L-Cysteine Isolation of Fleece and The Application in White Bread

Anna P. Roswiem [a,b,1,*], Dedy Suseno [b] , Triayu Septiani [b]

[a] Halal Research Center – Lembaga Penelitian Universitas YARSI, Jakarta, Indonesia
[b] Faculty of Dentistry, Universitas YARSI, Jakarta, Indonesia
[1] annap_ros@yahoo.com
* corresponding author

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ABSTRACT

Nowadays L-Cysteine is widely used in the manufacture of food, drug and cosmetic products. In food industry, L-Cysteine usually used as a food additive in instant noodle which serves as a flavor enhancer for meat, in wheat flour and/or bakery products, it is functions as an improving agent and bread improver. On industrial scales, L-Cysteine is made from the hydrolysis of protein from feathers and/or animal hair such as pig, sheep, or the other poultry feathers and even human hair. Halal status can be doubted (syubhat) and could be also haram. Therefore, the aims of this study were: (1) to isolate L-Cysteine from sheep hair that was sheared while the sheep were still alive (2) to compare the physicochemical properties of L-Cysteine from the sheep with the commercial L-Cysteine, and to compare the quality of white bread with L-Cysteine isolated from fleece, white bread with commercial L-Cysteine and white bread without L-Cysteine added. The results of this study showed that L-Cysteine could be isolated from fleece with a yield of 0.20%; the physicochemical properties of fleece L-Cysteine which comprises shape, isoelectric point, ninhydrin test and Rf value are the same as the physicochemical properties of commercial L-Cysteine, such as form of white crystals: Isoelectric point of 5.07; positive ninhydrin test with the formation of a purple colored complex solution; and the value of Rf is 0.34. The application of the result in the addition of L-Cysteine from fleece in the manufacture of white bread improve the quality of white bread and increasing the panelists preference for the white bread, and the expired date is longer than the expiride date white bread without the addition of L-Cysteine . In other words, L-Cysteine from fleece could be potential as a substitute for non-halal L-Cysteine.

1. Introduction

The main component of animal hair is protein. Proteins are macrobiopolymer molecules which composed of monomers called amino acids. One of the amino acids that composed protein is L-Cysteine. L-Cysteine is widely used in the food, pharmaceutical, and cosmetic industries. In the food industry, L-Cysteine is generally used as an additive in products : Wheat flour as improving agent; Instant noodles that has main role in triggering the taste of meats and Bakery as improving agent.

Since decades ago until now, amino acid producers, in order to meet consumer demand for L-Cysteine, make it by hydrolyzing the protein (keratin) of human and or animal hair (pork and or

poultry) also by fermentation technology methods. L-Cysteine from human hair and pig is definitely "haram", L-Cysteine from fleece is "halal" as long as the fur is removed (shorn) while the animal is still alive. Meanwhile, the halalness of L-Cysteine from the microbial product depends on the halalness of the ingredients process such as the production media and the additive ingredients in the fermentation process. (Karlhein; et al (2007); Roswiem, 2015; MUI, 2011)

Until now, fur from lamb is usually used for the wool industry. However, the lamb breeders in Indonesia have never used their fleece waste to make wool or isolate the Cysteine. Therefore, the aims of this study was to isolate the amino acid L-Cysteine from fleece that was sheared alive and to compare the physicochemical properties of fleece L-Cysteine with commercial L-Cysteine, also to compare the quality of white bread added with fleece waste L-Cysteine, white bread added with commercial L-Cysteine and white bread without L-Cysteine added.

2. Materials and methods

The materials used for the isolation of L-Cysteine are : ammonium hydroxide, aquadest, biuret reagent, fleece, congo red reagent, HCL, Pb acetate, activated carbon or norite, natrium acetate, sodium hydroxide. The fleece used in this study is fleece that was sheared from live lamb and was obtained from lamb breeders in the village of Ciambar-Sukabumi, West Java Indonesia

The ingredients used for produce bread are wheat flour, refined sugar, margarine (Palmia), yeast (fermipan), UHT milk, aquadest, and L-Cysteine isolated from fleece, commercial L-Cysteine (producer X). The composition of these ingredients are :

Yudane Dough

125 grams of high protein flour, 100 grams of boiling water and stir until smooth then put in cilling wrap, refrigerate in 2 hours or one night.

