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Addition of Green Pepper Enhanced Antioxidant Potential and Overall Acceptance of Beer

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Abstract: Beer is one of the most widely consumed alcoholic beverages all over the world. Different studies have been carried out to enhance the quality and acceptance of beer. Various raw materials including herbs and spices have been used as a beer adjunct or flavoring for centuries, especially for specialty beer. The objective of this study was to investigate the effect of green pepper on antioxidant potential and quality characteristics of beer. Addition of green pepper enhanced the antioxidant potential and the overall acceptance of the beer brewed with different proportion of pepper without deteriorating its basic chemical properties. Based on the overall acceptance as well as the antioxidant potential, the optimum proportion of green pepper to be added was 60 g in 10 L of water. Results of the present study imply that green pepper could be used as an effective enhancer for antioxidant potential and overall acceptance of beer.

Keywords: Antioxidant Potential, Beer, Green Pepper, Quality Characteristic

Introduction

Beer is one of the famous alcoholic beverages all over the world (Sohrabvandi et al., 2011) and is prepared from starch-containing raw materials as a source for maltose and glucose and is fermented by brewers' yeast. Barley is used as the main ingredient of starch-containing aides or extenders for fermentable sugars. In order to influence taste, color, and body of the finished beer product, mixing of different varieties of barley and their malts like Munich malt, Vienna malt, British pale ale malt, or roasted black malt is also in practice. In addition to barley as the main ingredient; other cereals like, wheat, wheat malt, corn, rice, and millet are also used as starch-containing adjuncts or extenders and sources for brewing beer. In most cases hops are added to beer to enrich latter's flavor mainly bitterness and they also act as natural preservative.

Fruits like cherry, raspberry, and peach have been commonly used with Belgian lambic styles of beer as a beer adjunct or flavoring for centuries. Among the different starch-containing adjuncts, fruits are popularly used in wheat based beer in which the latter provides a lot of texture but little flavor to the final product. For instance; Fruli, a wheat based Belgian fruit beer, is made of 70% wheat and 30% fruit juice (Fedora and Fedora, 2014). Fruits are used as key ingredients in some beer styles; however they are also used to contribute to the flavor and aroma profile of other styles. Fruits like cherries and raspberries are added to the finished beer followed by a further fermentation to produce a special fruit beer (De Keersmaecker, 1996). In addition; vegetables, honey, molasses, candy sugar, or other fermentable sugars are also found to be used to add their distinct flavors to beer.

Herbs and spices are also used in some beer styles. Spices like allspice, anise, cardamom, caraway, cinnamon, cloves, coriander, gingerroot, juniper berries, and vanilla bean as well as herbs like sweet gale, heather tips, and mint could be used to enhance the flavor of the brews (Nachel, 2008). For example, some beer styles like Belgian Witbier uses coriander, Finnish Sahti is brewed with juniper berries, and

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traditional beers in Britain are brewed with honey and spices. As beer is a worldwide beverage, many countries have been practiced brewing their own local beer specialties for centuries. German beer such as Pilsener, Düsseldorfer Altbier, and Bavarian Weissbier are famous ones. Similarly, beer from Great Britain named Ales and Stouts are also popular.

Pepper is a very important spicy food with different nutrients and phytochemicals having health promoting effects like anti-obesity activities (Jeon et al., 2010), antioxidant activity (Domínguez-Martínez et al., 2014; Jang et al., 2015). Although pepper is a widely used spice crop, no scientific study on the use of peppers to brew beer has been reported so far. Therefore, the objective of the present study was to analyze the influence of addition of peppers on the antioxidant potential and other physicochemical characteristics of beer.

Materials and Methods

Chemicals and materials Folin-Ciocalteu phenol reagent and DPPH were purchased from Sigma-Aldrich (St. Louis, USA). All other reagents were of analytical grade. Beer mix (The Great American Beverage Company, Reno, Nevada, USA) was obtained from a local store in Daegu, Korea. Pepper (*Capsicum annuum* L.) cv. Longgreenmat grown at Yeongyang Pepper Experiment Station, Gyeongsangbuk-do, Korea was used for this study. There was no specific reason for using this particular cultivar.

