JLSB
Journal of Life Science and Biomedicine

J. Life Sci. Biomed. 4(5): 416-420, 2014

ISSN 2251-9939

Cleaning Method by Keratinase Enzyme for Improving Quality Edible Bird Nest

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ABSTRACT: One indicator of the quality of Edible Bird's Nest (EBN) is the cleanliness of feathers attached, so it is necessary to find a method to remove the hair washing EBN but not damage glycoprotein as the main content. Enzimkeratinase is an enzyme that works specifically to destroy the fur, so expect feathers attached to the ediblebird's nest can be eliminated and the main content can still be maintained. The purpose of this study were to determine the quality of the bird's nest before and after leaching in terms of glycoprotein content, nitrite content, proximate content, microbial contamination (TPC, E.coli and Salmonella) and organoleptic properties. This study was a laboratory study with two treatments done in one step. Analysis of bids nest was organoleptic test, the content of glycoproteins, and microbial contamination. The test wareconducted to determine the level of public acceptance of cleanliness and the color of EBNby20 panelists using proximate Hedonik.Glycoprotein and proximate analysis was used to determine the effect of washing on the main content of EBN. Microbial analysis was to determine the effect of washing on the quality of EBN before and after leaching.Theresearsch method was descriptive. Results showed that bird nest after leaching has better quality than before washing.
Keyword: Quality, Edible Bird Nest, Keratinase

ORIGINAL ARTICLE
Received 10 Jan. 2014
Accepted 10 Mar. 2014

INTRODUCTION

Bird's nest is made by the male swallow (Collocaliaspp) from the saliva secreted by glands salivalis during the breeding and nesting season. Male swallow saliva hardens when dry and form a half bowl attached to the wall [1, 2].

Bird's nest still contains a lot of impurities, especially feathers from birds' feathers that fall out during nest manufacturing. Eggs fraction, bird droppings, dirt fleas, flea dirt and sand is contained in a bird's nest and all the debris to be cleared because the birds nest this swallow is intended for human consumption and all the dirt is not fit for human consumption.

The quality bird's nest determines the price and the international market acceptance of the bird's nest products. Personal interviews with exporters, quality bird's nest must be met were not contain salmonella bacteria, content limit E.colli 1 x 101 cfu / g.Swallow nest's unadulterated by other materials such as sea grass and karayagum[2] known by no change of the nutrients found in birds' nests before and after washing. No changes in the color of the bird's nest before and after washing.

Keratinase enzyme is a specific enzyme that functions as a feather degrading enzymes. It is not known specifically how this enzyme can degrade feathers, but these enzymes are known to degrade hair without cutting disulfide bond [3]. Keratinase enzyme applications is one alternative in the washing process bird's nest. Keratinase enzyme washing does not need the use of immersion so that no possible glycoprotein dissolved in water and most of the fur does not have to be taken one by one because it has been destroyed or disconnected from the bird's nest by the activity of the enzyme keratinase. The enzymatic method has not been tried before, so it is necessary to investigate how the quality of the bird's nest before and after washing. The parameters used in the determination is the test protein, dry matter, ash, crude fiber, crude fat glycoprotein, amino acids, test TPC, E.coli, salmonella and, cleanliness test with "sensory testing".

MATERIAL AND METHODS

Bird nest (Collocaliafuchiphaga) taken from Temuguru Village, District tile, Banyuwangi in December 2010. Study aimed to compare the quality of the bird's nest before and after leaching using keratinase enzyme. This study analyzed descriptively.

To cite this paper: Utomo B., Rosyidi D., Eka Radiati L., Tri Puspaningsih N., Diah Proborini W. 2014. Cleaning Method by Keratinase Enzyme for Improving Quality Edible Bird Nest. *J. Life Sci. Biomed.* 4(5):416-420.
Journal homepage:http://jlsb.science-line.com/

Bird nest comes from one harvest to ensure the bird's nest is homogeneous. Bird's nest that had been harvested soaked with warm water (40°C) for 1 minute. The second step was to wash the bird's nest with keratinase enzyme. Preparation of samples to be used in a test carried out after the completion of washing glycoprotein or until EBN clean of feathers. Test of Sensory Analysis performed for samples that have been washed. Analysis of glycoprotein content using "Glycoprotein Carbohydrate Estimation" [4], and Sensory conducted using [5]. Analysis of microbiological quality (TPC, E. coli and Salmonella) using selective media [6]. Proximate complete test method American association of cereal chemists [7].

