

RESEARCH ARTICLE

Effect of pH modulation on the physicochemical characteristics of chicken bone extract

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ABSTRACT

This study investigated the effect of brown rice-vinegar-induced changes in pH on the physicochemical properties (ash, moisture, protein, fat, collagen, chondroitin sulfate, sugar, and mineral content, color, salinity, and pH) of chicken bone extract. The following treatments were employed: Control (purified water, 62%; chicken bones, 30.47%; chicken feet, 6.88%; garlic, 0.58%; pH 7), T1 (purified water, 62%; chicken bones, 30.47%; chicken feet, 6.88%; garlic, 0.58%; organic brown rice vinegar, 0.01%; pH 6), T2 (purified water, 62%; chicken bones, 30.47%; chicken feet, 6.88%; garlic, 0.58%; organic brown rice vinegar, 0.02%; pH 5), and T3 (purified water, 62%; chicken bones, 30.47%; chicken feet, 6.88%; garlic, 0.58%; organic brown rice vinegar, 0.23%; pH 4). Protein, ash, and chondroitin sulfate contents and the brightness tended to increase, while the yellowness and redness, sugar content, salinity, and pH tended to decrease when the percentage of brown rice vinegar was increased. The collagen content was significantly higher in the control and T3 groups. Further, the Ca content tended to increase, while K, Mg, N, and P contents tended to decrease with increasing percentage of brown rice vinegar. The Ca/P ratio improved as the percentage of brown rice vinegar increased. At a concentration of 0.02%, brown rice vinegar effectively improved the overall quality of chicken bone extract in terms of the investigated physicochemical properties (minerals being the exception). Therefore, use of 0.02% brown rice vinegar during the preparation of chicken bone extract would result in the most ideal physicochemical properties.

Keywords: Chicken bone; Brown rice vinegar; pH; Collagen; Chondroitin sulfate.

INTRODUCTION

In recent years, the amount of meat consumed per capita is increasing worldwide due to population growth and an increase in average personal income (Godfray, 2010). As the rate of economic growth varies from region to region, meat consumption in low-income countries is low on average, but is significantly increasing in middle-income countries, and is stagnating in high-income countries (Godfray, 2010). The population of Asia accounts for more than 60% of the world's population, and meat production increased by 252.47% (from 38.5 mt to 135.7 mt) from 1985 to 2014 due to rapid economic and population growth. In particular, from 2004 to 2014, the rate of chicken meat production was 52.73%, which was a significant increase compared to that for other meats (Zhang, 2017).

Owing to the increasing demand for processed chicken meat, many chicken by-products, such as chicken feet,

bones, and feathers, are disposed off or used as feed or fertilizer for livestock. In addition to waste generation and environmental pollution, the disposal of these by-products leads to the loss of biological resources, such as useful fats and proteins. Researchers have studied various techniques for utilizing these chicken by-products (Lasekan, 2013). For example, in a previous study, researchers attempted to extract Ca and bioactive peptides from the bone and meat residues obtained by processing chicken feet (Malison, 2021) and also conducted studies to extract calcium from chicken bones and use it as food fortificant (Kettawan A et al., 2002). In another study, researchers extracted keratin from chicken feathers (Gupta, 2011), while in another, researchers converted chicken bone into peptone using pancreatic enzymes (Wang, 2016).

Among chicken by-products, bones contain many functional compounds, such as collagen, chondroitin sulfuric acid, essential amino acids, minerals, and vitamin

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B12, which are widely used as food and medicine for food-producing animals and pets (Jayathilakan et al., 2012; Rivera et al., 2000; Schiraldi et al., 2010).

As the main component of bone and cartilage, collagen extracted from chicken bones is an important source of useful bioactive peptides (Saiga et al., 2008). In particular, collagen has a gel-forming property and is widely used in the food and pharmaceutical industries (Gómez-Guillén et al., 2011). To efficiently extract and utilize collagen and gelatin, researchers have studied the quality of gelatin extracted from chicken head bones (Ee et al., 2019) and the functional properties of collagen and gelatin extracted from chicken head bones (Du et al., 2013).