Preparation of pepper beer samples Pepper beer was prepared as described in previous study (Cho et al., 2015) with some modifications. The beer was prepared by 4-step fermentation process using a beer machine (Model 2000, The Beer Machine Co, Reno, NV, USA). A commercially available beer mix (0.9 kg), yeast (2.5 g), water (10 L), and different proportions of fresh green pepper cut into small pieces were allowed for fermentation at 25°C for 6 days, then storage at 4°C for 3 days, again fermentation at 25°C for 7 days with 5 g added sugar per liter of fermenting product, followed by storage at 4°C for 3 days. Different varieties of pepper beer were named as B-0: ordinary beer containing no added green pepper, B-30: pepper beer containing 30 g of green pepper; B-60: pepper beer containing 60 g of green pepper; and B-100: pepper beer containing 100 g of green pepper in 10 L of water before fermentation. Addition of various proportions of green peppers was expected to have differential influences on the physicochemical properties of beer. A range of green pepper concentrations was used for brewing so that an optimum quantity of pepper could be identified to prepare superior quality pepper beer. After preparation, the beer samples were stored at 4°C until subsequent analyses at different intervals.

Chemical characteristics The pH of pepper beer was measured with a pH Meter (Model 250, Beckman Coulter Inc., Fullerton, CA, USA). Titratable acidity (lactic acid g/L) was measured by adding 5 mL of pepper beer sample to 125 mL of deionized water and titrating with 0.1 N sodium hydroxide to an endpoint pH 8.2. Alcohol concentration was measured as described by Ough and Amerine, (Ough and Amerine, 1988). All chemical measurements were replicated three times and the average values were reported.

Total phenol content The total phenol contents were measured according to the Folin-Ciocalteau method (Singleton et al., 1999) with some modifications. Fifty microliters of beer samples and 1 mL of 2% (w/v) aqueous Na_2CO_3 were mixed in vortex and kept for 3 min. Fifty microliters of 1 N of Folin-Ciocalteau reagent was added to the mixture and kept at room temperature under dark condition for 30 min. The absorbance was measured at 750 nm (Multiskan GO Microplate Spectrophotometer; Thermo Fisher Scientific Oy, Vantaa, Finland). Total phenols were determined as gallic acid equivalents (μg GAE/mL pepper beer), and the values are presented as means of triplicate analyses.

DPPH radical scavenging activity DPPH radical scavenging potential was measured according to the methods described by Cheung et al. (2003) with some modifications. One hundred microliters of 0.1% DPPH methanol solution was mixed with 0.1 mL of sample. The mixture was allowed to react at room temperature in dark for 30 min. The absorbance was measured at 517 nm (Multiskan GO Microplate Spectrophotometer; Thermo Fisher Scientific Oy) and the radical-scavenging activity (RSA) was calculated as a percentage inhibition using the equation: $\%RSA = (1-S_{ab}/C_{ab}) \times 100$, where S_{ab} is the absorbance of the sample and DPPH solution; C_{ab} is absorbance of DPPH solution without sample. The assays were carried out in triplicate and the results are expressed as mean values.

Sensory properties evaluation Pepper beer samples prepared with different amounts of green pepper were rated for color, flavor, and overall taste, respectively, on the scale of: 1= very bad, 2= bad, 3= moderate, 4= good, and 5= very good (Kim et al., 2015). All the sensory properties were evaluated by 20 volunteer panelists (10 women and 10 men) selected from the list of graduate students of College of Agriculture and Life Sciences of Kyungpook National University, Daegu, Korea. The results showed are the average values of 20 evaluations for each sensory property.

Statistical analysis Data were subjected to analysis of variance (ANOVA) using SAS 9.4 (SAS Institute, Inc). Differences between means at $p<0.05$ were identified using Tukey test. Average values are presented as mean±SD.

Results and Discussion

Chemical characteristics The chemical compositions of ordinary (B-0) and pepper beer varieties (B-30, B-60, and B-100) during storage at 4°C are shown in Table 1. The pH values of beer varieties were in order of B-100>B-60>B-0>B-30 on 0 day of storage. The values of B-0 and B-30 were not significantly different ($p>0.05$) on 0 day of storage. The value of B-100 was the highest whereas that of B-0 was the lowest on 30 days of storage as compared to other samples. It was also highest for B-100 on 60 days of storage but the lowest was for B-30. In general the pH value of particular beer sample has been found to increase slightly increased on 30 days of storage but again decreased on 60 days. The high pH value of pepper beer varieties might be due high pH value of green pepper fruits than that of B-0 (Derossi et al., 2010; Charlo et al., 2012). Slight increase in pH value of low alcohol beer was also found by Sohrabvandi et al. (2010) during storage at 5°C for 20 days.

The alcohol concentration was also significantly different ($p<0.05$) among different beer varieties. Sample B-60 contained the highest whereas B-0 did the lowest alcohol value for 0, 30, and 60 days of storage. The alcohol concentration was found to slightly be increased with storage except for B-0. The results of the alcohol concentration in pepper beer during storage also agrees with the results of Entwisle et al. (2008).