RESULTS

1. Protein and Glycoprotein Content

Table 1 showed the results of the analysis of proteins and glycoprotein of EBN before and after washing.

Table 1. Protein and Glycoprotein content of EBN

Tratment	Protein (%)	*Glycoprotein* (%)
Before washing	53,39 ± 0,51 a	11.38 ± 0,58 a
After washing	53,15 ± 0,56 a	11.10 ± 0,25 a

Note : Average followed by the same letter in the same column ware not significantly different (P> 0.05)

Table 1 shows that the content of protein and glycoprotein EBN without washing and after washing with keratinase enzyme did not change. This happens because the main protein of EBN is glycoprotein [1, 2] and the washing was done by using the enzyme keratinase, that the enzyme does not react cut glycoprotein structure [3].

Lin et al. [3] suggested that the keratinase enzyme not only work on keratin proteins, but also can work on some other proteins which can be hydrolyzed by the enzyme, namely BSA, casein, collagen, and elasin. Therefore, the enzyme keratinase not interact with glycoprotein. Keratinase enzyme is a protease enzyme belonged endopeptidase, since this enzyme in the group of serine, which is the way it works to cut the bond of the peptide substrate binding polypeptide [8]. Lin et al. [3] stated that the way these enzymes that degrade fur is not known in detail, but just note that this enzyme degrades feathers without breaking the disulfide bonds that were held by the feather keratin.

Average content of EBN glycoprotein before and after washing did not change significantly. Changes glycoprotein content occurs but was very small. This was because the enzymes used were not pure (purity 60%), so that other proteases were mixed in the enzyme will react with a glycoprotein which was the dominant type of protein in the EBN. More complete explanation can be followed at the discussion of amino acid level of EBN.

2. Amino Acid

The result of amino acid analysis given on Table 2.Amino acids content of EBN before and after washing by keratinase enzyme described. EBN amino acid content after washing did not differ between the two treatments. Some EBN amino acid content did not change when compared between before and after washing. Some amino acids have increased, such as tyrosine, arginine and alanine. Amino acids decreased after washing include cysteine, glutamic acid, serine, and threonine.

Table 2. Amino Acid Content EBN and Swallow Bird Fur

Amino Acid	Amino Acids of EBN		Amino Acids of Fur
	Before washing	After washing	
Proline	2,14±0,36	1,76±0,19	0.48
Tyrosine	0,65±0,07	0,89±0,02	0.22
Valine	1,12±0,05	1,10±0,06	0.06
Methionine	0,53±0,10	0,40±0,10	0.13
Cystine	0,43±0,04	0,37±0,03	0.55
Isoleucine	1,01±0,23	0,89±0,24	0.44
Leucine	2,31±0,12	2,04±0,23	0.55
Phenylalanine	0,67±0,03	0,62±0,04	0
Lysine	2,11±0,54	1,62±0,22	0.32
Aspartic Acid	1,74±0,12	1,36±0,22	0.68
Glutamic Acid	4,56±0,11	3,39±0,82	1.76
Serine	1,13±0,19	0,75±0,08	0.27
Glycine	0,42±0,02	0,41±0,04	0
Histidine	0,59±0,06	0,45±0,13	0.27
Arginine	0,60±0,07	0,75±0,05	0.34
Threonine	1,08±0,27	0,66±0,13	0.26
Alanine	0,45±0,07	0,71±0,13	0.13

The decrease in amino acid content EBN after allegedly laundering as a result of the loss of feathers attached to the EBN. Ramnani, Sign and Gupta, [9] reported that the main content of the feather is protein. Protein content of about 80% feather, so the loss of feathers attached to the EBN will have an impact on the decrease in amino acids.EBN changes before and after washing were presented in Figure 1.

