Cosmeceutical potency of functional ripe buni cider

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Abstract. Buni fruit (Antidesma bunius) is a local seasonal sour fruit in Indonesia and commonly used as street foods such as rujak buni. In this study, we formulated cider drink from ripe buni fruits and determined its efficacy for cosmeceutical potency via antioxidant and collagenase inhibitory activities. Buni ciders were fermented with both single and combine cultures of Acetobacter xylinum and Saccharomyces cerevisiae. Ciders were further identified by gas chromatography-mass spectrometry (GC/MS) and tested for its antioxidant activity by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical inhibitory assay, collagenase inhibitory assay, alcohol content by Conway method, total soluble phenolic content, and sensory analysis. Buni cider had a pH of about 3.0 and alcohol content up to 3.6%. Compound profiling showed that buni ciders contained flavonoids, alkaloids, and organic acids. The antioxidant activity of buni ciders was reached in the range of 73.11-78.12 %, and the total phenolic content in ciders was 7.34-22.55 mg gallic acid/mL. Buni cider fermented from single A. xylinum had a potential inhibitory effect against collagenase. Sensory profiling showed that most panelists preferred to drink buni cider fermented from single A. xylinum rather than that of other buni ciders and juice. These data indicate that ripe buni cider fermented from single A. xylinum had potential antioxidant and collagenase inhibitory effects. Besides, it could be applied for cosmeceutical application for the management of skin aging.

1. Introduction

Buni (Antidesma bunius) is a seasonal sour fruit that can be found in Indonesia, with limited availability and only obtainable at certain areas. The limited availability is caused not only by the fact that it is a seasonal fruit, but also due to the decline in cultivation efforts. Even though the availability of buni fruit is declining, there is a lot of untapped potential from it. Buni fruit contains a lot of useful nutrition such as vitamins, fibers, and minerals [1]. People commonly eat this fruit raw like other fruits or by further processed such as salad, jam, an ingredient in fruit-flavored drink, and flavored enhance in fish soup [2, 3]. Functional drinks have become a trend demanded by consumers because it is believed to have health benefits, for example, fermented drinks. Cider is a fruit-based fermented drink made using microorganisms like yeast or bacteria [4]. Apple cider is one of the most popular types of cider. However, cider can be made with other types of fruits as well. Regular consumption of cider will benefit a person’s health, such as reducing blood pressure, a source of antioxidant, reducing diabetes risks, preventing cardiovascular diseases, and possessing antibacterial properties.

Humans are susceptible to aging as they grow older. Contributing factors to aging can be split into intrinsic and extrinsic factors. Intrinsic factors are those caused by a reduction in skin elasticity and increasing activity of enzymes such as collagenase. Extrinsic factors are caused by pollutions such as
those from tobacco products and motor vehicles, also from ultraviolet rays produced by the sun [5]. Skin wrinkling is a common indication of aging, and reactive oxygen species (ROS), which is accumulated in the cell. It causes oxidative damage to cellular components, such as DNA, mitochondria, and lipid membranes. Furthermore, ROS can induce activator protein and matrix metalloproteinase-1 activity. However, it inhibits transforming growth factor activity, which generates collagen degradation largely. Later, this can lead to decreasing in the structure and elasticity of the skin, the appearance of a solar scar, and generating wrinkles on the skin [6]. Nowadays, anti-aging products are in trend, with a lot of beauty clinics offering treatments at a premium, putting it out of reach from the general masses. Cosmetic products also offer anti-aging properties, focusing primarily on the face. Anti-aging treatments can be done by a well-regulated diet. Diets rich in antioxidants are very effective in combating the aging process. Natural antioxidants can be obtained from fruits and vegetables [7]. This research aimed to formulate cider beverages from ripe buni fruits and determine its efficacy as a cosmeceutical agent via antioxidant and collagenase inhibitory activities.

2. Materials and methods

2.1. Sample preparation
Ripe buni fruits were obtained from a local traditional market in Parung, West Java Province (Indonesia). A total of 384 g ripe buni fruit was washed and then blended using a mixer. Then, blended ripe buni fruit was dried into powder using a freeze drier at -45 °C for three days. Ripe buni powder was filtered and stored for further cider production.