Chondroitin sulfate, which is commonly extracted from chicken bones, is one of the major components of cartilage and is abundant in invertebrates and mammals. It plays a role in maintaining cartilage structure and function (Schiraldi et al., 2010; Watanabe et al., 1998; Malavaki et al., 2008). Researchers employed hydrothermal static adsorption extraction to effectively extract chondroitin sulfate from chicken bones (Wang et al., 2019). In another study, researchers also determined whether a sufficient amount of chondroitin sulfate can be obtained from the keel of chickens (Luo et al., 2002).

Different extraction conditions are employed to extract collagen and other proteins from bones depending on the pH (Du et al., 2013; Gerzhova, 2016; Kazeniak, 1961; Park et al., 2005; Zeugolis, 2007). As a result, studies have been conducted to determine the factors that affect flavor by subjecting chicken bones to extraction at different pHs (Pippen, 1965). In addition, a study was conducted to analyze the amino acid composition of protein coagulum precipitated in discarded bovine bone extract prepared at varying pHs (Golan & Jelen, 1979).

Among organic natural substances, brown rice vinegar has a pH of 2.55–3.24; has a higher content of glutamic acid, alanine, valine, isoleucine, leucine, and arginine than other vinegars; and contains organic acids, such as acetic, oxalic, tartaric, and malic acids. In addition, brown rice vinegar is a grain-fermented vinegar that is effective in promoting digestion and food consumption (Jeong, 2009; Moon et al., 1997). Brown rice vinegar exhibits anti-diabetic, anti-obesity, blood pressure-lowering, anti-aging, and anti-tumor effects (Joo et al., 2009) and can enhance the flavor of the chicken bone extract (Lu, 2018).

Although studies have been conducted to extract functional substances by adjusting pH when preparing the bone extract, studies analyzing changes in the physicochemical properties of extracts—when preparing the chicken bone

extract—based on pH changes using natural or organic substances that are acceptable to modern consumers are limited. Therefore, this study analyzed how the physicochemical parameters of the chicken bone extract vary according to the changes in pH during the preparation of the chicken bone extract using organic brown rice vinegar. We further identified the optimal pH of chicken bone extraction and determined the suitability of chicken bone extract for use as a food or functional substance.

MATERIALS AND METHODS

Materials and preparation of chicken bone extract

Organic chicken bones and chicken feet provided by ORGE Co., Ltd. were mixed at a 5:1 ratio and immersed in water at 32° C for 40 min to remove blood (Fig 1A). The primary extract was prepared by mixing chicken bones (30.47%), chicken feet (6.88%), garlic (0.58%), and purified water (62.00%) using a pressure extractor at 121°C for 8 h (Fig 1B). Distilled water (pH 7) was used as purified water. To compare and analyze how the physicochemical parameters of the chicken bone extract varied according to changes in pH, the pH of the purified water was adjusted using brown rice vinegar (Hansalim Korean Co., Ltd.) (Fig 1C). The second extract was prepared under the same conditions used for the first round of extraction and employed the residual bone left after the primary extraction. Based on the adjustment of the pH of purified water, the following treat groups were used: Control (purified water, 62%; chicken bones, 30.47%; chicken feet, 6.88%; garlic, 0.58%; pH 7), T1 (purified water, 62%; chicken bones, 30.47%; chicken feet, 6.88%; garlic, 0.58%; organic brown rice vinegar, 0.01%; pH 6), T2 (purified water, 62%; chicken