Ingredients A

500 grams of high protein flour, 40 grams of granulated sugar, 8 grams of instant yeast (fermipan), 2.5 grams of L-Cysteine from fleece and commercial L-Cysteine (manufacturer X), 380 grams of cold UHT milk.

Ingredients B

30 grams of cold Margarine, 10 grams salt.

The instruments was used in this research are Florence flask, Buchner funnel, desiccator, sand bath, thin layer chromatography (TLC), mixer, baking sheet and oven.

2.1. Procedure

2.1.1 Sample Preparation

Sample preparation in this study used modified method of Harrow, et al (1960) in Margriet, (2003).

2.1.2 Isolation of L–Cysteine

Isolation of L–Cysteine from fleece was carried out according to the modified method of Harrow, et al (1960) and Jandik, et al (2001). The L-Cysteine isolation process using isolation method from animal protein.

The clean fleece was placed in a 500 ml Florence flask, crushed in 200 ml of 6 M HCL solution on a steam bath. Adding 10 grams of fleece until it reaches (100 grams). After all of the solids had dissolved, a further high temperature heating (reflux) was carried out with a sand bath until 5 ml of the sample was decolorized with norite and the biuret test reaction was negative. The solution is filtered, and the insoluble solids are rinsed twice with a small amount of distilled water. The solid formed was dissolved in 20 ml of 40% NaOH solution. The solution then cooled, stored for three days at room temperature. The solids and liquids are separated by suction, then the solids are dissolved in 150 ml of 1M HCI solution. Filtered and decolorized in 2-5 times with treatment was using 4-6 grams of norit, and filtered again. The clear and colorless solution is stored for 5-6 hours at room temperature. The filtrate formed was tested for isoelectric point. The crystallized of L–Cysteine was filtered, rinsed, and to dried

2.1.3 Isoelectric point test

The isoelectric point test was carried out in parallel with the filtration of 0.5 M NaOH. In the final solution (before crystallization) which was almost colorless, the titration was stopped when the sample solution tested by congo red gave negative reaction and precipitate was formed.

2.1.4 Ninhydrin Test

Ninhydrin test is general test for the identification of amino acids. With this test, amino acids except proline, hydroxy proline, and asparagine will be react with ninhydrin to form purple complex solution.

2.1.5 Thin Layer Chromatography Test

In the Thin Layer Chromatography (TLC) method, the eluent is made from a mixture of: (n-butanol: acetic acid: aquadest = 20 : 10 : 10), was put in chromatography chamber. The eluent was incubated for ± 1 hour until the chromatographic chamber was saturated with the eluent.

On the chromatographic plate (Silica gel 245), a solution of L-Cysteine (the result of hydrolysis of fleece and commercial L- Cysteine) was spotted. This L- Cysteine solution was prepared by dissolving 5 mg of L-Cysteine per ml of NaOH which was then diluted 5 times with distilled water until became a mixed solution of 1 mg/ml L-Cysteine. Then put into a chromatographic chamber was containing the eluent. Let the eluent move up to the mark (ascending chromatography). Remove the plate from the chromatography chamber. Then dried the plate in oven at 60 °C. The dried plate was sprayed with ninhydrin reagent and dried again in the oven at 100 – 110 °C. The purple stain of L–Cysteine is calculated for Rf value.

R_f (Retention flow) value showed by formula

$$R_f = \frac{a}{b}$$

a : distance of the spot from the starting line
b : eluent final boundary distance (mobile phase) from the starting line

2.1.6 Making of White Bread

Mix all of the A ingredients, adding the Yudane until well blended (approximately 10 minutes)

- Then mix of B ingredients into the dough, wait until it forms a gluten window (elastic)
- Divide into several parts, wait and roll out the dough to remove the trapped air.
- Put it in a baking dish that has been smeared with margarine and wait until it expands and then bake until cooked
- Bread that has been cooked is chilled, then the mass density test is carried out (Faridi, 1993) and the development ratio test (Tanuwijaya, 1990) in Margriet (2003)
- Bread that has been cold, then packed in clear plastic (food grade) which is tightly closed with a seal. Furthermore, organoleptic tests were carried out which included of taste, color, and texture which is used a preference test (hedonic test). Organoleptic test was done by 3 panelists with 3 repetitions.

