PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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Pakistan Journal of Nutrition 14 (3): 174-179, 2015
ISSN 1680-5194

Chemical Characteristics of Collagen Extract from Scapula of Bali Cattle (*Os scapula*) Produced Using Different Extractant

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Abstract: Collagen is a hydrocolloid products of animal protein resulted in partial hydrolysis. Cow bones abattoir by-product is a product that is rich in collagen proteins, thereby it's potentially as a source of raw materials. The protein collagen is now widely used as a dietary supplement. This study aimed to evaluate the chemical properties of the extract collagen product from *os scapula* of Bali cattle through the application of some types of extractant in the production process. Laboratory experimental method was used in this study. A total of four types of extractant was applied, namely: (1) distilled water, (2) ethanol 60% (v/v), (3) CH_3COOH 0.5 M and (4) $Ca(OH)_2$ 0.5 M. The pattern of Complete Randomized Design (RAL) of Undirectional pattern and the analysis of variance was used as the study design and data analysis, while Duncan's Multiple Range Test (DMRT) was used as a further test if it was significant. The results showed that the use of different extractant extracts on the collagen production processes using raw materials from *os scapula* of Bali cattle affected ($p<0.05$) the value of the yield, moisture content, protein content and calcium levels, but there was no significant effect ($p>0.05$) on lipid levels and phosphorus levels. Extract collagen from *os scapula* of Bali cattle produced using four types of extractants have functional properties identical functional groups based on the presence of CO, C-O, OH, CH and N-H bond.

Key words: Collagen zextra, *os scapula*, Bali cattle, extractant, chemical aspect

INTRODUCTION

The structure of cattle bone is rich of protein collagen compounds that are strongly bound to the minerals calcium (Ockerman and Hansen, 2000). Collagen is a structural protein that is only produced by the body tissues of cattle (Zeugolis *et al*., 2008) and recently, it has been widely used as a food supplement to prevent bone disease (*osteoporosis*), joint pain (*osteoarthritis*) and premature aging (Rogart *et al*., 1999; Tian *et al*., 2011). Until now the use of abattoir waste such as bone as the cheap collagen protein source in fact has not been widely explored.

The exploration of bone waste as a source of collagen is possible, because the collagen compound contained in cow bones appeared to have similarities in terms of chemical composition, morphology, distribution, function and pathology with the collagen compound found in the human body (Junqueira *et al*., 1998). The exploration process of bioactive compounds such as collagen extract derived from bovine bone until now has not been much publicized yet. Therefore, this exploratory research has been conducted in the form of the optimization of the production processes of bone collagen compounds, especially Bali cattle bones in the shoulder blade (*os scapula*) in the form of extracts instant readily consume. The results of the initial study showed that the shoulder blade (*os scapula*) of Bali cattle have the potential for higher protein content (39.4%) compared to other parts such as the spinal bones (*os vertebrae*) (37.56%), thigh bone (*os femur*) (24.06%) and lower leg bone (*os tibia-fibula*) (Said *et al*., 2012).

The optimization of the production process allows the bioactive compounds in the form of collagen can be done through an appropriate extractant engineering use. The use of an appropriate extractant in the production process of collagen extracts, will possibly produce the maximum quantity and quality of the product (Wang *et al*., 2008; Zeugolis *et al*., 2008). This study aimed to evaluate the chemical properties of the product of extract collagen from the raw materials of shoulder blade (*os scapula*) of Bali cattle through the application of some type of extractants in the production process.

MATERIALS AND METHODS

Materials: (1) distilled water, (2) ethanol 60% (v/v) (Merck), (3) CH_3COOH 0.5 M (Merck) and (4) $Ca(OH)_2$ 0.5

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M (Merck) was used as extractant. Water bath (Memmert Type WPE-45), digital oven (Memmert), analytical balance (PS RADWAG 600/C/2), beaker glass (Duran), glass funnel, measuring cup (Duran), thermometer and the devices for proximate analysis.