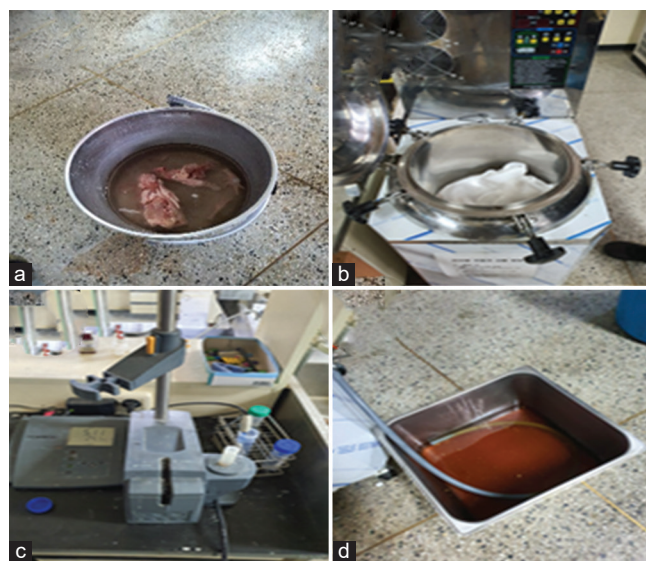


Fig 1. Blood removal (a), extraction (b), pH adjustment (c), Chicken bone extract (d)

bones, 30.47%; chicken feet, 6.88%; garlic, 0.58%; organic brown rice vinegar, 0.02%; pH 5), and T3 (purified water, 62%; chicken bones, 30.47%; chicken feet, 6.88%; garlic, 0.58%; organic brown rice vinegar, 0.23%; pH 4) (Fig 1D). Afterward, the chicken bone extract was stored at -25°C and used for subsequent experiments.

Physicochemical analysis of the extract

Analysis method

In this study, three replicates were established for each sample, and the average value of the results of the three was reported as the result.

Measurement of general components

Moisture, crude protein, crude fat, and ash (%) contents were measured in accordance with the AOAC method (1995).

Collagen measurement

Approximately 4 g of sample and 30 ml of sulfuric acid were mixed in an Erlenmeyer flask, covered, and heated in a dry oven at 105°C for 16 h. The obtained sample was then placed in a 500 ml volumetric flask, diluted with triple-distilled water for homogenization, and filtered using Whatman No. 2 φ150 mm filter paper. Next, 5 ml of the filtrate was diluted to 100 ml and then 2 ml of the diluted solution was added to a test tube, followed by the addition of 1 ml of oxidant solution, shaking, and allowing to stand for 20 min at room temperature (20~25°C). Next, 1 ml of a color reagent was added to each test tube and mixed, followed by incubation in a 60°C water bath for 15 min and cooling with running water for at least 3 min. The absorbance was then measured at 558 nm using a spectrophotometer. For standard curve generation, 2 ml of the working standard solution was subjected to color development and color measurement, and then absorbance was measured. The collagen content (g/100 g) was analyzed by substituting it into the regression equation.

Chondroitin sulfate measurement

Chondroitin sulfate content was measured according to the method of food code (1999). Briefly, 5 ml of sodium borate sulfuric acid reagent (sodium borate 1.0 g/sulfuric acid 200 ml) was added to the test tube and cooled on ice for 10 min. Thereafter, 1 ml of the sample was carefully added, mixed, cooled on ice for 2 min. Next, 1 ml of the mixture was heated in boiling water for 10 min and cooled again in ice water for 5 min, followed by the addition of 0.2 ml of carbazole (0.125 g/100 ml ethanol) and heating for 15 min in boiling water. After cooling for 2 min in ice water, the absorbance was measured at 530 nm using distilled water as a control. The measured absorbance value was extrapolated into the glucuronic acid standard curve to obtain the amount

of glucuronic acid in the sample, followed by the calculation of the chondroitin sulfate content (%) using the following equation: Chondroitin sulfate content (%) = [$\frac{\text{volume of glucuronic acid in the sample}}{\text{sample volume}} \times 2.593 \times 100$].

Chromaticity measurement

For chromaticity measurement, L (brightness), a (redness), and b (yellowness) were measured using a spectrophotometer (Model JX-777, Color Techno. System Co., Japan) standardized using a white plate (L, 89.39; a, 0.13; b, -0.51)

Salinity and sugar content measurement

One millimeter of the sample was taken and salinity was measured using a salinity meter (TM-30, 0–30%, TAKEMURA, Japan). For sugar content measurement, 1 ml of the sample was dropped onto a sugar content meter (PR-101, ATAGO, Japan). The experiment was performed in triplicate and the average value is presented.

pH measurement

The pH was measured after adding 100 ml of distilled water to 10 g sample. All samples were homogenized at 7,000 rpm for 30 s using a Homogenizer (Bihon seiki, Ace, Japan), and then the pH was measured using a pH meter (Mettler Delta 340, Mettler Toledo, Ltd., UK).

