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Chemical Quality of Dried Stingray (*Dasyatis Sp.*) Marinated with Belimbing Wuluh (*Averrhoa Blimbi L.*)

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Department of Fish Product Technology

Abstract:- This study aims to examine the effect of marination of Stingray (*Dasyatis sp.*) meat by Belimbing Wuluh extract (*Averrhoa blimbi L.*) with a different concentration towards chemical quality and urea content of dried Stingray meat. The study employed experimentally in the laboratory by treatments, i.e., marination of stingray meat by belimbing wuluh extract by randomized design non factorial with twice replications. The factor concentration of 25% (A), 50% (B) and 75% (C). The data analysis steps involved ANOVA (Analysis of Variance) and Duncan's Multiple Range Test (DMRT) if significantly different to measure the tested parameters, i.e., urea content, protein content, and water content respectively. The urea content analysis by LC-MS indicates that the urea content of control sample, sample A, B, and C is 0.76 g/mL, 0.65 g/mL, 0.48 g/mL, 0.16 g/mL respectively, which signifies that higher concentration of blimbing wuluh extract contributes to the lower urea content. The ANOVA analysis result shows that the treatment does not affect towards protein content of dried stingray with a value of 13.7%-16.04% ($p>0.05$) in each sample. Likewise, the treatment also does not contribute significantly towards water content of dried stingray with a value of 16.21-17.36.

Keywords:- *Belimbing wuluh; dried stingray, LC-MS, water, protein, urea.*

I. INTRODUCTION

As one of the Indonesian marine biological resources, stingray fish (*Dasyatis sp.*) is found abundant in territorial waters, ocean waters, and in Economy Exclusive Zone. The Indonesian statistical data of fisheries on 2008 highlights that the fish landing rate reached a huge amount of 35,784 tons (Research and Development Team of Credit and UMKM (Micro, Small, and Medium Enterprises), 2010). Generally processed in fresh form, stingray contains fairly high nutritional contents, particularly protein. However, it is included in non-economical fish that are less favored by some Indonesians. Mardiah (2008) states that the protein content in the stingray is 16.86% and the fat content is quite low, at 0.42%. The development of processed stingray product is still limited; generally due to the high urea content causing stinky odor when the fish is processed too late because the urea content decomposes into ammonia during the pre-rigor phase. This highly contributes to the acceptance of stingray products.

As Yasin (2005) states, stingrays are included into cartilaginous fish class (Elasmobranchii) with considerably high urea content of 2.33%. This is supported by Lagler et al., (1977) who note that all types of cartilaginous fish on average contain high urea content, i.e., 2.0 - 2.5% of total meat, compared to those in bony fish with 0.05% of urea content. Moreover, research by Yunizal, et al. (1998) reported that physical treatment was done in several ways to reduce or eliminate urea content in stingray meat, i.e., cold water washing process which is capable of reducing 50% of urea content, and boiling process with superheated steam which can reduce the content up to 2.25%. In addition, chemical treatment is also applied, e.g., by soaking in a vinegar solution to reduce 80% urea content and heating under alkaline condition using KOH solution, which is capable of removing 70% urea levels. This complies with Suparno (1992) who states that acid solution is applicable to reduce urea content in meat.

Further, urea removal treatment can also involve acid solution extracted from natural ingredients, one of which is by using acid extract from belimbing wuluh (*Averrhoa blimbi L.*). Complying to the notion, research by Mursito (2010) claims that belimbing wuluh (*Averrhoa bilimbi L.*) contain citric acid, oxalic acid, acetic acid, formic acid, saponin acid, tannic acid, phenolic acid, and various minerals. On top of that, Suparmi et al. (2010) found that urea content in stingray (*Trigon sephen*) can be reduced by soaking in 2% citric acid solution and 80 ml extract of bean sprouts, with reduction of 59.51% and 42.63% respectively.