2.2. Ripe buni cider production
Cider production was done according to the Sugito experiment with alterations [8]. The inocula used in this study included \textit{Saccharomyces cerevisiae} and \textit{Acetobacter xylinum}. \textit{S. cerevisiae} was obtained from commercial instant yeast. A total of one g yeast was diluted with 100 mL warm water and mixed before being inoculated. \textit{A. xylinum}, in the form of liquid culture, was used in the experiment. Ripe buni powder with 2% (w/v) concentration was added with sugar 10% (w/v), and then water was added until the volume reached 30 mL. The mixture was stirred until homogenous. Concentration and volume were similar for all ripe buni cider treatments. A total of 10% inoculum was inoculated into the mixture. Cider fermentation was under the anaerobic condition for \textit{S. cerevisiae} and aerobic conditions for \textit{A. xylinum}. The type of inoculum and experimental design is described in Table 1. The design of the experiment was divided into a single (Ax and Sc) and mixed culture (MC1.1 and MC1.2) with different fermentation times (Table 1). The fermentation process in mixed cultured treatment was started with \textit{S. cerevisiae} until the determined time. Then, the cider was centrifuged at 4032 x g for 3 minutes to get a clear solution. Next, the supernatant was moved into a new vessel. \textit{A. xylinum} was inoculated into it and fermented again until the determined time. Ripe buni cider was pasteurized at 65 °C for 30 minutes after the fermentation process, followed by centrifugation calibrate again at 4032 xg for three minutes. The pH of the ripe buni cider was measured using a pH meter. Ripe buni cider was stored in the freezer during experiment time.

| Table 1. Treatment for buni cider fermentation. |
|-----------------------------------------------|
| Treatment with inoculum | Fermentation time (weeks) |
|                            | Sc | Ax | MC1.1 | MC1.2 |
| \textit{Saccharomyces cerevisiae} | 1  | -  | 1     | 1     |
| \textit{Acetobacter xylinum}      | -  | 2  | 2     | 1     |

2.3. Compound identification by gas chromatography mass spectrometry
The compound of ripe buni cider and the juice was identified by pyrolysis gas chromatography-mass spectrometry (py-GC/MS) using Thermo Scientific ISQ 7000 Single Quadrupole GC-MS based on Mulyono \textit{et al.} with alterations [9]. The sample was injected into the capillary. The temperature of the instrument and injector was set. The results showed that the mass spectrogram was matched by the
instrument automatically into certain compounds based on the similarity of the m/z pattern to the mass spectroms instrument database.

2.4. Alcohol content assay
Alcohol content assay of ripe buni cider was determined using an assay modified by Sandi and Zubaidah [10]. K$_2$CO$_3$ saturated solution, which was prepared by a total of 117 g K$_2$CO$_3$, was diluted in 75 mL distilled water. K$_2$Cr$_2$O$_7$ solution was prepared by diluting a total of 0.37 g K$_2$Cr$_2$O$_7$ in 15 mL distilled water. Then, a total of 28 mL concentrated sulfuric acid was slowly added while being stirred. Afterward, the solution was diluted until the volume reached 50 mL. Buni cider sample and chamber were placed on the Conway diffusion cell and closed tightly. Conway was spun with a figure of eight movements to homogenize the sample and K$_2$CO$_3$ solution. The mixture was incubated for two hours at 30 °C. The positive result was measured spectrophotometrically at 605 nm. The alcohol content was measured using the alcohol standard curve.

2.5. Total soluble phenolic content assay
Ripe buni cider and juice were investigated for their phenolic content using the Folin-Ciocalteu method described by Agustinah et al. with a slight modification [11]. Using 96 well, we transferred 25 µL of the sample into it and added 25 µL of 95% ethanol, 125 µL of distilled water, and 12.5 µL of 50% (v/v) Folin-Ciocalteau reagent, respectively. The mixture was left to incubate for five minutes, followed by the addition of 25 µL of 5% Na$_2$CO$_3$. After thorough mixing, the reaction mixture was incubated in the dark for one hour, and the absorbance was read at 725 nm. Standard curves were generated using the increasing concentrations of gallic acid in 95% ethanol. Absorbance values were converted to total phenolics and expressed as mg of gallic acid per mL volume of the ripe buni cider and juice.