Preparation process of extractant: A total of 450 ml of 4 (four) types of extractant (1) distilled water, (2) ethanol 60% (v/v), (3) CH_3COOH 0.5 M and (4) $Ca(OH)_2$ 0.5 M was prepared. Each extractant was put in a glass beaker 1000 mL size and placed in a water bath with the temperature setting on it.

Production process of collagen extract: The multi extraction technique (Ockerman and Hansen, 2000) (modified) is used to produce collagen extract. The raw materials: shoulder blade (*os scapula*) was cut with the size of 1-2 cm and then washed. 60% (v/v) ethanol was used as a decreasing material (fat removal process) for 2x2 h with bone ratio: 60% ethanol (1:1.5) 0.5M H_2SO_4 solution is used as a material demineralization (removal of mineral components) for 48 h with bone ratio: 0.5M H_2SO_4 (1:1.5). an ossein is then neutralized with $Ca(OH)_2$ 10% for 24 h with bone ratio: $Ca(OH)_2$ 10% (ratio of 1:1.5). A total of 300 g sample of the bone was used and then put in 4 types of extractant solution that was created prior to bone ratio: extractant (1:1.5) (v/v). Extracted bone samples were stratified for 48 h (stage 1:24 h, temperature 55-60°C) resulted fraction 1 and stage 2:24 h, temperature 65-70°C resulted fraction 2. Results fractions 1 and 2 are combined and then filtered with a flannel cloth to produce a filtrate. The filtrate was then dried with an oven temperature of 55-60°C for 48 h to obtain a dense collagen extract. The collagen extract pulverized in a blender to further evaluate its properties).

Method of analysis: Yields (Y) (%) (Gimenez *et al*., 2005b). Yields were determined by the formula Y = ECW/BS x 100%, where Y = Yields (%); ECW = Extracts Collagen Powder (g); BS = Bone Samples (g).

Water content (Wc) (%) (AOAC, 2005). Water content was determined by the oven method. Container was emptied in oven (100-105°C, +1 h), cooled in a desiccator for 30 min, then weighed. A total of ±0.5 g of sample ECW was put into the cup, oven (100-105°C, 24 h) until its weight was constant. The sample in the cup was cooled in a desiccator for 15 minutes and then weighed. Water content was determined by the formula:

$$Wc = Ws\text{-}Ds/Ws \times 100\%$$

where, Ws = ECW wet (g),
Ds = ECW dry (g)

Protein content (Pc) (%) (AOAC, 2005). It was determined by the Kjeldahl method. A total of 0.5 g of pulverized sample was put in a 100 mL Kjeldahl flask and the mixture of 1 g of selenium and 10 mL of concentrated H_2SO_4 was added. The solution was then destructed until clear. Once cool, then poured into 100 mL volumetric flask, rinsed with distilled water. A total of 5 mL of solution was pipetted and added 5 mL of 30% NaOH solution and distilled water. The flask reservoir containing 10 mL of 2% H_3BO_3 plus 4 drops of indicator prepared and distilled to a volume of approximately 50 mL. The result was then titrated with 0.0222 N HCl or H_2SO_4. Pc defined by the formula = V x N x 0.014 x 6.25 x P/weight of sample x 100%, where V=volume of titration, N = Normality of HCl or H_2SO_4, P = Factor diluents (100/5).

Fat content (Fc) (%) (AOAC, 2005). It was determined by the Soxhlet method. A total of 2 g of the sample was wrapped in filter paper and put into the Soxhlet flask. The flask was previously dried in an oven with the temperature of 105°C for 2 h and cooled in a desiccator for 30 min. Petroleum ether solution was put in a flask soxhlet already filled with the sample. Reflux process was done using a water bath for ±3 h. The flusk contains the results of reflux oven for ±1 h at a temperature of 105°C and cooled in a desiccator for further weighed. Fat content was calculated using the formula:

$$Fc = Wf/Ws \times 100\%$$

where, Wf = weight of fat (g),
Ws = weight of sample (g) x 100%.