Based on the previous studies, a study is conducted to determine the reduction of urea content in dried stingrays by using natural ingredients, i.e., acid compounds extracted from belimbing wuluh (*Averrhoa blimbi L.*). This study aims to produce dried stingrays with expected specifications, i.e., products with low urea content, to encourage the number of consumption and interest of processed stingray products.

Research objectives:-

This study intends to measure the influence of marination of Stingray (*Dasyatis sp.*) meat by Belimbing Wuluh extract (*Averrhoa blimbi L.*) with different concentrations towards the chemical quality and urea content of dried Stingray meat.

➤ *Research Method:-*

The study employed experimentally in the laboratory by treatments, i.e., marination of stingray meat by belimbing wuluh extract by randomized design non factorial with twice replications. The factor concentration of 25% (A), 50% (B) and 75% (C). The data analysis steps involved ANOVA (Analysis of Variance) and Duncan’s Multiple Range Test (DMRT) if significantly different to measure the tested parameters, i.e., urea content, protein content, and water content respectively. The urea content analysis by LC-MS (Ganjar and Rohman,2007), protein content analysis by Hjeldahl method (BSN,2006a) and water content analysis by (BSN, 2006b).

• *Research tools and materials*

This research involved various tools; for preparation of stingray meat and extract of belimbing wuluh, i.e., knife, container, cutting board, spring scales, blender, 2000 mL measuring cup, filter cloth, spoon, mechanical dryer; and for documentation, i.e., camera and stationery. In addition, the tools used for chemical testing of urea levels implemented digital scales, wire clamp, measuring flask, test tube, dropper pipette, UPLC-MS (62 QToF Waters), analytical balance (Shimadzu), 1 mL syringe, 10-100 µl micropipette, vial LC (waters), 3D shaker, and glass tools used in pharmaceutical laboratories. Moreover, the Kjeldahl method protein test encompassed tools, i.e., a digital scale, steam distillator, boiling rocks, acid chamber, clock timer, Erlenmeyer flask, paper, destruction tube, measuring cup, and spatula. Further, to conduct the water content test, the study included tools, i.e. cup, desiccator, a thermometer, a digital scale, and open-ended pliers.

The materials used to produce dried stingray were: 4kg of fresh stingray meat (supplied from Torosiaje village, Popayato district, Puhuwato regency), clean water, and 5kg of belimbing

The result of comparison of concentration, retention time, and peak area from chromatogram data shows that the mean of urea retention time is 0.37 minutes, as obtained in control sample and all three samples A, B, and C. The retention time is the time it takes for the analyte to enter the column at the beginning of the injection until it exits the column and the signal is maximally captured by the detector.

The shift in retention time that occurs during the analysis is caused by the pressure in the column during the elution process. The comparison of concentration, retention time, and peak area is shown in Table 1.

No	Sample	Concentration sample (µg/mL)	Retention time	Area peak (x 10 ³)
1.	Control	25	0.41	1.90
2.	Sample A (25%)	25	0.36	1.37
3.	Sample B (50%)	25	0.37	1.14
4.	Sample C (75%)	25	0.37	1.01

Table 1. The comparison of concentration, retention time, and peak area.

wuluh fruit. Additionally, materials needed for urea test were: 4 samples of dried stingray puree, aquades, methanol pro analysis (MERCK), methanol hyper grade for HPLC (MERCK), 70% alcohol, and whatman filter paper. Moreover, protein chemical test involved four samples of dried stingray puree, H₂SO₄, HgO, Na₂S₂O₃, aquades, H₂BO₃, indicator mixture (a mixture of red methyl and blue methylene), and HCl. For the water content test, it used samples of dried stingray.

II. FINDINGS AND DISCUSSION.

➤ *Chemical characteristic of dried stingray*

• *Analysis of urea content in dried stingray*

The urea content analysis results in chromatogram data of control sample and sample A, B, and C, which shows relatively similar retention time. Noegrohati (1994) asserts that qualitative analysis on HPLC (High-Performance Liquid Chromatography) is carried out by comparing the retention time of pure compounds with the retention time of the compounds referred to in the sample. Below is the chromatogram data of control sample and sample A, B, and C showing an increase in peak area at a retention time of 0.37 minutes.