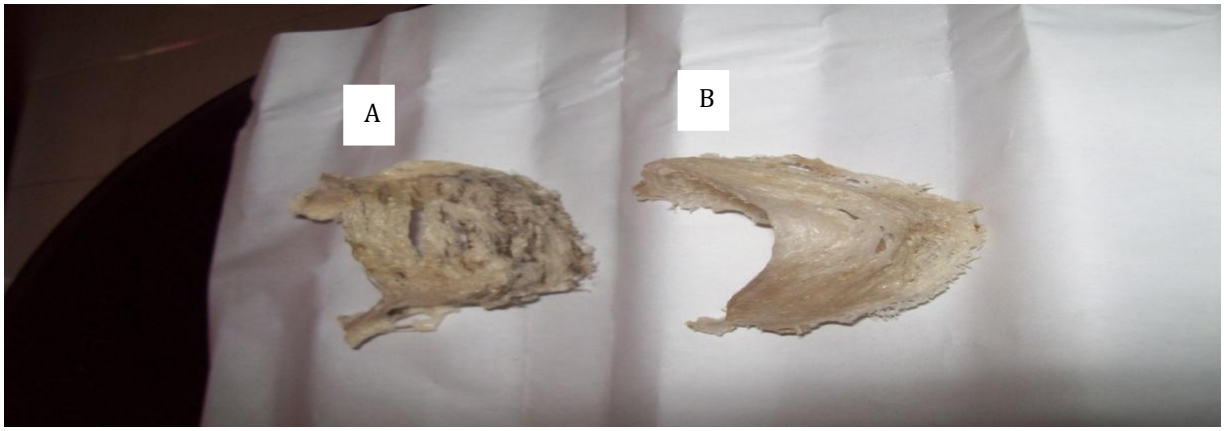

Figure 1. EBN before (A) and after (B) washing

Some amino acids increased after washing and some did not change. This was caused by the interaction between the enzyme proteins on EBN. Purity of the enzyme that has been used is 60% because pure keratins enzymes are very expensive and were used in the medical course. In addition, to obtain a pure enzyme takes a long time.

Protease extracted from bacteria by Dow et al. [10] can react with the matrix glycoprotein. The enzyme used was mixed with other protease enzymes. Results of research conducted by Rahayu [11] showed that the type of protease produced by Bacillus bacteria of many kinds, among other types of neutral proteases, protease metals and alkaline protease, so supposedly there is interaction between the protease enzyme polypeptide chains in glycoproteins EBN. Interaction between the protease enzyme keratinase other than indicated by the decrease in protein content and glycoproteins EBN before washing and after washing with keratinase enzyme.

3. Proximate Analysis

Table 3 shows the proximate analysis EBN before and after washed use a keratinase enzyme concentration of 1:2 (v / v) did not change. This occurs because the washing was done uses the enzyme keratinase. This enzyme is a protease enzyme that works specifically on keratin protein so it will not interfere with the content of other nutrients contained in bird's nest [3]. Keratinase enzyme leaching by immersion that is not going to be able to use the nutrients enurunkan nest which is soluble in water [1].

Tabel 3. Proximate and Fiber of Edible Bird Nest

Treatment	Dry matter	Ash	Fiber	Fat
Before washing	79.99±0.18 a	5.7±0.29 a	0.43±0.30 a	0.74±0.14 a
After washing	79.99±0.18 a	5.7±0.29 a	0.43±0.30 a	0.74±0.14 a

4. Cleanness

The results of organoleptic test showed that the average panelist on the cleanliness of combination treatments before washing and after washing was 2.1 to 3.56. The greater the value of panelist then EBN better cleanness. A panelist mean on cleanness EBN was presented in Figure 2.

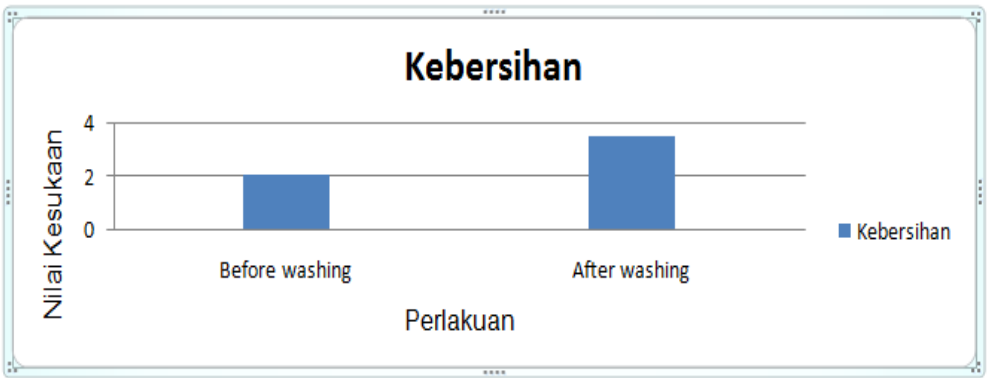

Fig 2. Organoleptic Test Diagrams Cleanness in EBN

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Friedman test results indicate that washing EBNhad significant effect (P ≤ 0.05) to the cleanliness of EBN. This was because the fur is on EBN has been broken down by the enzyme keratinase and soluble, so EBNafter washing was clean of feathers. Based on personal interviews with employers EBN (2010), fur is one of the impurities in the bird's nest that is the most difficult to remove. Washing with keratinase enzyme may facilitated in removing feathers from the nest. This was because the enzyme keratinase worked specifically to destroy the hair by cutting the keratin which was the main component of hair on the tip of the amino peptide, so that the bristles become obsolete and easily washable [3].