Mineral measurement

The Ca, P, Na, K, and Mg contents were measured by inductively coupled plasma light emission spectroscopy using ICP-OES (Optima 5300DV, PerkinElmer, USA).

Statistical analysis

All experiments were repeated at least three times, and the statistical processing program SAS 9.4 (Windows, USA) was used to test the significance of the measurements. To compare the significant difference between the measured values, significance was tested at the 5% level using the Duncan's multiple range test.

RESULTS AND DISCUSSION

Physicochemical characteristics of the chicken bone extract according based on the percentage of brown rice vinegar used

Ash, moisture, protein, and fat contents

Table 1 shows the general components observed on preparing chicken bone extract with different percentages of brown rice vinegar. The crude ash content was the lowest in the control group, but significantly higher in the T1 and T2 groups. The moisture content did not show a significant difference between the extracts. The content of

crude fat was the highest in T2, but there was no significant difference between extracts. The content of crude protein was the highest in T2, and the content of crude protein tended to increase as the percentage of brown rice vinegar increased. This difference in crude protein content of chicken bone extract was similar to the results obtained by Cho(1989) who reported that the protein content increased as the pH decreased; however, in our study, the T3 group (lowest pH) had less protein content than the T1 and T2 groups. The protein in chicken bone extract is not only derived from bones, but also from the skin and muscles

Table 1: Proximal analysis of chicken stock with pH modulation by brown rice vinegar (%)

Treatments	Ash	Moisture	Fat	Protein
C	0.06±0.00 ^b	98.56±1.01	0.04±0.01	1.33±0.02 ^d
T1	0.12±0.00 ^a	97.90±0.83	0.03±0.00	1.95±0.00 ^b
T2	0.11±0.01 ^a	97.33±0.33	0.05±0.00	2.51±0.01 ^a
T3	0.07±0.00 ^b	98.23±0.19	0.03±0.00	1.66±0.00 ^c

a-d Means with different superscripts represent differences in treatments (p<0.05)

Table 2: Collagen and chondroitin sulfate of chicken stock with pH modulation by brown rice vinegar

Treatments	Collagen (g/ml)	Chondroitin sulfate (mg/ml)
C	1.35±0.05 ^a	133.79±11.59 ^b
T1	1.18±0.07 ^{bc}	139.38±4.51 ^{ab}
T2	1.12±0.03 ^c	144.78±1.87 ^{ab}
T3	1.25±0.03 ^b	144.58±7.55 ^a

a-c Means with different superscripts represent differences in treatments (p<0.05)

Table 3: Hunter's color values of chicken stock with pH modulation by brown rice vinegar

Treatments	L	a	b
C	35.18±0.08 ^d	2.90±0.25 ^a	19.85±0.51 ^a
T1	37.95±0.03 ^b	1.22±0.06 ^b	15.30±1.20 ^c
T2	38.78±0.07 ^a	1.36±0.09 ^b	17.05±0.26 ^b
T3	36.72±0.11 ^c	0.50±0.19 ^c	13.26±0.51 ^d

a-d Means with different superscripts represent differences in treatments (p<0.05)

Table 4: pH, salinity and sugar content of chicken stock with pH modulation by brown rice vinegar

Treatments	pH	Salinity (%)	Sugar content (%)
Control	6.01±0.02 ^a	3.07±0.21 ^a	3.87±0.12 ^a
T1	5.52±0.01 ^b	2.13±0.15 ^c	2.5±0 ^c
T2	5.44±0.05 ^c	2.06±0.1 ^b	2.87±0.12 ^b
T3	4.98±0.01 ^d	2.03±0.06 ^c	2.63±0.23 ^{bc}

a-d Means with different superscripts represent differences in treatments (p<0.05)

Table 5. Mineral contents of chicken stock with pH modulation by brown rice vinegar (mg/ml)