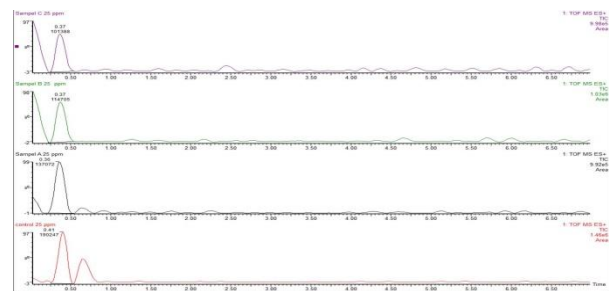


Fig 1:- Chromatogram result of the control, sample A, B, and C.

The previous table displays concentration, retention time, and a peak area of four samples of dried stingray. In addition to the retention time on the chromatogram, the elution result also contains the value of peak area. The value serves to determine the urea content in the sample by using the calibration curve. The peak area visible on each chromatogram shows a linear relationship between concentration and peak area.

The peak area obtain is used to measure the area content in the sample by using the calibration curve, substituting the value as y value in equation $y = ax + b$. The chromatogram data in Table 2 shows the similarity of retention time. Thus, it is confirmed that the samples contain urea. The retention time of dry stingray samples (A, B, C) also illustrates an almost similar result to the retention time of the control sample, i.e., during the first minutes. As a result, this means that the three samples contain urea compounds, as the retention time is relatively close to that of the control sample.

Indeed, the retention time result has indicated the suspected compounds in the sample. However, it is necessary to identify specific compounds using mass spectrometer in order to analyze

the ion molecules and fragmentation pattern of the compounds. The result of the compounds observed in the chromatogram from the separation result by HPLC (High-Performance Liquid Chromatography) is further analyzed by mass spectrometer detector. The mass spectrometer detector is able to identify the compounds eluted from the HPLC column by ionization and mass ratio calculation (m/z), and separation of the molecular fragments into small pieces.

Method of liquid chromatography with mass spectrometer detector (LC-MS) is chosen to provide accurate results qualitatively and quantitatively. The notion is in line with Agilent (2001), which states that liquid chromatography (LC) method separates sample components and then brings them to the mass spectrometer in order to create and detect charged ions. The LC-MS data is applicable as a reference to weight, molecules, structure, identity, and quantity of certain sample components.

The molecular mass analysis employs a mass spectrometer with the ESI (Electron Spray Ionization) method, in which the sample is carried by a liquid, and the mode employs positive ion. The analysis result of the mass spectrometer shows a correlation between the magnitude of the voltage energy and the model of the fragmentation; thus, the higher the voltage energy given will produce many fragments from the compound. It shows the presence of urea compounds, as evidenced by the peak at 60 m/z with similar molecule weight to urea of 60 msi.

The data of the mass spectrum of urea content is depicted in Figure 2 as follows.

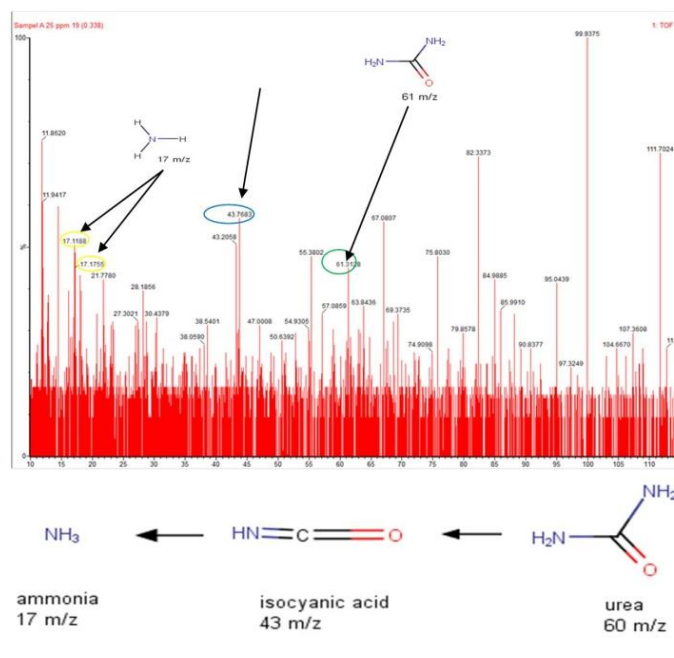


Fig 2:- Mass spectrum of urea content.