2.6. Antioxidant assay
The antioxidant assay was done according to the modified method of Ghimeray et al. [12]. Stock solutions ten mM 1,1-diphenyl-2-picrylhydrazyl (DPPH) was prepared and calculated according to the following formula:

$$\text{Scavenging activity} \ (\%) = \left[ \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \right] \times 100\%$$

2.7. Collagenase inhibitory activity assay
Collagenase inhibitory activity assay was determined by using collagen zymographic analysis according to the modified method of Iswara et al. [13]. Ripe buni cider and juice were prepared by treating the collagenase enzyme (2mg/mL) with 8 µL of the samples individually and incubated for half an hour at 37 °C. A number of 8 µL collagenase enzymes along with 8 µL distilled water and 4 µL enzyme buffers were considered as control. After the incubation period, 15 µL was loaded to each well and running gel for two hours (100 V and 400 mA). After it finished, the gel was washed with 2.5 % Triton X-100 for 30 minutes in an orbital shaker and then washed three times with distilled water and incubated in an enzyme buffer solution (50 mM Tris-Cl, and 5 mM CaCl$_2$ pH 7.4) for overnight at 37 °C. Followed by incubation, the enzyme buffer solution was drained and washed with distilled water for one minute and then stained with (0.25% w/v) Coomassie blue for two hours. The destaining procedure was done for 30 minutes after the gel was stained. The area of the light translucent zone over a blue background was photographed. Percentage of collagenase inhibitory activity was calculated according to following formula:

$$\% \ \text{Inhibition} = \left[ \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \right] \times 100\%$$

2.8. Sensory analysis
Sensory analysis was conducted by a hedonic test with 9 points hedonic scale, which was done according to the method described by Anjani et al. with a slight modification [14], to determine product based on panelist’s preferences. The panelists used in this analysis were 61 semi-trained
Panelists (students of Faculty Biotechnology, Atma Jaya Catholic University, Indonesia). Panelists had to give a score towards the sample given. The attributes tested were taste, color, aroma, aftertaste, and overall. Each attribute was analyzed and scored with a range from 1 until 9. The value of 1 is for extremely dislike, 2 for very dislike, 3 for dislike, 4 for rather dislike, 5 for neutral, 6 for rather like, 7 for like, 8 for every like, and 9 for extremely like.

2.9. Statistical analysis
Triplicate experiments were performed throughout this study. All data were presented as mean ± standard deviation. Significant differences were determined using one way ANOVA at P<0.05 and Duncan Multiple Range Test (DMRT) in the IBM SPSS version 23.

3. Results and discussion

3.1. Profiling of ripe buni ciders by GC/MS
The GC/MS chromatograms of ripe buni ciders and juice are shown in Figure 1. The components in cider Ax from a week A. xylinum fermentation consisted of phospholipids (0.55%), organic acids (2.42%), alkaloids (0.29%), flavonoids (1.9%), and phenolics (3.79%). Chemical constituents in cider Sc made from a 1-week S. cerevisiae fermentation were grouped into phospholipid (0.45%), alkaloid (3.28%), flavonoid (5.79%), and antioxidant (0.11%) classes. Furthermore, cider MC1.1 made from a 1-week S. cerevisiae fermentation and followed by a 1-week A. xylinum fermentation contained phospholipid (0.46%), organic acid (28.59%), flavonoid (1.61%), phenolic (0.69%), and coumarin (3.66%) groups. While chemical constituents in cider MC1.2 produced from a combination of 1-week S. cerevisiae fermentation and 2-week A. xylinum fermentation were grouped into phospholipids (1.20%), organic acids (7.36%), flavonoids (6.75%), and phenolics (0.41%). Besides, ripe buni juice was characterized and grouped into phospholipids (1.52%), alkaloids (10.68%), flavonoids (3.78%), and phenolics (2.22%).

Buni fruits are berry-type fruits that commonly grow in Asian countries, including Indonesia. This fruit can be consumed directly or by further processed of other food products. Ripe buni fruit has a reddish color and slightly sour, while the raw ones are green and tend to be acidic. The red color of mature fruit comes from anthocyanin pigments, which act as antioxidants [15]. The availability of buni fruit now is limited. It can only be obtained in certain areas and under-utilized in Indonesia. Research on this fruit is also relatively limited, but in fact, there is a lot of untapped potential from it that can be developed. Buni fruit is rich in nutrients, such as carbohydrates, sugar, organic acids, proteins, vitamins, minerals, anthocyanin, flavonoids, and phenolic acids. Ripe buni ciders had a reddish color, and the compound is identified by GC/MS analysis (Figure 1).