Calcium level (Ca) (%) (AOAC, 2005). It was determined from the results of the determination of ash content. Ash sample was added 3 mL of concentrated HCl, diluted with 0.5 mL of distilled water. The solution was poured into 100 ml flask using a funnel and filter paper, distilled water added to the limit. A solution of 20 mL pipetted into a glass 100 mL, plus a red indicator and NH_4OH 1:1 to orange or yellowish. Added a solution of HCl 1: 3 to a red again. The solution was heated and added 15 cc of ammonium oxalate 4%, then heated, the precipitate was next filtered. Filter paper was rinsed with hot water, dried, then was put into erlenmeyer 100 mL and added 5 cc of concentrated H_2SO_4. The solution was heated at 70-80°C and titrated with 0.1000 N $KMnO_4$ to a red color. Calcium level was calculated based on the formula:

$$(P \times a \times N\ KMnO_4 \times 20)/(mg\ of\ sample) \times 100\%$$

where, P = Dilution (100/20 = 5):
a = volume of titrant, N = 0.1000.

Phosphorus level (Ph) (%) (AOAC, 2005). It was determined from the results of the determination of ash content. Ash sample was added with 3 mL of concentrated HCl, diluted with 0.5 mL distilled water. The solution was poured into 50 mL volumetric flask and added with 3 mL of a solution of ammonium molibdad

and 2.5 mL of vitamin C, plus distilled water to the limit, then homogenized. The solution was read with a spectrophotometer (λ = 570 ηm). Phosphorus level was calculated with the formula:

(A x 10,97)-0.0475 x 500/weight of sample (mg)

where, A = absorbance in a spectrophotometer.

Functional groups (Sastrohamidjojo, 1992); (Sastrohamidjojo, 2001). KBr pellet method (Potassium Bromide) with the Spectrophotometry Fourier Transform Infrared (FTIR) (Shimadzu PC-8201) at wave numbers 4000-650/cm was used as a determinant of functional groups. A total of 0.1-2% by weight of pulverized samples under infrared lamps together with KBr, pressed at a pressure of 8-20 tons/unit area to obtain the form of pellets, vacumised. The result of the wave spectrum was then read on the monitor.

Data analysis: Laboratory experimental method was used in this study. A total of four types of extractant was applied, namely: (1) distilled water; (2) ethanol 60% (v/v); (3) CH_3COOH 0.5 M and (4) $Ca(OH)_2$ 0.5 M. Complete Randomized Design (RAL) Unidirectional pattern and analysis of variance (ANOVA) was used as the study design and data analysis, while Duncan's Multiple Range Test (DMRT) (Steel and Torrie, 1980) was used as a further test if the treatment significantly influenced the result.

RESULTS AND DISCUSSION

Yields: The yield of collagen was defined as the amount of collagen extracts produced from a number of raw materials in a clean state obtained through the extraction process (Gimenez *et al*., 2005a). The results of the extraction process through the different types of extractant showed the value of the yields as in Fig. 1.

Results of the data analysis of variance in Fig. 1 shows that the application of different types of extractant was highly significant ($p<0.01$) on the yields value. The use of 0.5M CH_3COOH extractant resulted in the highest yields value (12.95%), compared to the other solvents was 4.31% (distilled water), 1.90% (60% ethanol) and 5.84% ($Ca(OH)_2$ 0.5M). Therefore, it can be explained that the use of 0.5M CH_3COOH extractant in the production of collagen extract is the most efficient production process. The yields of the extract of collagen is the amount of collagen produced from a number of raw materials through the extraction process (Gimenez *et al*. (2005a). Sucrose content generated depends on the process used. The high yields value at the use of solvent acid (CH_3COOH 0.5M) can be caused by the ability of the material of the acid to loosen the bonds of the molecules constructed the bone collagen, so the collagen extracted becomes more leverage. The yields resulted from a production process is greatly influenced by the extraction process (solvent) on the activity of the