5. Colour

The results of organoleptic test showed that the average preferences of the panelists on the color combination treatments before washing and after washing is 3.11 to 4.22. A value greater panelists, means the better the color produced. A panelist on the colors meanEBN is presented in Figure 4.

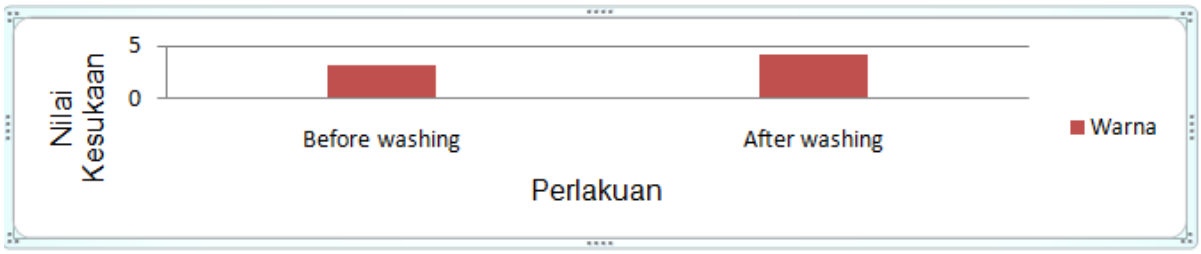

Figure 4. Organoleptic Colour Test Diagram on EBN

Figure 2 shows that the average value of the color preferences of the panelists to EBN before washing lower (3.11) than it had been washed (5.33).Friedman test results indicate that leaching EBN significant effect (P ≤ 0.05) to the cleanliness of EBN. This was because the fur is on EBN has been cleared, which according [13], black in color due to the EBN feathers attached to EBN, so the nest appears to be black, and with the loss of hair color will look original nest is white.

Keratinase enzyme used was crude enzyme or impure enzymes, so it still contains other protease enzymes. The protease enzyme according to Kumar and Takagi, [14] were ditergen, so that this enzyme can dissolve impurities other than bird's nest.

6. Best Result Based on Organoleptic Test

Determination of the best treatments of various combinations of EBN treatment between before washing and after washing by using effectiveness index [14]. This method was done on the organoleptic parameters. The organoleptic parameters used include; cleanliness and color. The best treatment assessment are presented in Table 4.

Table 4. Best Treatment Organoleptic Test

Treatment	Cleanness	Colour	Value
Before washing	2,11	3,11	0,000
After washing	3,56	4,22	0,462*

*** = best result**

The analysis shows that the best treatment was obtained on after washing sample EBN with the following characteristics: mean 5.44 a panelist to cleanliness and color 5.38.

7. Microbial Content

The results of the microbiological content analysis showed that the content of Total Plate Count (TPC), E.coli and Salmonella bird nest (EBN) before and after washing using enzyme concentrations 1:2.

Tabel 6. Analysis of Mmicrobiological Qquality

Treatment	Total Plate Count	*E.coli*	*Salmonella*
Before washing	3 log 41.33±1.15 a	0	0
After washing	3 log 19.67±0.58 b	0	0

Table 6 shows that the content of E. coli and Salmonella EBN without washing, and use a 1:2 concentration of the enzyme did not change. This happens because before washing bird nest does not contain both bacteria. TPC content test results showed a decrease in total number of microbes contained in a bird's nest. This is because when washing birds' nests, droppings are a source of microbial contamination has been cleaned.

DISCUSSION

The quality bird's nest cleaning results using keratinase enzyme has oranoleptik quality and better microbiological quality when compared with the bird's nest without washing. Chemical constituents leaching results of bird's nest with keratinase enzyme did not change when compared with the bird's nest without washing, it can be seen from the content of its main bioactive glycoproteins and other proximate content, so it can be concluded that the quality of the bird's nest after washing better than without washing.

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