Chemical compounds in ripe buni ciders and juice were grouped into some group compounds, including flavonoids, alkaloids, and phenolics. The results were similar to Butkhup and Samappito study, which stated that ripe buni fruit contained flavonoids, alkaloids, and phenolics [15]. The concentration of each compound was different because of its various cider fermentation treatments. The fermentation process produced various secondary metabolites with different concentrations [4]. Our results are in line with the study of Mukherjee *et al.* which stated that several herbs, particularly fruits, vegetables, and whole grains, contained antioxidants, polyphenols scavenging free radicals and eliminate by-products of metabolism [6]. They are also used as a diet, which can be healthy for the body and can be helpful to avoid aging. Phytoconstituents have been reported to have skin aging prevention, including esculetin, 4-hydroxycinnamic acids, anthocyanin, catechin, epicatechin, curcumin, and others [6]. These constituents are also grouped as antioxidant agents. Interestingly, some of these constituents were also found in cider Ax, such as esculetin and 4-hydroxycinnamic acid derivatives (methyl caffeate) (Figure 1b). Meanwhile, specific phytoconstituents, which have been known to have skin aging prevention, were not found in cider Sc (Figure 1c).
Figure 1. Chemical profiling of buni ciders (Ax (a), Sc(b), MC1.1 (c), and MC1.2 (d)) and buni juice (e) by GC/MS. Ax: two weeks fermentation using *A. xylinum*, Sc: one-week fermentation using *S. cerevisiae*, MC1.1: one-week fermentation using *S. cerevisiae* continued with one-week fermentation using *A. xylinum*, MC1.2: one-week fermentation using *S. cerevisiae* continue with two weeks fermentation using *A. xylinum*. 
Buni fruits are berry-type fruits that commonly grow in Asian countries, including Indonesia. This fruit can be consumed directly or by further processed of other food products. Ripe buni fruit has a reddish color and slightly sour, while the raw ones are green and tend to be acidic. The red color of mature fruit comes from anthocyanin pigments, which act as antioxidants [15]. The availability of buni fruit now is limited. It can only be obtained in certain areas and under-utilized in Indonesia. Research on this fruit is also relatively limited, but in fact, there is a lot of untapped potential from it that can be developed. Buni fruit is rich in nutrients, such as carbohydrates, sugar, organic acids, proteins, vitamins, minerals, anthocyanin, flavonoids, and phenolic acids. Ripe buni ciders had a reddish color, and the compound is identified by GC/MS analysis (Figure 1). Chemical compounds in ripe buni ciders and juice were grouped into some group compounds, including flavonoids, alkaloids, and phenolics. The results were similar to Butkhup and Samappito study, which stated that ripe buni fruit contained flavonoids, alkaloids, and phenolics [15]. The concentration of each compound was different because of its various cider fermentation treatments. The fermentation process produced various secondary metabolites with different concentrations [4]. Our results are in line with the study of Mukherjee et al. which stated that several herbs, particularly fruits, vegetables, and whole grains, contained antioxidants, polyphenols scavenging free radicals and eliminate by-products of metabolism [6]. They are also used as a diet, which can be healthy for the body and can be helpful to avoid aging. Phytoconstituents have been reported to have skin aging prevention, including esculetin, 4-hydroxycinnamic acids, anthocyanin, catechin, epicatechin, curcumin, and others [6]. These constituents are also grouped as antioxidant agents. Interestingly, some of these constituents were also found in cider Ax, such as esculetin and 4-hydroxycinnamic acid derivatives (methyl caffeate) (Figure 1b). Meanwhile, specific phytoconstituents, which have been known to have skin aging prevention, were not found in cider Sc (Figure 1c).

3.2. pH values and alcohol content of ripe buni ciders
The fermentation process affected the pH and alcohol contents of ripe buni cider at various treatments. The pHs of buni ciders (Ax, Sc, MC1.1, and MC1.2) and juice were reached at 3.07, 2.97, 3.06, 2.92, and 3.11, respectively. In terms of alcohol content, the results showed that ripe buni juice had no alcohol content. In contrast, ripe buni ciders at various treatments (ciders Ax, Sc, MC1.1, and MC1.2) showed various levels of alcohol from 0.84 to 3.68% (Figure 2). Buni cider made from one-week fermentation using S. cerevisiae continue with one-week fermentation using A. xylinum (cider MC1.1) had the highest alcohol content (3.68%). This study employed bacteria, yeast, and mixed culture of bacteria and yeast as major cultures for the fermentation of cider (Table 1). Mixed culture was chosen because it had the ability to enhance cider flavor [16]. The fermentation process produced some organic acids that lead to the decrease of pH value [4]. Ripe buni juice, which was not involved in the fermentation process, showed the highest pH value among all ciders. Fermentation produced alcohol as well as gas and organic acids. The alcohol content in ciders as the result of glucose molecule broke down into alcohol by yeast (S. cerevisiae) [4]. In a single culture, fermentation treatment using A. xylinum was found alcohol content; however, there was no yeast addition. These data might be correlated with natural yeast found in ripe buni fruit, which was done in the fermentation process. In fruit skin, naturally found yeast species are classified in genus Candida, Debaryomyces, Torulaspora, Rhodotorula, Saccharomyces, and Pichia [17]. In a recent study, there are no specific genus or yeast species in buni fruit, so further study about that is needed.