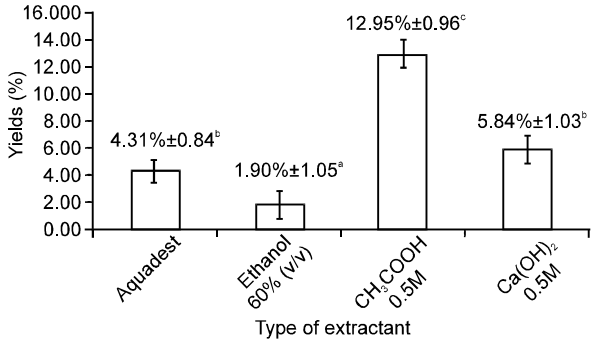

Fig. 1: Graph of the yields value (%) collagen extract of Bali cattle scapula produced using different types of extractant. a, b, c: significant differences ($p<0.01$)

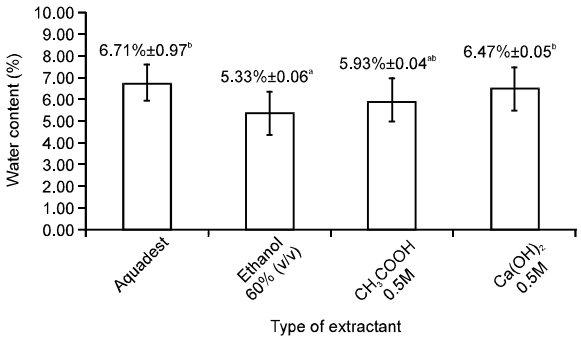

Fig. 2: Graph of the water content (%) collagen extract of Bali cattle scapula produced using different types of extractant. a, b: significant differences ($p<0.05$)

protein collagen (Kasankala *et al*., 2007). The increased acid concentration led to an increase in the concentration of H^+ ions in curing solution that will ultimately accelerate the process of hydrolysis. The faster rate of hydrolysis tends to increase the number of collagen molecule which is converted into gelatin that have an impact on increasing the value of the yields.

Water content: The overview on the comparison of water content of collagen extracts produced using different extractants is presented in Fig. 2.

Results of the data analysis of variance in Fig. 2 shows that the application of different extractant significantly influenced ($p<0.05$) the water content of collagen extract that use shoulder blade (*os scapula*) of Bali cattle as raw material. The application of different extractant related to changes in the content of the collagen extracts moisture during the production process. Extractant molecules function to hydrolyze the structure of amino acids that construct the bone protein so that it becomes very weak and eventually undergo the denaturation process (Muyonga *et al*., 2003). Denaturation process leads to changes in the molecular bond and the amount of

bound water, so that the molecular bond becomes weaker and decrease (Soeparno, 2005). It will then cause water molecules easily separated at the time of the subsequent drying process and lower the value of water content of the collagen.

Protein content: Collagen is a group of structural protein derived from extracellular matrix. Collagen extract has been widely used in the food and pharmaceutical industries (Karim and Bhat, 2008). Protein content of the collagen extract produced using different extractants is presented in Fig. 3.

Results of the data analysis of variance in Fig. 3 shows that the application of different types of extractant was significant ($p<0.05$) on the protein content of collagen extract that used shoulder blade (*os scapula*) of Bali cattle as the raw materials. The dilution process of collagen is affected by the solvent used. The differences of the extractant and an increase in the concentration of extractant increases the dissolved collagen (Wang *et al*., 2008). The use of acid as an extractant can cause the collagen fibrils of bone swell and broke and then hydrolyzed to the unit fibrils or macromolecular tropocollagen) (Leeson *et al*., 1995).

