The influence inter-day colorimetric method in quantification of chicken meat’s total cholesterol with lipid extraction - saponification pretreatment

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Abstract. Unskinned chicken meat’s cholesterol consisting of thigh, breast, and wing meat were determined inter-daily by ferric chloride reagent spectrophotometric methods. The pretreatments before cholesterol analysis were conducted which involved with lipid extraction, saponification, and subsequent extraction of unsaponified matter. Afterwards, spectrophotometry was carried out. For understanding precision of quantification, the total cholesterol was measured inter-daily in which the difference of measurement time were one day for each same part of chicken meat. The research showed unskinned thigh meat had significantly higher total cholesterol 186.19 mg/100 g as compared to breast, and wing meat (156.05 mg/100g and 135.38 mg/100 g; p<0.01 for both). Inter-daily spectrophotometry analysis insignificantly affected on thigh, breast, and wing meat’s cholesterol content. This method can become solution for analysis time limitation problem in cholesterol spectrophotometric analysis.

1. Introduction

Cholesterol is fat-like substance of mammalian tissue containing 27 atom carbon [1]. Their combination of steroid and alcohol make the substance usually bounded with lipoprotein and can be esterified, besides free-form cholesterol is also found [2]. The cholesterol plays role in composing cellular membrane and becoming a precursor of bile acid, vitamin D, and essential hormones, such as cortisol, progesterone, testosterone, etc. During growth stage of human life, cholesterol is essential substance for composing myelin, thus it aids in developing nervous system [3]. Cholesterol is commonly synthesized by body itself, but it can be served by dietary intake such as egg, meat, fish, etc. Cholesterol in optimum amount give advantages for human. However, its exceed level is correlated to arteriosclerosis and cardiovascular disease, so that the cholesterol dietary intake to our body should be controlled [4].

To estimate cholesterol dietary intake, the precise determination is essentially needed. The spectrophotometric or colorimetric method is often used in cholesterol calculation because of low cost and simplicity, so that it can be applied in all vary laboratory [5]. However, the pretreatments before spectrophotometer analysis including lipid extraction and saponification spend more time and they were complicated. To overcome it, many researchers proposed saponification without lipid extraction...
pretreatment (direct saponification in other word). This pretreatment is effective and efficient because it doesn’t consume time, labor, and apparatus [6]. It is commonly known that saponification has role to change esterified cholesterol to free cholesterol, so that total cholesterol is resulted from the analysis [7]. However, based on the fact that direct saponification is unavailable for sample extract whose the color disturb the turbidity, the lipid extraction coupled with saponification technique is still remain to be applied in cholesterol analysis.

This study was aimed to assess the quantification of total cholesterol by lipid extraction coupled with saponification technique. The broiler chicken meat was used as analyzed sample because it was consumed worldwide, moreover its cholesterol content is rather high based on some literatures [8]. The extract sample was analyzed for its cholesterol by FeCl₃ reagent - spectrophotometric methods in triplicate repetition but at different day. The aim was to prove that total cholesterol quantification by lipid extraction coupled with saponification technique resumed by inter-day colorimetric method is available or not.

2. Material and Methods

2.1. Material

Broiler chicken meat was purchased from local market in Jogjakarta. The number of broiler was single with consideration that the cholesterol will be differ among the individual chickens, moreover the research focused in inter-day testing. Part of meat analyzed were thigh, breast, and wing. Standard cholesterol C_{27}H₄₅OH was purchased from Sigma Aldrich, USA. Chloroform, KOH, ethanol, sulfuric acid, FeCl₃, 6H₂O, acetic acid, petroleum ether were purchased from Merck.

2.2. Methods

2.2.1. Cholesterol standard calibration curve preparation. Ferric chloride reagent (FeCl₃ reagent) was initially prepared by dissolving FeCl₃.6H₂O by glacial acetic acid at portion 10% (w/v), then followed by diluting FeCl₃ solution in H₂SO₄ with dilution factor of 10. Meanwhile, cholesterol stock solution was prepared by dissolving cholesterol standard by glacial acetic acid to get final concentration 0.1 mg/ml. Then serial dilution was carried out to stock solution by glacial acetic acid for getting cholesterol concentration of 0.075; 0.050; 0.025; 0.0125; and 0.0050 mg/ml. The cholesterol stock and its diluted solution were reacted by FeCl₃ reagent in volume ratio (1:1). Absorbance of solutions were measured by UV-Vis spectrophotometer (Dynamica HALO RB-10 Spectrophotometer) at wavelength 515 nm. Cholesterol standard curve depicted the relationship of cholesterol concentration and its absorbance value. The linearity of standard curve was high with R square value was 0.9997.

2.2.2. Pretreatment sample before colorimetric analysis. Chicken meats comprising thigh, breast, and wing were unskinned and then dried in cabinet dyer at temperature 105°C for overnight. The pretreatment sample before colorimetric analysis followed method by Ismail et al. [9] with slight modification. The dried sample was ground for obtaining fine powder. The meat powdered was weighed for 2 gr and was conducted for fat extraction by soxhlet method with petroleum ether 40 ml for 8 hr. Afterwards, the fat extract was diluted by chloroform 10 ml. A portion of 2 ml solution was saponified by reflux boiling with alcoholic-KOH 10 ml for 1 h in order to convert esterified cholesterol to free cholesterol. It was cooled, then water was used for rinsing it. The unsaponified matter was separated by extraction with petroleum benzene for three times. Then, cholesterol extract was separated from the solvent by evaporation at 60°C. The extract sample was ready to be analysed. For samples would analysed at different day, samples were stored in desiccator at ambient temperature.