The detection result of urea compounds by mass spectrometer indicates that the four samples of dried stingray contain urea compounds based on molecular weight and detected

fragmentation patterns. The molecular weight of the detected urea compound is 61 m/z, as a result of urea fragments hydrolyzed to ammonia from MS data; moreover, the detected ammonia molecule is 17 m/z. In this case, the molecular weight of urea content shifts from 60 g/mol to 61 g/mol, due to the positive ion mode (M⁺) employed in the mass spectrometer detector. This complies with Watson (2009), that M⁺ signifies that the molecule ion brings a positive charge since the ion loses an electron, thus, the resulted molecular weight is increased by one (1).

The chromatogram result of Figure 1 elaborates the correlation in a decrease of urea content of every treatment. This is highlighted by the decreasing value of Area Under Curve (AUC) at a retention time of 0.37 minutes on each marination treatment using an extract of belimbing wuluh with different concentration during the analysis process. The LOD (Limit of Detection) and LOQ (Limit of Quantitation) values of instruments are 9.100 µg/mL and 27.57 µg/mL respectively. The following Table 2 displays the concentration value and method validation.

Concentration (µg/mL)	Peak area x10 ⁵	Found Concentration (µg/mL)	Recovery %
5	0.77	5.631578947	112.6315789
10	1.03	10.19298246	101.9298246
15	1.21	13.35087719	89.00584795
20	1.64	20.89473684	104.4736842
25	1.90	25.45614035	101.8245614
Mean (n=5)			101.9730994
SD			8.485718252
SE of Intercept			0.069859383
SD of Intercept			0.157183611
LOD			9.100103782
LOQ			27.57607207

Table 2. Concentration value and method validation.

The table 2 shows that the calibration curve of the standard solution is depicted in the graph of concentration curve (x) and peak area (y) with linear regression equation of $y = ax + b$ or $y = 0.0574x + 0.449$ with the correlation coefficient (r^2) is 0.984. The calibration curve of the standard solution is observable in figure 3 as follows.

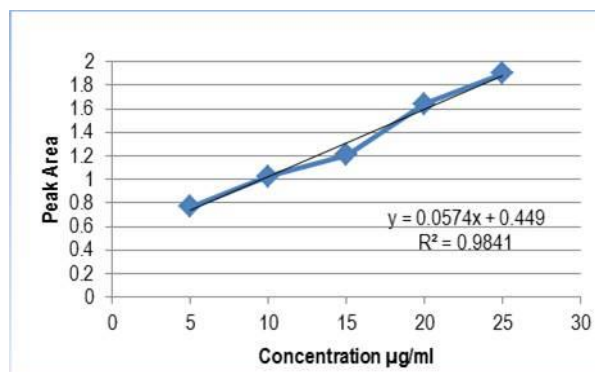


Fig 3:- Analysis of calibration curve

Of the five concentration variations made in the range 5-25 µg/mL, the value of the area obtained is plotted into the y-axis, while the standard series is plotted into the x-axis, thus creating a

calibration curve with the equation of the line $y = 0.057x + 0.449$. From the equation, the intercept b value is 0.449; this affirms that the curve intersects the y -axis at 0.449.

Meanwhile, a value (0.057) represents the slope of the curve. Moreover, the r -value in $r^2=0.984$ is the coefficient correlation value. As Harmita (2004) affirms, the coefficient correlation is only accepted when $r \geq 0.900$. The linearity data signifies a linear correlation between level and width of peak area.

