2. Aronia concentrates inoculated with mixed starter culture

Aronia concentrates were inoculated with the mixed starter culture (*Lactobacillus bulgaricus, Streptococcus thermophilus* and *Saccharomyces cerevisiae*). The mixed starter culture consisted of lactic acid bacteria (the mixture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, Lyofast Y 450 B, Sacco, Lombardy, Italy) and yeast (*Saccharomyces cerevisiae*, Saf-instant yeast, Lesaffre, Lille, France) at a weight ratio of 1:1. The mixed starter culture was kept below −18 °C in a freeze-dried direct-to-vat set form before inoculation treatment. The samples of this study were prepared as four types according to the different ratios of mixed starter culture: non-inoculated aronia concentrate (NA), aronia concentrate inoculated with 0.05 mg/100 g mixed culture (AI05), aronia concentrate inoculated with 0.1 mg/100 g mixed culture (AI10), and aronia concentrate inoculated with 0.15 mg/100 g mixed culture (AI15). Samples were inoculated with mixed starter cultures and stored at 37 °C for 30 min. The reaction was finally stopped at 85 °C for 5 min by pasteurizing. All of the samples were stored at 4 °C until analysis.

2.3. Chemical properties

2.3.1. Total soluble solid and pH determinations

pH meter (2115102 Dual StarTM Benchtop pH Meter, Thermo Scientific Orion, Massachusetts, USA) previously calibrated with buffer solutions (4.0 and 7.0) was used to evaluate the pH of the samples. Total soluble solids were determined in °Brix using a handheld refractometer (PAL-α, ATAGO Co. LTD., Tokyo, Japan) previously adjusted to zero with distilled water. The prism of the refractometer was cleaned with distilled water after each analysis.

2.3.2. BSA precipitation assay

Bovine Serum Albumin (BSA) precipitation method is a modified version of the protocol described by Harbertson, Yuan, Mireles, Hanlin, and Downey (2013). The protein precipitation assay is based on the association of tannins to BSA under static conditions with buffer at various pH (Harbertson et al., 2008). This method is usually used to evaluate a concentration of precipitable tannins in sample after dissolution of the tannin-protein complexes and colorimetric reaction with ferric chloride. Briefly, 1 mL aliquots of a methanolic aronia solution diluted into a buffer containing 5 g/L potassium bitartrate adjusted to pH 3.3 with HCl were added to 2 mL of pH 4.9, 200 mmol/L acetic acid, 170 mmol/L NaCl containing 1.5 mg/mL BSA and incubated at room temperature for 15 min. Samples were then centrifuged at 13,500 rpm for 5 min to form a pellet with a clear supernatant using a centrifuge (Micro 17R, Hanil Science Industry Co., Ltd., Gimpo, Korea). The supernatant was discarded, and the remaining pellet was incubated for 10 min after adding 1.75 mL triethanolamine (TEA) buffer containing 5 g/100 g TEA and 5 g/100 g sodium dodecyl sulfate (SDS) adjusted to pH 9.4 with HCl. After the incubation period the sample was mixed mechanically to dissolve the tannin-protein pellet. To each sample, a 250 μL aliquot of ferric chloride reagent containing 10 mmol/L $FeCl_3$ in 0.01 mol/L HCl was added to the tube and allowed to stand at room temperature for 15 min. After the incubation period, the absorbance was determined at 510 nm in the Multiskan Go spectrophotometer (Thermo Scientific, Vantaa, Finland) for each sample and compare to tannic acid standards (600, 700, and 800 μg/mL) prepared daily. TEA buffer was used as a blank. Tannin values were reported in mg tannic acid equivalent (TAE) per mL of aronia concentrate.

2.3.3. Determination of condensed tannins

Determination of condensed tannins was based on the vanillin assay reported by Broadhurst and Jones (1978). A 0.5 mL of aqueous extract, contained in a test tube, was mixed with 3 mL of 4 g/100 g vanillin-methanol solution and then mixed with 1.5 mL of 36 g/100 g hydrochloric acid. The mixture was allowed to stand for 15 min at 20 °C in the dark. The absorbance of the mixture was measured at 500 nm using the Multiskan Go spectrophotometer. A catechin aqueous solution (1 mg/L) was used for calibration. The final results were expressed as mg catechin equivalent (CE) per mL of aronia concentrate.

2.3.4. Acetaldehyde measurement

Acetaldehyde was measured enzymatically using a commercial test kit (Megazyme, UK) according to the instructions of test kit. Aronia samples were analyzed in triplicate as described previously (Sheridan & Elias, 2015).

2.4. Sensory properties

2.4.1. Consumer testing

The degree of liking of four samples (NA, AI05, AI10 and AI15) was evaluated using consumers. Consumers (n = 50) above the age of 19 were non-probabilistically recruited at different locations, according to their willingness and availability to participate in the study.

A paper-and-pencil questionnaire was administered to consumers. 10 mL of each sample was served in clear paper glasses (identified by a random, 3-digit code) and presented to participants in a sequential monadic mode, according to a complete balanced experimental design. The test was conducted in an open area under a fluorescent lighting system. Conversation was strictly prohibited throughout the entire test, and monetary compensation was provided after the test. Water was supplied to clean the palate between samples. Sample acceptability was assessed for overall liking, astringency, bitterness, sweetness, sourness and berry taste ratings provided on a 9-point verbal hedonic scale (1 = “dislike extremely”, 5 = “neither dislike nor like”, 9 = “like extremely”) (Bamidele & Fasogbon, 2017).

2.4.2. Ranking descriptive analysis

Ranking descriptive analysis was used to assess the sensory intensities of the four samples (NA, AI05, AI10 and AI15). The panelists of trained judges consisted of 18 individuals, trained according to the consensus method described by Lawless and Heymann (2010).

References used for the evaluation of all attributes were decided upon panel consensus (Table 3). Samples were served in a monadic sequence according to a complete randomized block design (that is, block = replication) and the assessors were asked to assess the relative rank of the attributes based on the references provided. The assessors were trained to use rank scales and a warm-up sample prior to the test was evaluated. It was presented to each panelist as a means to review attribute definitions. The evaluation of the samples by ranking descriptive analysis was completed for each attribute, samples were ordered by increasing intensity. The results were analyzed using Friedman test (Newell & MacFarlane, 1987) to evaluate sample