2.1.7 Analysis of White Bread Product

In the final product, analysis was carried out which included mass density test, develop ratio test, and organoleptic test (taste, color, and texture). The basis for sampling is to determine the acceptance panel to improve the quality of bread using the addition of L-Cysteine isolated from fleece.

2.1.7.1. Mass Density Test (Faridi, 1993) in Margriet (2003)

Bread that has been cooked is removed from the tin, then the maximum height, length, width are measured and also the bread is weighed

$$\text{Mass Density} = \frac{\text{Bread weight (g)}}{a \times b \times c \text{ (cm)}}$$

Note :
a = the width of bread
b = the lenght of bread
c = the height of bread

2.1.7.2. Expansion Ratio Test (Tanudjaja, 1990) in Margriet (2003)

Using a vernier caliper, measure the thickness and width of the bread before and after baking

$$\text{Expansion Ratio} = \frac{a1 \times b1}{a \times b}$$

Note :
a = thickness before baking
b = width before baking
a1 = thickness after baking
b1 = width after baking

2.1.7.3 Organoleptic Test (Soekarto, 1985) in Margriet (2003)

The organoleptic test includes a preference test for taste, color, and texture on bread products using a preference test (hedonic). The samples are presented randomly with certain code which there are 3 panelists, each of panelist was asked to provide an assessment.

The hedonic scale used has a range from:

- Like extremely (1.00 – 1.999)
- Like very much (2.00 – 2.999)
- Like (3.00 – 3.999)
- Dislike slightly (4.00 – 4.999)
- Dislike moderately (5.00 – 5.999)

2.1.7.4 The Expired date Test

The expired date test was carried out to test the resistance of bread against of the mold growth. Each bread is wrapped in plastic packaging (food grade), stored at room temperature, and observed for mold growth every day

2.1.7.5 Experiment Design and Data Analysis

In this experiment, fixed completely randomized design (CRD) was used, the number of repetitions was the same, with three treatments. This design is used for the data analysis of the **mass density test** and the **expansion ratio test**,

The details of the treatment combinations are as follows:

P1 : Bread without the addition of L–Cysteine
P2 : Bread with the addition of L–Cysteine Commercial
P3 : Bread with the addition of L–Cysteine from fleece

Each treatment had three repetitions. Data analysis was carried out by ANOVA (Gasperzs, 1991 *in* Anggriawan 2013).

If there is a significant difference between the treatments was used, it will be followed-up with Duncan test (Steel & Torrie (1989) in Angriawan 2013) using the SPSS program ver 20.

3. Results and Discussion

3.1. Isolation of L–Cysteine from fleece

The result of L–Cysteine isolation from fleece showed in table 1.

Table 1. Ethanol and sugars content in fruit juice samples.

Repetition	Materials (gram)	
	Fleece	L–Cysteine
1	100.00	0.192
2	100.00	0.196
3	100.00	0.202
4	100.00	0.205
5	100.00	0.199
6	100.00	0.196
7	100.00	0.205
8	100.00	0.192
9	100.00	0.199
10	100.00	0.103
Average		0.199 (0.20%)

From these results, L-Cysteine isolated from fleece was less than L-Cysteine isolated from human hair (approximately of 2.50%) and also less than L-Cysteine isolated from fleece in the previous studies (approximately of 1.25%) (Fieser and Fieser (1960) in Margriet (2003). The difference in the amount (yield) of L-Cysteine isolated in this study could be caused by the differences in the ingredients for washing fleece. In this study, the ingredients for washing fleece using liquid detergent, while in previous studies using bar soap (Harrow, et al 1960) in Margriet (2003).

Notes :

The main raw materials used to make soap are as follows.

a. Bar soap : KOH / NaOH and Vegetable oil
b. Liquid soap : bar soap and water

The raw materials for making liquid detergent in this research are follows: bar soap, water, baking soda, and natrium carbonate.

According to Harrow, et al (1960), in Margriet (2003), natrium carbonate was used to wash animal hair or human hair for isolating of L-Cysteine, the yield obtained will be very small and maybe not be isolated. Therefore if in this research used bar soap without natrium carbonate, the yield be more than 0.20 %.

Apart from the difference in fur washing ingredients, the difference in the amount (yield) of L-Cysteine isolated in this study could be caused by differences in the ingredients or types of feed eaten by their domesticated sheep or lamb, especially the type of feed or the source of the feed ingredients. If the type of feed or the source of the feed ingredients contains high protein, the yield of L-Cysteine produced can be higher.