From the precision test it is obtained that SD of precision is 0.1571, in which the acceptance criteria according to Harmita (2004) is $SD < 2$; thus affirming that the test result is still under the acceptance zone of SD value. On top of that, the final parameter validation step involves LOD (Limit of Detection) and LOQ (Limit of Quantitation) value. The calculation result obtains the limit of detection of 0.09 mg/L and the limit of quantitation of 0.27 mg/L; which means that the validation test of the method of analysis has met the requirements set. The notion reaffirms that the method of analysis of urea content in dried stingray by LC-MS is valid and applicable.

The analysis result illustrates that the urea content in each sample of dried stingray (control, A, B, C) is 0,0076 g/mL (0,76%), 0,0065 g/mL (0,65%), 0,0048g/mL (0,48%), and 0,0016 g/mL (0,16%) respectively, signifying that each sample contains different urea content. The following figure 4 describes data of urea content in each sample.

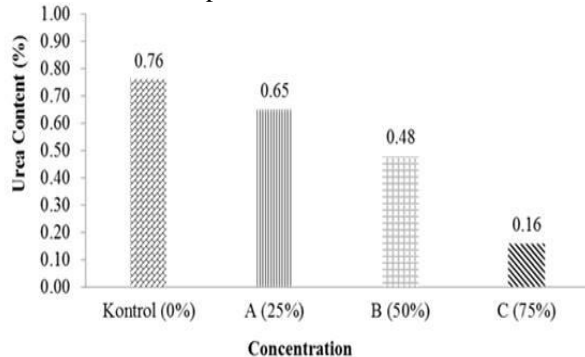


Fig 4:- Histogram of urea content in dried stingray.

The highest and the lowest urea content are in a control sample (0.76%) and sample C (0.16%) respectively. As highlighted in the histogram data in Figure 4, there is a decrease of urea content in each sample marinated by an extract of belimbing wuluh with different concentration; it shows that the higher concentration that is given to stingray meat, the urea content in the meat decreases significantly.

This is due to the chemical reaction between acid solution and urea during marination in stingray meat. During the marine process, the acidic solution will permeate into the stingray meat, resulting in the neutralization of urea. This is in accordance with Irfan (2000), who states that the reduced urea content by the lime solution is due to the basic urea compounds and the acidic lemon

solution that reacts to each other, triggering the urea neutralization.

Citric acid is also found in belimbing wuluh, hence, the extract is also applicable to reduce urea content in stingray meat. On top of that, belimbing wuluh also contains saponin, acting as a detergent to reduce urea content (Muchtadi, 1989).

In line with the notion, Mursito (2002) states that studies have found active substances in belimbing wuluh, i.e., saponin, tannin, flavonoid, glucoside, formic acid, citric acid, minerals, calcium oxalate, and potassium. Similar research by Erungan et al. (2005) on reducing urea content in the Cucut fish meat finds out that by using saponin compounds from 80 mL of sprout filtrate for 3 hours, it can reduce urea content up to 53.41%.

Validation test is conducted to validate the parameters employed in order to meet the requirements set. According to the United State Pharmacopeia (USP), method validation is performed to ensure that analytical methods are accurate, specific, reproducible, and resistant to the range of analyte. The parameter test involves the accuracy test, a test of precision of limit of detection and limit of quantitation, and linearity test. The test further engages accuracy test based on reproducibility test (% recovery), stated as the ratio of the resulted rate with the exact rate, resulting in 101.97%. Referring to Harmita (2004), the reproducibility test result (% recovery) has met the requirements of 98-102%.