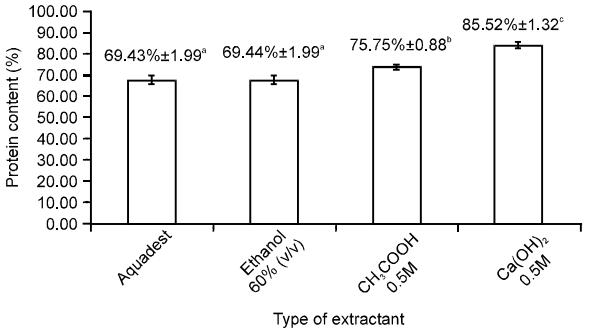

Fig. 3: Graph of the protein content (%) collagen extract of Bali cattle scapula produced using different types of extractant. a, b, c: significant differences ($p<0.05$)

Fat content: Level of fat in food product is directly related to the quality of the food (Winarno, 1997). Comparison of fat content of collagen extracts produced using different types of extractants is presented in Fig. 4.

Based on the data analysis of variance in Fig. 4, the use of different types of extractant does not affect ($p>0.05$) fat content of collagen extract (*os scapula*) of Bali cattle significantly. Fat contained in the extract collagen dominated by fat particles bound with proteins (lipoproteins) (Sarkar, 1995). The use of different extractant did not result in different levels of fat extracts. This can be caused by the influence of the washing process after immersion in the extractant. When the extraction process is carried out in the extractant solution, protein dissolved occurs followed by dissolving fat (Zeugolis *et al*., 2008; Wang *et al*., 2008). Fat soluble then was wasted with protein so that the results showed no difference for the four types of extractant.

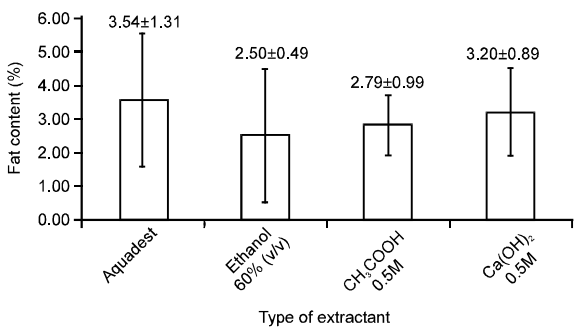

Fig. 4: Graph of the fat content (%) collagen extract of Bali cattle scapula produced using different types of extractant. a, b: significant differences ($p<0.05$)

Calcium level: Calcium and collagen is one of the minerals that can be used to help renovate the bone due to *osteoporosis* (English, 2011). Characteristics of calcium level in the extracts made from collagen shoulder blade (*os scapula*) of Bali cattle produced using four types of extracts is presented in Fig. 5a.

Figure 5 shows a comparison of calcium level of the collagen extract from shoulder blade (*os scapula*) of Bali cattle as raw material. Results of data analysis of variance showed that the differences in the type of extractant in the production process significantly influenced ($p<0.05$) the level of calcium in the collagen

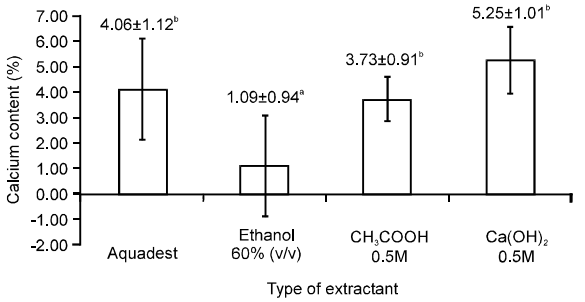

Fig. 5: Graph of the calcium content (%) collagen extract of Bali cattle scapula produced using different types of extractant

extract. Calcium is a major constituent of beef bones that is bound to the collagen component (Ockerman and Hansen, 2000). The use of a solution of $Ca(OH)_2$ as an extractant resulted in the highest level of calcium compare to other extractant, because in the extraction process, there is a portion of the extractant molecule of calcium and bone constituent molecules are deposited and extracted together with collagen.