The needed for L-Cysteine in various industries in the world is mostly met by L-Cysteine isolated from human hair and only slightly met by L-Cysteine resulting from fermentation technology.

Based on that, L-Cysteine isolated from sheep or lamb can replace L-Cysteine isolated from human hair which is forbidden for consumption by Muslims, as long as the fleece is washed with soap not with another soap containing natrium carbonate.

Apart from that, according to Jaswir, et al. (2020) the availability of fleece from sheep or lamb breeders in Indonesia is pretty much and it makes non-halal L-Cysteine products/materials can be substituted by L-Cysteine from halal fleece.

3.2. Physicochemical Properties of L-Cysteine

3.2.1 L–Cysteine Isoelectric Point Test

The basic principle in determining the isoelectric point of amino acid is in the electrophoresis process an amino acid does not show electron migration towards the positive or negative electrodes, so that at the isoelectric point the amino acids will be precipitate.

According to Fessenden (1986), in Margriet (2003), the isoelectric point of an amino acid in one of the categories:

1. Isoelectric point neutral amino acid (pH 5.00–6.00)
2. Isoelectric point of acid amino acids(pH 3.00)
3. Isoelectric point of base amino acids(pH 9.00–10.00)

The result of the isoelectric point test of the L-Cysteine of the fleece in this study was 5.07, so that the L-Cysteine of the fleece was classified as a neutral amino acid. The isoelectric point values of L–Cysteine isolated from this fleece suitable to the isoelectric point value of commercial L–Cysteine (manufacture X)

3.2.2 Thin Layer Chromatography (TLC) Test

The results of the Thin Layer Chromatography (TLC) test of fleece L–Cysteine are shown in Table 2.

Table 2. Rf Value of L–Cysteine Test

L–Cysteine Test	Rf
L–Cysteine from fleece	0.34
Commercial L–Cysteine (manufacture X)	0.34

From the Table 2 above, it was found that L-Cysteine isolated from fleece (wool keratin) hydrolyzate had the same Rf value as commercial L-Cysteine (manufacture X).

These results are different from the Rf value of L-Cysteine from chicken feathers (white color) (Margriet, 2003) and the Rf value of L-Cysteine from Ajinomoto (Margriet, 2003). The difference is due to the different types of raw material sources. According to Margriet, (2003) the source of the raw material is white chicken feathers, and the difference in the titrant material. In the research of Margriet, (2003), the titrant material is natrium acetate, while in this study the material is 0.5 M NaOH.

3.3 Application of L-Cysteine from fleece in the produce of white bread

3.3.1 Mass Density Test

Based on the calculation of the mass density test according to the formula from Faridi (1993) in Margriet (2003), the mass density of bread was obtained as shown in Table 3.

Table 3. Result of Bread Mass Density Test

Treatment	Average of Mass Density in White Bread (g/cm3) *
P1	0.245 c
P2	0.384 a
P3	0.357 b

P1 : bread without L-Cysteine
P2 : bread with commercial L-Cysteine
P3 : bread with L-Cysteine from fleece
**Numbers followed by the same letter not significantly different at the 95% confidence level*

The results of the mass density variance of bread, showed that statistically there was a significant effect on the addition of commercial L-Cysteine and L-Cysteine from fleece. From the results of Duncan test, has known that the mass density of each bread is different from one another. The mass density of each white bread is shown in Table 3. The differences of the mass density between non-Cysteine white bread with Cysteine and white bread caused Cysteine could makes gluten (the main protein in wheat flour) more soften (the main protein in wheat) so that the wheat flour dough becomes softer. therefore the mass density becomes greater even though the texture is less smooth.

3.3.2 Result of Mass Expansion Ratio Test in White Bread

Based on the mass expansion ratio test according to Tanudjaja (1993) in Margriet (2003), the expansion ratio of bread was obtained as shown in Table 4.