Treatments	Ca	K	Mg	Na	P	Ca/P
C	15.25±4.40	383.88±0.65 ^a	19.91±0.08 ^a	233.38±0.51 ^a	191.53±0.17 ^a	0.08±0.02 ^b
T1	22.28±8.97	352.55±2.24 ^b	15.42±0.36 ^c	191.87±2.56 ^b	168.12±0.94 ^b	0.13±0.05 ^{ab}
T2	17.17±1.33	272.94±1.60 ^c	18.87±0.11 ^b	161.12±0.98 ^c	136.12±0.76 ^c	0.13±0.01 ^{ab}
T3	20.56±5.84	191.10±5.71 ^d	15.45±0.38 ^c	119.42±2.39 ^d	117.70±3.23 ^d	0.18±0.05 ^a

a-d Means with different superscripts represent differences in treatments (p<0.05)

(Wang, 2016). Therefore, the result that T3, which had the lowest pH, had less protein content than did T1 and T2 may be attributed to the difference in the degree of bone growth of the chicken bones received from the factories that provided the raw materials.

Collagen-derived protein and chondroitin sulfate content

Table 2 depicts the collagen-derived protein and chondroitin sulfate contents in chicken bone extract prepared using different percentages of brown rice vinegar. Collagen is present in large amounts in the connective tissue and bone and is a major bone protein. Hydroxyproline, an amino acid found in collagen—by constituting a certain ratio (12.5–14%) with hydroxylysine—is used as an index to quantify collagen (Weiss JB, 1982). In this study, the collagen content was determined by measuring the hydroxyproline content, so it was expressed as a collagen-derived protein. The content of collagen-derived protein in chicken bone extract was significantly higher in the control and T3 groups and significantly lower in the T1 and T2 groups. The chondroitin sulfate content in chicken bone extract was significantly higher in all treatments compared to that in the control, and the content of chondroitin sulfate tended to increase as the percentage of brown rice vinegar decreased. Researchers have shown that collagen can be easily extracted from rat tail tendons when the rat tail tendons are pretreated under weak acidic conditions (Zeugolis, 2008), and when chicken bones were treated at neutral pH (pH, 7) with a single acid, the content of collagen increased (Ee, 2019), which is consistent with the results of this study in which the control (pH, 7) and T3 groups had higher collagen contents than did the other two treatments. Liu et al. (2007) reported that a weakly acidic pH is optimal for the proteolytic extraction of chondroitin sulfate. This is similar to the observation in this study, in which the chondroitin sulfate content was significantly higher in the weakly acidic treatment group than that in the neutral control group.

Chromaticity

Table 3 depicts the chromaticity of chicken bone extract prepared with different percentages of brown rice vinegar. The L value was the lowest in the control group and significantly higher in the T2 group. The a value was the highest in the control, and value tended to decrease as the percentage of brown rice vinegar decreased; the lowest a value was observed in the T3 group. The b value was the

highest in the control and the lowest in the T3 groups. Consistent with the results of Woo (2010) who found that the L value increased owing to increased acidity when chicken bone extract was prepared by adding tomatoes. In addition, the results of this study were similar to those of a study in which the L value of all groups increased and the b value decreased compared to those in the control group when duck bone extracts were prepared by adding malic acid (Kim, 2011). It is thought that the tendency of the L value to increase in the brown rice vinegar-added group (compared with the control group) was due to the increase in solid content in response to the addition of brown rice vinegar.

pH, salinity, and sugar content

Table 4 depicts the pH, salinity, and sugar content of chicken bone extracts prepared using different percentages of brown rice vinegar. The pH of the extract tended to decrease as the percentage of brown rice vinegar increased, and significant differences were observed in all extracts with respect to the pH. This difference in pH is similar to that observed by Kim et al. (1999); they showed that the pH of the broth decreased as the amount of organic acid added increased. The salinity of the extract was the highest in the control group and tended to decrease as the percentage of brown rice vinegar decreased. This difference in salinity is attributed to a decrease in the content of minerals, such as Na, in the extract as the pH decreases. The sugar content of the extract was significantly higher in the control group, and the lowest content was observed in the T1 group. Eastoe (1954) identified that the presence of various amino acids, such as alanine, valine, isoleucine, leucine, and arginine, decreased the sugar content of the hydrolysate during the preparation of chicken bone extract. As brown rice vinegar is rich in amino acids, such as alanine, valine, isoleucine, leucine, and arginine, it is thought that the treated groups tended to have a lower sugar content than the control group without brown rice vinegar.