Titratable acidity (TA) value of pepper beer samples were significantly high ($p<0.05$) as compared to B-0 for fresh as well on 30 and 60 days of storage. Among the pepper beer varieties TA values were not significantly different ($p>0.05$) for fresh and stored for 30 days, however B-60 showed significantly high ($p<0.05$) TA value on 60 days of storage. The TA values were found to increase with storage time irrespective of the beer varieties. Pearce et al. (2016) also found TA value increased for some beer samples during storage at 4°C, however the mechanism of increased TA could not be explained.

DPPH radical scavenging activities and total phenol content The DPPH radical scavenging activities and total phenolic contents of pepper beer varieties were significantly ($p<0.05$) high as compared to B-0. (Table 2). The DPPH radical scavenging potential of B-100 was the highest for 0, 30, and 60 days of storage. The value for B-30 and B-60 was not significantly different ($p>0.05$) for fresh beer sample but was high for B-60 on 30 and 60 days of storage. The higher DPPH radical scavenging activities of pepper beer varieties might be due to higher antioxidant potential of peppers (Domínguez-Martínez et al., 2014; Jang et al., 2015). The reduced DPPH radical scavenging potential of beer varieties during storage might be due to lowered reducing power. The results were in agreement with those of the previous studies (Li et al., 2016; Yang et al., 2017).

The total phenol content (TPC) of pepper beer were significantly different ($p<0.05$) among different samples and also with storage time (Table 2). The TPC of B-100 was the highest on 0 and 30 days of storage whereas the lowest value was found for B-0. The TPC of fresh beer sample was increased with the amount of pepper added to fermentation mixture. With storage the TPC decreased for all samples variedly. The highest TPC on 60 days of storage was found for B-30 followed by B-60. High total phenol contents of pepper beer were probably caused by addition of green pepper which contained large amounts of phenolic compounds (Nagy et al., 2015; Byun et al., 2016). The polyphenols, flavonoids, and flavonols in alcoholic beverage are the reasons for antioxidant activities (Park and Lee, 2002). Since there are multiple mechanisms involved in alcoholic beverage preparation, no single assay could accurately measure all the antioxidants within the mixed or complex system (Hong et al., 2009). The reduced TPC of beer varieties during storage were in agreement with those of the previous studies (Li et al., 2016). The reduced TPC during storage might be related to raw materials and brewing processes involved (Piazzon et al., 2010) as well as the oxidation reaction of phenolics with free radicals or polymerization with proteins (Kuchel et al., 2006). Addition of green pepper prominently enriched the antioxidant potential and total phenolic contents of pepper beer.

Sensory characteristics Sensory characteristics of pepper beer varieties are significantly different ($p<0.05$) with the amount of green pepper used as well as the storage time (Table 3). Measurement of sensory characteristic is a key in evaluating the beer quality. Color values of B-0 for 0 and 30 days of storage was the highest among the beer varieties whereas the values were not significantly different ($p>0.05$) on 60 days of storage. The flavor value of pepper beer varieties on 30 days of storage were not significantly different ($p>0.05$), however the value in B-60 was found to be significantly high ($p<0.05$) on 60 days of storage. The overall acceptance of B-60 was significantly high ($p<0.05$) among beer varieties on 0 and 30 days of storage. In general, the overall acceptance of B-0 and B-100 were lower than those of B-30 and B-60. The results of overall acceptance showed that B-60 is the best pepper beer variety for fresh consumption as well as after storage for 30 or

60 days. Flavor differences suggest important factor for development and marketing opportunities of the alcoholic beverage products (Pickering and Cullen, 2008). The perception of oral sensations is complex and can be influenced by various factors like ethnicity (Prescott and Bell, 1995), gender and age (Mojet et al., 2003), overall health/medication (Schiffman, 2007), and other psychological and physiological factors.

Conclusion

Addition of green pepper into the beer brewing mixture enhanced the antioxidant potentials and the overall acceptance of pepper beer without producing any adverse effect on the basic chemical properties of beer varieties. As the overall acceptance value of beer is a key determinant for quality preference, the optimum proportion of green pepper to be added was 60 g per 10 L of water. The antioxidant potential of the pepper beer brewed with the optimum amount determined in the present study was also considerable. Results of this study suggest that green pepper could be used to enhance the antioxidant potential and the overall acceptance of beer.

Conflict of interest

The authors declare no conflict of interest.