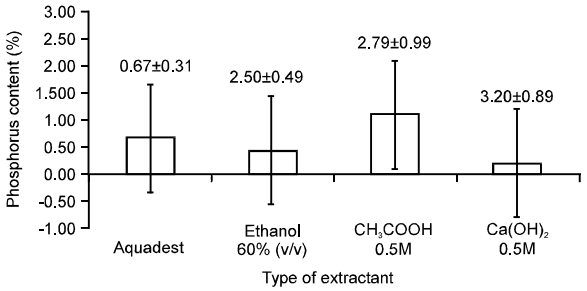

Fig. 6: Graph of the phosphorus content (%) collagen extract of Bali cattle scapula produced using different types of extractant

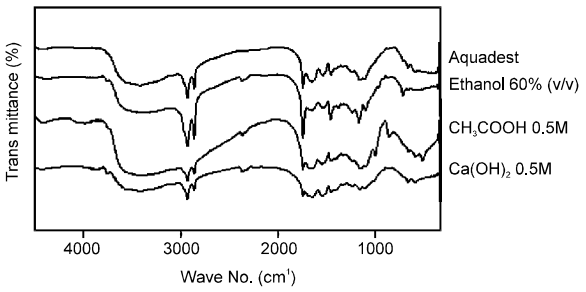

Fig. 7: Comparison of functional groups spectrum of the collagen extract of Bali cattle *os scapula* produced using different types using FTIR extractant

Phosphorus content: The comparison of phosphorus content of the collagen extract blades (*os scapula*) of Bali cattle produced using four types of extractant is presented in Fig. 6.

The results of data analysis of variance in Fig. 6 shows that the application of four different types of extractant in the production process of collagen extracts had no effect ($p>0.05$) on the level of phosphorus in the extract. Phosphorus is one of the compounds contained in bone which has a lower proportion of calcium. According to Trilaksani *et al.* (1997), the process of bone recycling, hydrolysis of non ash components occurs such as protein. This led to the increase of ash component including phosphorus which is the component of bone.

Profile of functional properties: The functional group is a group of special forces on the atoms in a molecule that plays a role in giving the characteristic of chemical reactions in the molecule. Profile spectrum of functional groups of collagen extracts produced using different extractants is presented in Fig. 7.

Profile spectrum of functional groups showed that the product of collagen extract is manufactured using the extractant distilled water, ethanol 60% (v/v), CH_3COOH and $Ca(OH)_2$ absorbs infrared light at each wave number 1159.22/cm, 1172.72/cm, 1147.65/cm and 1166.93/cm. This suggests that each of the four products of the extract has a C-O functional group on the molecule chain. Besides the four products also absorb infrared light at each wave number 1743.65/cm, 1745.58/cm, 1743.65/cm and 1743.65/cm. It shows also that the product of the collagen extract has functional groups C = O (*carbonyl*) in the molecular chain.

For the functional group of O-H (base) and C-H (aldehyde), all four products also detect the presence of the group, but its intensity is very small, which is detected at the wave number 2250.93/cm. For the functional group N-H (amide) in the molecular chain, all of the products absorb the infrared light at wave number 2922.16/cm. The presence of N-H groups (amide) indicates that the product is a component of a protein with similar properties. The covalent bonds that consist up a product will absorb the different frequencies of electromagnetic radiation in the infrared region of the spectrum. If the two spectral peaks at the right compound in many ways the two compounds are identical (Sastrohamidjojo, 1992).

Conclusion: The use of different extractants at the production process of collagen extract using raw materials shoulder blades (*os scapula*) of Bali cattle affected the value of the yields, water content, protein content and calcium level, but had no effect on fat level and phosphorus level Collagen extract from the shoulder blade (*os scapula*) of Bali cattle produced using four types of extractants have the identical functional properties based on the presence of functional groups C-O, C = O, O-H, C-H and N-H.

ACKNOWLEDGEMENT

I acknowledge Directorate General of Higher Education, Ministry of Education dan culture for the financial support through the National Priority Research Grant program Master Plan for the Acceleration and Expansion of Indonesian Economic Development 2011-2025 (PENPRINAS MP3EI 2011-2025) Corridor Sulawesi.

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