3.3. Total soluble phenolic content of buni ciders
Total soluble phenolics content in various ripe buni ciders is shown in Figure 3. Among all ripe buni ciders, cider MC1.1 was found to exert the highest total phenolic content (22.55 mg gallic acid/mL) compared to other ciders and juice. Total soluble phenolic content assay generally has a positive correlation with antioxidant activity. Some studies showed that the increase of antioxidant activity was linear with its total phenolic content [18]. Phenolics could act as free radical scavenging through hydrogen donors. Interestingly, our findings demonstrated that there was no correlation between total soluble phenolic content in ripe buni ciders and their antioxidant activities (Figure 3 and 4). It is due to the presence of the following factors. The antioxidant activity was not solely from phenolic contents.
but could be due to the presence of other phytochemicals [19]. Total soluble phenolic content was also affected by the fruit ripening, which decreased during the ripening time. The sweet and bitter taste of the fruit decreased because the total soluble phenolic content during the ripening time decreased. A recent study found that the total phenolic content of buni fruit accounted for was decreasing from 19.60 to 8.66 mg GAE/g during ripening [20].

Figure 2. The alcohol content in buni ciders and juice. Ax: two weeks fermentation using *A. xylinum*, Sc: one-week fermentation using *S. cerevisiae*, MC1.1: one-week fermentation using *S. cerevisiae* continue with one-week fermentation using *A. xylinum*, MC1.2: one-week fermentation using *S. cerevisiae* continue with two weeks fermentation using *A. xylinum*.

Figure 3. Total phenolic contents of ripe buni cider and juice. Ax: two weeks fermentation using *A. xylinum*, Sc: one-week fermentation using *S. cerevisiae*, MC1.1: one-week fermentation using *S. cerevisiae* continue with one-week fermentation using *A. xylinum*, MC1.2: one-week fermentation using *S. cerevisiae* continue with two weeks fermentation using *A. xylinum*.

Figure 4. Antioxidant activity of buni ciders and juice. Ax: two weeks fermentation using *A. xylinum*, Sc: one-week fermentation using *S. cerevisiae*, MC1.1: one-week fermentation using *S. cerevisiae* continue with one-week fermentation using *A. xylinum*, MC1.2: one-week fermentation using *S. cerevisiae* continue with two weeks fermentation using *A. xylinum*. 
3.4. Antioxidant activity of buni ciders

The antioxidant activity of buni ciders and juice was tested by the DPPH method, and the result is demonstrated in Figure 4. All buni ciders and juice exerted the potential antioxidant activity at a range of 73.11 to 78.12 % as well as ascorbic acid standard. The antioxidant activity of ripe buni ciders was tested using DPPH radical scavenging assay. It is noted that this assay has been widely applied for the quick screening of plant extracts due to its simplicity, reproducibility, and being independent of sample polarity [11]. Our data showed that ripe buni ciders and juice had potential antioxidant activity at a range of 76-78% (Figure 4). Antioxidant activity was affected by compounds that acted as antioxidant agents in the sample. Ripe buni fruits are known to contain antioxidant group compounds, such as flavonoids, phenolics, cathecins, anthocyanins, and carotenoids. It seems that fermentation also produces various secondary metabolites, which can be classified as antioxidants. In our study, ripe buni ciders contained several secondary metabolites, such as flavonoid, phenolic, and alkaloid [15].