Protein content of dried stingray:-

Histogram of the average protein content of dried stingray

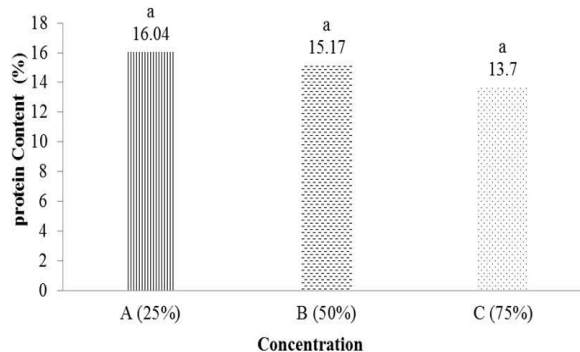


Fig 5:- The average protein content of dried stingray.

The figure 5 depicts that the range of protein content of stingray meat is 13.70-16.04%. Based on ANOVA variance analysis (Appendix 7), treatment of belimbing wuluh extract concentrate have no significant effect ($p < 0.05$) on the protein content of dried fish. However, the protein test analysis describes that marination of stingray meat using the extract with different concentrations can lead to a significant decrease in protein content, as follows: in sample A, protein content is in 16.04%, while in samples B and C it decreases to 15.17%-13.70%.

The decrease of protein content in dried stingray meat is affected by the reaction of marination by belimbing wuluh extract with different concentration, i.e., 25%, 50%, and 75%. The higher concentration used in the marination leads to decreasing content of protein in the meat. This is down to the acid reaction contained in the belimbing wuluh extract is capable of loosening the binding capacity of meat protein to its liquid and triggering the amino acid racemization in the meat. Racemization is a phenomenon that occurs when proteins are needed in acidic solutions, thus affecting protein nutritional value (Palupi et al., 2007). It can also occur during acidic or roasting conditions, especially when there is a lipid or reducing sugar.

Water content of dried stingray:-

Histogram of water content in dried stingray

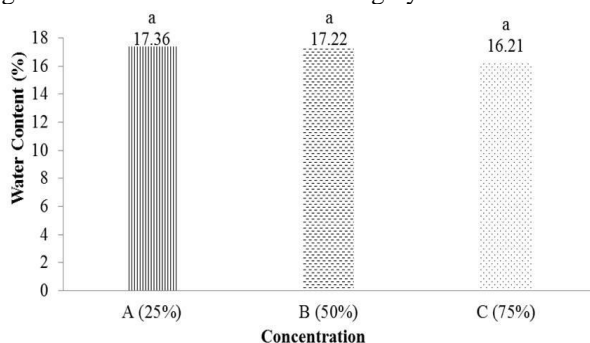


Fig 6:- The average protein content of dried stingray.

The previous figure signifies that the water content of the dried stingray is 16.21-17.36%. Based on ANOVA variance analysis, treatment of belimbing wuluh extract with different concentration have no significant effect ($p < 0.05$) on the water content of dried fish.

As observable from the histogram, there is a decrease in water content in each treatment. Although the laboratory test results did not show a significant difference, nonetheless, differences in the water content of the three samples is considered to be affected by the treatment of belimbing wuluh extract containing acidic compounds. This results in decreasing chemical quality of the water content in stingray meat. It is supported by Ernawati (2008) that the smaller pH level causes the binding energy of the meat to decrease and causing the meat to lose fluid; this causes the meat texture to be mushy and not compact. In addition, reduction of water content in stingray meat is down to the treatments, i.e., marination and drying; consequently, there is no significant difference in the water content between each sample.

III. CONCLUSION

The findings and discussion of the chemical quality of dried stingray marinated by belimbing wuluh extract (Averrhoa blimbi L) are concluded as follows: Marination of stingray meat using belimbing wuluh extract with different concentrations is proven to reduce urea content in stingray meat throughout all treatments. Prior to the treatment, the urea content in control sample and

sample A, B, and C is 0.76 g/mL (0.76%), 0.65 g/mL, 0.48 g/mL, and 0.16 g/mL respectively. The findings illustrate that higher concentration of belimbing wuluh extract is capable of reducing more urea content of the meat. Moreover, the treatments also result in a decrease in the protein content of the dried stingray meat, i.e., 16.04% - 13.7%, and in its water content, i.e., 16.21% - 17.36%.

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