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Table 1. Chemical characteristics of pepper beer during storage at 4℃

Sample[1)]	Storage time (day)	pH	Alcohol (%, v/v)	Titratable acidity[2)] (g/100 mL)
B-0	0	4.12±0.02[c3)]	4.02±0.02[d]	0.11±0.01[b]
	30	4.21±0.03[c]	4.09±0.03[d]	0.12±0.03[b]
	60	4.20±0.02[c]	4.08±0.02[c]	0.16±0.02[c]
B-30	0	4.10±0.01[c]	4.17±0.03[b]	0.17±0.01[a]
	30	4.13±0.01[d]	4.19±0.02[c]	0.18±0.02[a]
	60	4.01±0.01[d]	4.35±0.03[b]	0.19±0.02[b]
B-60	0	4.31±0.02[b]	4.21±0.04[a]	0.18±0.01[a]
	30	4.33±0.02[b]	4.43±0.01[a]	0.20±0.01[a]
	60	4.30±0.02[b]	4.48±0.02[a]	0.24±0.01[a]
B-100	0	4.65±0.02[a]	4.16±0.01[c]	0.16±0.02[a]
	30	4.61±0.04[a]	4.30±0.01[b]	0.18±0.01[a]
	60	4.51±0.03[a]	4.31±0.01[b]	0.20±0.01[b]

[1)]B-0: Ordinary beer containing no added pepper, B-30: pepper beer containing 30 g of pepper in 10 L of water, B-60: pepper beer containing 60 g of pepper in 10 L of water, and B-100: pepper beer containing 100 g of pepper in 10 L of water.

[2)]As lactic acid.

[3)]Values are expressed as Mean±SD of three replicates. Values followed by different superscripts for same day of storage within column indicate significant difference (p<0.05).

Table 2. DPPH radical scavenging activities and total phenols content of pepper beer during storage at 4℃

Sample[1)]	Storage time (day)	DPPH[2)] (% Inhibition)	Total phenol content (μg GAE[3)]/mL of sample)
B-0	0	65.71±1.96[c4)]	723.18±4.21[d]
	30	52.78±2.71[b]	566.98±7.21[d]
	60	40.77±2.33[c]	320.11±2.99[d]
B-30	0	77.51±2.31[b]	1,009.70±2.77[c]
	30	55.26±1.67[b]	821.27±4.12[b]
	60	51.71±3.17[b]	666.21±3.71[a]
B-60	0	78.27±1.23[b]	1,190.91±6.71[b]
	30	70.00±2.92[a]	767.31±28.21[c]
	60	60.16±2.71[a]	650.21±7.21[b]
B-100	0	83.97±1.33[a]	1,321.82±3.32[a]
	30	70.17±1.19[a]	892.12±4.32[a]
	60	66.00±2.31[a]	612.31±2.71[c]

[1)]Samples are defined in Table 1.

[2)]DPPH free radical scavenging activity.

[3)]Gallic acid equivalent.

[4)]Values are expressed as Mean±SD of three replicates. Values followed by different superscripts for same day of storage within column indicate significant difference (p<0.05).

Table 3. Sensory characteristics of pepper beer during storage at 4℃

Sample[1)]	Storage time (day)	Sensory characteristics[2)]		
		Color	Flavor	Overall acceptance
B-0	0	4.21±0.11[a3)]	3.61±0.11[c]	3.61±0.17[c]
	30	4.31±0.21[a]	3.60±0.09[b]	3.65±0.05[c]
	60	4.22±0.16[a]	3.69±0.21[b]	3.66±0.04[b]
B-30	0	4.01±0.21[b]	3.99±0.03[b]	4.12±0.08[b]
	30	4.00±0.31[c]	3.89±0.31[a]	4.17±0.04[b]
	60	4.21±0.21[a]	3.77±0.34[b]	4.20±0.15[a]
B-60	0	3.87±0.10[c]	4.23±0.16[a]	4.31±0.09[a]
	30	4.12±0.21[b]	4.22±0.18[a]	4.51±0.03[a]
	60	4.30±0.11[a]	4.34±0.09[a]	4.41±0.20[a]
B-100	0	3.88±0.17[c]	4.00±0.12[ab]	3.51±0.15[c]
	30	4.12±0.16[b]	4.11±0.01[a]	3.21±0.11[d]
	60	4.21±0.19[a]	4.00±0.05[b]	3.23±0.09[c]

[1)]Samples are defined in Table 1.

[2)]Quoted values are mean±SD of triplicate experiments (n=20) based on 5 points score (very poor, 1; poor, 2; fair, 3; good, 4; and very good, 5).

[3)]Values are expressed as Mean±SD of three replicates. Values followed by different superscripts for same day of storage within column indicate significant difference (p<0.05).