2.2.3. Spectrophotometric analysis of sample. Cholesterol extract sample was diluted with glacial acetic acid 4.5 ml. One ml portion of sample solution diluted again with glacial acetic acid until the
volume reached 10 ml. The next step was similar to that of standard cholesterol. Cholesterol sample solution was also reacted by reacted by FeCl$_3$ reagent with volume ratio (1:1) and then absorbance was measured at 515 nm. Spectrophotometric analysis for a cholesterol sample extract was in triplicate but repetition was conducted at different day. Total cholesterol was calculated as following formula:

\[
\text{Cholesterol (mg/100g)} = \frac{(C_s \times DF \times V_s \times F)}{(m \times 100)}
\]

Where: \(C_s\) is cholesterol concentration based on standard calibration curve, \(DF\) is dilution factor (in this case, the \(DF\) value is 10), \(F\) is factor because of one fifth of fat extract that was saponified (\(F=5\)), \(m\) is dried chicken meat weight. \(V_s\) is total volume of cholesterol extract, in this case \(V_s\) was 4.5 ml.

2.2.4. Statistical analysis. Collected data were statistical analysed by SPSS 12. One way Analysis of Variance (one way ANOVA) was used for compared mean of thigh, breast, and wing meat cholesterol with significance level of 0.01. Furthermore, effect of inter-daily analysis on cholesterol content was studied by Pearson correlation. Pearson correlation was considered to be chosen because it is used for parametric test.

3. Results and Discussion

It was considered that chicken skin contained high cholesterol, about 71 mg / raw chicken skin [10]. Before chicken meats were analysed, they were unskinned for avoiding ambiguous result. The analytical data for cholesterol content of thigh, breast, and wing chicken meat can be seen in Table 1 and Table 2.

**Table 1. The result of inter daily cholesterol analysis of chicken meat by spectrophotometric method**

| Part of meat | Day of analysis after extraction | Cholesterol content (mg / 100 g meat)* |
|-------------|---------------------------------|---------------------------------------|
| Thigh       | 1st                             | 188.94                                |
|             | 2nd                             | 186.18                                |
|             | 3rd                             | 183.45                                |
|             | 1st                             | 155.90                                |
| Breast      | 2nd                             | 157.71                                |
|             | 3rd                             | 154.53                                |
|             | 1st                             | 141.25                                |
| Wing        | 2nd                             | 130.25                                |
|             | 3rd                             | 134.63                                |

*All measurement were conducted as dry weight basis

**Table 2. The result of correlation test inter day cholesterol analysis spectrophotometric method on cholesterol content**

| Part of meat | Cholesterol content (mg / 100 g meat) | Effect of differ day analysis on cholesterol content |
|-------------|---------------------------------------|-----------------------------------------------------|
| Thigh       | 186.19 ± 2.75*                        | insignificant                                       |
| Breast      | 156.05 ± 1.60*                        | insignificant                                       |
| Wing        | 135.35 ± 5.54*                        | insignificant                                       |

*Means within a column with no common superscript differ significantly (P < 0.01)

All measurement were conducted as dry weight basis, values are means ± standard deviation for 3 meats per group.

From Table 1 and Table 2, it can be shown that thigh chicken contained more cholesterol than breast and wing. It agreed with results obtained by Anandhi et al.[11] that thigh chicken cholesterol was higher than that of breast. Salma et al.[12]found that thigh cholesterol (194.2 mg /100 g) was
higher than breast (93.6 mg/100 g). The cholesterol determination was carried out by enzymatic method with direct saponification pretreatment and the result gave cholesterol content was almost similar to the range of cholesterol content in this research.

The value total cholesterol from this research was also similar to range of by Ponte et al. [13] research in which the breast cholesterol was 185.04 mg/100 kg dried meat. Ponte’s cholesterol quantification was conducted by HPLC method with direct saponification pretreatment. Dinhet al. [14] also investigated that chicken thigh had more cholesterol than breast.

Meanwhile, cholesterol spectrophotometric analysis conducted in different day (1st, 2nd, 3rd day) for every sample did not affect cholesterol content significantly. It proved that inter-day colorimetric method was precise for cholesterol quantification. It means that the method achieve acceptable repeatability. As we known, that pretreatment before spectrophotometric analysis spend more time, so the analysis at the day after is available. The reproducibility of inter-day also obtained acceptable repeatability for cholesterol analysis by gas chromatography – tandem mass spectrometry [15].

4. Conclusions
Analysis of cholesterol content by FeCl₃ colorimetric method revealed that thigh meat had significantly higher total cholesterol 186.19 mg/100 g as compared to breast, and wing meat (156.05 mg/100g and 135.38 mg/100 g; p<0.01 for both). Inter-day spectrophotometry analysis achieve acceptable repeatability because the different-day analysis insignificantly affected on thigh, breast, and wing meat’s cholesterol content. The value total cholesterol from this research was in range of other research in which different method were used, i.e. HPLC method and enzymatic method with direct saponification pretreatment.

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