3.5. Collagenase inhibitory assay of ripe buni ciders

Collagenase inhibitory effects of buni ciders and juice by SDS-PAGE are shown in Figure 5. The results demonstrated that buni ciders made from single culture treatments (Ax and Sc) were found to inhibit collagenase activity at the moderate inhibitory activity (Table 2). Among all buni ciders, cider Ax exerted the highest collagenase inhibitory activity (~65.29 %). Collagen is the major component of the skin that can degrade by the collagenase enzyme. Inhibition of collagenase activity could delay the process of collagen degradation to maintain skin elasticity, strength, and flexibility. Collagenase inhibitory assay of ripe buni cider and juice was further confirmed by the zymographic method (Figure 5). Those were subjected to collagenase activities. The results are summarized in Table 2. Among all ripe buni ciders, cider Ax was shown to exhibit the highest inhibitory activity against collagenase (65.29%). Cider Sc was the other potential sample that exerted collagenase inhibitory activity (54.83 %). There was another mechanism of collagenase inhibition involving the Zn ion active site on collagenase. Collagenase has a structural Zn ion at its active site, which plays a major role in facilitating interaction with an inhibitor. Ripe buni fruit contains polyphenol compounds that are known to be metal chelators and may bind to a Zn ion active site and prevent the substrate from enzyme activation. In addition to the polyphenols, flavonoids are also chelated Zn metal by its 3-hydroxy flavone structure [18]. Previous studies showed that polyphenols, flavonoids, and other antioxidant compounds were responsible for collagenase inhibition.

| Sample          | Volume (Intensity) | Inhibition (%) |
|-----------------|--------------------|----------------|
| aControl        | 653,730            | 100            |
| bCider Ax       | 226,940            | 65.29          |
| cCider Sc       | 295,260            | 54.83          |
| dCider MC1.1    | 694,050            | 0              |
| eCider MC1.2    | 573,020            | 12.35          |
| fRipe buni juice| 179,508            | 0              |

*Control: collagenase; *Ax: two weeks fermentation using *A. xylinum; *Sc: one-week fermentation using *S. cerevisiae; *MC1.1: one-week fermentation using *S. cerevisiae continue with one-week fermentation using *A. xylinum; *MC1.2: one-week fermentation using *S. cerevisiae continue with two weeks fermentation using *A. xylinum.

3.6. Sensory results of ripe buni ciders

The sensory test of ripe buni ciders was done by 61 semi-trained panelists, and the profile result is shown in Figure 6. Among all samples, buni juice had the highest score for parameters of aftertaste (6.4) and overall (6.6). The sensory profile also showed that most panelists preferred buni juice and cider Ax in terms of taste, aftertaste, and overall parameters rather than that of other buni ciders and commercial apple cider. In contrast, buni ciders MC1.1 and MC1.2 were preferable by panelists due to their color parameters. Ripe buni and commercial cider used in this study were sweetened with sugar.
and diluted with water. The addition of sweetener maintained the natural acidity taste of ripe buni and commercial cider. Panelists preferred ripe buni juice rather than ripe buni cider in case of aroma, aftertaste, and overall attributes (Figure 6). Meanwhile, panelists preferred the color of cider MC1.1 and the taste of cider Ax. Ripe buni cider had a strong acidic aroma and slightly alcohol. It indicated that the panelists did not like that aroma. Symoneaux et al. stated that alcohol content played a role in giving bitterness aftertaste slightly [21]. It might affect the score of ripe buni cider aftertaste, which was lower than ripe buni juice. Anthocyanins in ripe buni fruit play a role in giving red color and are relatively unstable in acidic conditions. In more acidic pH conditions, the reddish anthocyanin color fades to pink [22].

**Figure 5.** Collagenase inhibitory activity of buni ciders and juice by SDS-PAGE analysis. Ax: two weeks fermentation using *A. xylinum*, Sc: one-week fermentation using *S. cerevisiae*. MC1.1: one-week fermentation using *S. cerevisiae* continue with one-week fermentation using *A. xylinum*, MC1.2: one-week fermentation using *S. cerevisiae* continue with two weeks fermentation using *A. xylinum*.

**Figure 6.** Sensory profile of buni ciders and juice. Ax: two weeks fermentation using *A. xylinum*, Sc: one-week fermentation using *S. cerevisiae*. MC1.1: one-week fermentation using *S. cerevisiae* continue with one-week fermentation using *A. xylinum*, MC1.2: one-week fermentation using *S. cerevisiae* continue with two weeks fermentation using *A. xylinum*.

## 4. Conclusion

Buni ciders contained major active compounds (flavonoids, alkaloids, and organic acids) and had main characteristics with a pH of 2.92-3.11, alcohol content of 0.8-3.6%, and phenolic content of 7-22 mg GAE/mL. Buni ciders exerted cosmeceutical efficacy via antioxidant (73-78%) and collagenase inhibitory activities (55-65%). Among all buni ciders, cider Ax fermented from a single culture of *A. xylinum* showed the best characteristics, cosmeceutical efficacy, and the sensory result compared to
his cider might offer a potential application for a functional cosmeceutical beverage for the prevention of skin aging.

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