Table 4. Result of Mass Expansion Ratio Test in White Bread

Treatment	Mass Expansion Ratio in Bread *
P1	2.52500 [a]
P2	2.52900 [a]
P3	2.14333 [b]

P1 : bread without L-Cysteine
P2 : bread with commercial L-Cysteine
P3 : bread with L-Cysteine from fleece
**Numbers followed by the same letter not significantly different at the 95% confidence level*

The results of the variance in the mass expansion ratio of bread shown that has statistically significant effect on the addition of commercial L-Cysteine. From the results of Duncan test, known that the mass expansion of bread without the addition of commercial L-Cysteine is significantly different from the mass expansion of bread with the addition of L-Cysteine from fleece. Apart from the differences in the type of develop materials, it is also due to the differences in the thickness of the dough development process. Dough development of P1 dough is more elastic than dough of P2 and P3. the development ratio of P2 is slightly higher than that of P1, but the two are not significantly

different. The smallest development ratio of P3 is due to the L-Cysteine source used is from fleece and the dough development of P3 dough is very less elastic, so the ratio of P3 dough development is the least compared to the ratio of P1 and P2 dough development.

3.3.3. Result of White Bread Organoleptic Test

The organoleptic test in this study included panelist preference for color, taste, and texture. Test results is shown in Table 5, 6, and 7.

The hedonic scale used has a range from :

- Like extremely (1.00 – 1.999)
- Like very much (2.00 – 2.999)
- Like (3.00 – 3.999)
- Dislike slightly (4.00 – 4.999)
- Dislike moderately (5.00 – 5.999)

Table 5. Result of Organoleptic Test of Color in White Bread

Treatment	Color	Taste	Texture
bread without L-Cysteine	Like	Like very much	Like
bread with commercial L-Cysteine	Like very much	Like very much	Like very much
bread with L-Cysteine from fleece	Like very much	Like	Like very much

Table 6. Result of Organoleptic Test of Taste in White Bread

Treatment	Test Scales of Bread Taste				
	Panelis 1	Panelis 2	Panelis 3	Average	Notes
bread without L-Cysteine	2.00	3.00	2.00	2.333	Like very much
bread with commercial L-Cysteine	3.00	2.00	2.00	2.333	Like very much
bread with L-Cysteine from fleece	3.00	3.00	3.00	3.00	Like

Table 7. Result of Organoleptic Test of Texture in White Bread

Treatment	Test Scales of Bread Texture				
	Panelis 1	Panelis 2	Panelis 3	Average	Notes
bread without L-Cysteine	3.00	3.00	3.00	3.00	Like
bread with commercial L-Cysteine	2.00	2.00	2.00	2.00	Like very much
bread with L-Cysteine from fleece	2.00	2.00	2.00	2.00	Like very much

3.3.4 Compilation of Organoleptic Test Results L-Cysteine application in produce of White bread

The compilation of organoleptic test result is showed in Table 8.

Table 8. Compilation of Organoleptic Test Results from the addition of L-Cysteine in produce of white bread

Treatment	Test Scales of Bread Color				
	Panelis 1	Panelis 2	Panelis 3	Average	Notes
bread without L-Cysteine	3.00	3.00	3.00	3.00	Like
bread with commercial L-Cysteine	2.00	2.00	2.00	2.00	Like very much
bread with L-Cysteine from fleece	3.00	2.00	2.00	2.333	Like very much

From the data above, white bread with added of L-Cysteine from fleece and commercial L-Cysteine is more preferred than white bread without L-Cysteine added.

4.4 Shelf life Test (Expired Date Tets)

Table 9. Result of Shelf Life Test

Treatment	bread without L-Cysteine	bread with commercial L-Cysteine	bread with L-Cysteine from fleece
Shelf life Test	4 days	8 days	8 days

In the storage of white bread without the addition of L-Cysteine, the fungus began to grow on the fourth day, followed by hardened texture and sour smell. In the storage of white bread with the addition of commercial L-Cysteine and L-Cysteine from fleece, the fungus began to grow on the eighth day, followed by hardened texture, sour and slight ammonia smell in the white bread added with L-Cysteine from fleece. This may be due of the characteristic smell from lamb.

4. Conclusion

From the result of this study, the following conclusions can be drawn: L-Cysteine can be isolated from fleece with yield of 0.20%. The physicochemical properties of L-Cysteine from fleece which include: shape, isoelectric point and Rf are the same as the physicochemical properties of commercial L-Cysteine (manufacture X). L-Cysteine from fleece is a substitute product for non-halal L-Cysteine

In order to produce more L-Cysteine yield, will be recommended that the material used for washing the fleece is soap that does not contain of natrium carbonate. The halal status of the L-Cysteine produced is could be not doubtful, is very recommended that the L-Cysteine isolated from lamb or sheep is also has halal certified.

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