Minerals

Table 5 depicts the mineral content of chicken bone extract prepared using varying percentages of brown rice vinegar. There was no significant difference in Ca content in all samples, but as the percentage of brown rice vinegar increased, the Ca content in the treated groups increased compared to that in the control group, consistent with the results of Lee (2002) and others who reported that lowering the pH using organic acid (vinegar) during the preparation of chicken bone extracts results in increased Ca content. The K content was significantly higher in the control group and exhibited a tendency to decrease as the percentage of brown rice vinegar increased. The Mg content was significantly higher in the control and significantly lower in the T1 and T3 groups. The Na content was the highest in the control group and showed a tendency to decrease as the percentage of

brown rice vinegar increased. The P content was the highest in the control group and showed a tendency to decrease as the percentage of brown rice vinegar increased. Park et al. (1983) concluded that phytic acid, an ingredient in brown rice, combines with minerals to form insoluble substances, thereby explaining why the contents of minerals, such as K, Mg, Na, and P, showed a tendency to decrease. The Ca/P ratio was the highest in the T3 group and tended to increase as the percentage of brown rice vinegar increased. According to Park et al. (1983), intake of high levels of P inhibited the absorption of Ca. The results of this study showed that the P content was higher than the Ca content. It is thought that the amount of P extracted was more than that of Ca due to the heating of the bone for a long time (Kim, 2006). Bell et al. (1977) reported that skeletal loss occurs when P uptake is increased and Ca/P ratios are < 0.5 . In this study, the Ca/P ratio in all samples was < 0.5 , which is different from the ideal ratio of Ca/P; however, the Ca/P ratio improved as the pH decreased.

CONCLUSIONS

In this study, we employed varying amounts of brown rice vinegar (0, 0.01, 0.02, and 0.23%) for preparing chicken bone extracts, and analyzed the collagen, chondroitin sulfuric acid, sugar, and mineral contents, color, pH, and salinity of the extracts. The optimal pH for bone extract preparation and the beneficial physicochemical properties of chicken bone extract were investigated to determine whether the chicken bone extract could be used as a functional food. Results showed that the protein content of the control group without the addition of brown rice vinegar was 1.33 (the lowest) and the protein content of the chicken bone extract increased significantly as the percentage of brown rice vinegar increased. The ash content of the control group without brown rice vinegar was the lowest (0.06), and the ash content of chicken bone extract increased significantly as the percentage of brown rice vinegar increased. content was significantly increased in the control group without brown rice vinegar and in the T3 group with 0.23% brown rice vinegar. Chondroitin sulfate content was significantly increased as the percentage of brown rice vinegar increased. With respect to the chromaticity, as the percentage of brown rice vinegar increased, the L value increased, and the a, b value decreased. The pH of the chicken bone extract was significantly decreased as the percentage of brown rice vinegar increased. As the percentage of brown rice vinegar increased, the salinity and sugar content tended to decrease significantly compared to those in the control group. With respect to minerals, as the percentage of brown rice vinegar increased, the content of all minerals except that of Ca, tended to decrease, and the Ca/P ratio tended to increase.

Taken together, we found that the addition of an appropriate amount of brown rice vinegar during the preparation of chicken bone extract promotes the ash, protein, collagen, chondroitin sulfate, and Ca contents, making the chicken bone extract a useful functional food. This study provides a foundation for further studies as there are only few studies that have analyzed the physicochemical properties of chicken bone extracts prepared via pH adjustment. In future studies, a storage test according to pH control will be included when preparing chicken bone extracts to further support the benefits of adding brown rice vinegar during the preparation of chicken bone extracts, thereby addressing the limitations in preparing chicken bone extract (chicken stock) products.

Authors' contributions

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Writing - review & editing: Sanghun Lee, Yunhwan Park, Sanghun Park, Yun-a Kim, Gyutae Park, Sehyuk Oh, Yoonsik Kim, Jungseok Choi.

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