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Freeze-Dried Cattle Meat Improves the Measurement of Total Cholesterol

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Abstract

The objective of this study was to propose an adaptation to the measurement methodology using enzymatic kits with raw meat and compare the efficiency in the quantification of total cholesterol in raw and freeze-dried meat, in order to optimize the execution of the analysis and the estimation of the results obtained. We used the *longissimus thoracis* and *semitendinosus* Nellore muscles which were acquired in a commercial slaughterhouse. They were ground with a food processor and separated; one of these parts was freeze-dried and the other was kept raw. The methodology purposed in our research (using freeze-dried meat), obtained better measures for cholesterol levels in both muscles. The total cholesterol measures for *longissimus thoracis* and *semitendinosus* with freeze-dried meat were 12% and 13% higher comparing to raw meat, respectively. The utilization of freeze-dried meat was more effective on the extraction and measurement of total cholesterol in both muscles.

Keywords: Enzymatic kit; *Longissimus thoracis;* Raw meat; *Semitendinosus*

Introduction

Since its major portion is mainly constituted by muscle fibers, the meat is considered a source of high biological value proteins, because it carries in its composition essential amino acids, lipids, vitamins and minerals. Additionally, there is a great concern by the researchers to improve some characteristics of bovine meat related to cholesterol and saturated fat. Aiming to increase the proportion of unsaturated fat over saturated, there is a tendency to research the effects of the addition of vegetable oils, which are rich in unsaturated fats, on ruminants feed. The cholesterol is a small fraction of the animal tissue, which may appear on the free-form or esterified with a fatty acid. The bovine meat, on average, contains about 70 to 75mg of cholesterol/100g, with over 90% appearing on the free form [1]. The cholesterol levels and the saturated fat portion are closely related to coronary diseases showed by population. They are described by the doctors as great villains to the human health, since they can cause obstruction of coronary veins, compromising the normal functioning of the heart. The fraction that may increases the risk of arterial obstruction is the Low-Density Lipoprotein (LDL) that represents about 85% of total cholesterol, whereas the High-Density Fraction (HDL) helps on the clearing of tissues and arteries [2]. However, the cholesterol plays an important biological role because it acts as a precursor on the hormonal regulation and serves as a source of energy to the organism. Due the importance on the human health, the cholesterol measurement must be precise, practical and quick. So, we saw the need to adapt the currently methodologies to improve the total cholesterol quantification in cattle meat. There are several methodologies for quantification of total cholesterol in meat cuts, such as [3], enzymatic kit, by chromatography. Based on the results obtained by several authors in the literature and relating to the reference results, the results obtained by these authors are below, dispersion, with standard error of the mean and large coefficient of variation. As a result, we saw the need to adapt the methodology using the enzyme kit, to improve total cholesterol estimates, as well as standard error and coefficient of variation. The objective of this study was to propose an adaptation to the measurement methodology using enzymatic kits with raw meat and compare the efficiency in the quantification of total cholesterol in raw and freeze-dried meat, in order to optimize the execution of the analysis and the estimation of the results obtained.

Materials and Methods

This research was performed at the Laboratory of Technology of Animal Products in the Department of Technology, located at São Paulo State University in Jaboticabal, Sao Paulo, Brazil. We used the *longissimus thoracis* and *semitendinosus* muscles from Nellore cattle (300 animals) which were acquired in a commercial slaughterhouse. They were ground with a food processor and separated; one of these parts was freeze-dried and the other was kept raw. We made three hundred replications for each muscle (300 for *longissimus thoracis* and 300 for *semitendinosus*) and three hundred replications treatment (300 for raw meat versus 300 for freeze-dried meat), totalizing 1200 replications. Before proposing the adaptation in the [4] methodology, preliminary tests were performed to quantify the sample weight as well as the standard curve standardization. In the [4] methodology around 2 grams of raw sample is used. To quantify the total cholesterol, we weighed about 750 milligrams (mg) of the freeze-dried sample on falcon tubes; posteriorly, it were added 6 mL of ethanol and 4 mL of aqueous solution of potassium hydroxide (KOH); the tubes were taken to water bath, firstly at 40oC until the complete dissolution of the sample, then at 60°C for 10 minutes more; after this, we added 5mL of distilled water to cool the dissolved samples and 10mL of n-Hexane to separate the phases. The upper phase, containing hexan and cholesterol, was taken off and reserved; this process was repeated three times. From the reserved phase, we took triplicate aliquots with 3mL that was transferred to the test tubes; the tubes were submitted to drying with Nitrogen Gas (N_2) until the liquid was totally dried. After drying, we added 0.5mL of isopropyl alcohol to each tube that was agitated by vertexing until the complete solubilization, and then we added 3mL of color reagent of the Labor lab® Cholesterol COD-PAP Liquid Stable laboratorial kit that consists in Cholesterol Esterase (CHE), Cholesterol Oxidase (CHOD), Peroxidase (POD), 4-aminofenazona (4-AF) and Good buffer (phenol and sodium cholate). The samples were taken back to water bath at 37°C for 10 minutes and then they were submitted to "rest" for 90 minutes before the reading in spectrophotometer at 499nm. The standard curve was built from a pure cholesterol solution (1000mg/100mL), with the concentrations varying between 0,01 to 0,09mg/mL. The reading of the samples and standard curve was performed using a Shimadzu® spectrophotometer model UV-1800. We made three hundred replications for each muscle (300 for *longissimus thoracis* and 300 for *semitendinosus*) and three hundred replications treatment (raw meat versus freeze-dried meat), totalizing 1200 replications. Data were arranged in a completely randomized design with two treatments (sample type) and 300 replicates. A one-way ANOVA was performed, and, in case of significance, group means were compared by the Tukey's test, with the significance level set at $P < 0.05$, using software SAS [5].

Results and Discussion

The means and the coefficient of variation using raw meat and freeze-dried meat for the *longissimus thoracis* and *semitendinosus* muscles were found in Table 1. The means of cholesterol levels obtained by enzymatic method with Raw Meat (RM) and Freeze-Dried Meat (FZD) for *longissimus thoracis* (106.64mg/100g (FZD) and 97.87 mg/100g (RM)) and for *semitendinosus* (115.37mg/100g (FZD) and 98.11mg/100g (RM)), were higher than those found by USDA (62mg/100g). [4,6,7] observed values of 51mg/100g and 75mg/100g, respectively, which is lower comparing to the results found in this research. [8] working with drying methods on the quality of beef jerky, obtained values for high free fatty acid which increased the cholesterol level of the samples, which are very similar to our results (106.64mg/100g and 115.37mg/100g) for *longissimus thoracis* and *semitendinosus* in freeze-dried meat. [9] found values of 56.46mg/100g of cholesterol, using the *longissimus thoracis* muscle of 5/8 Nelore+3/8 Charles animals with the methodology purposed by Folch, which are lower comparing to our results. [10] working with half-blood Nelore/Simental young animals, found values for the cholesterol of 64mg/100g, results that are lower in comparison to the results in this paper. [11] obtained higher values for the cholesterol comparing to those found by [9,10] but lower than the results observed on our research. [12-14] they worked with castration in superpresences steers; precocious steers fed with protected fat and with pasture-terminated animals, observed about 60mg /kg, 68mg /kg and 40mg/kg of total cholesterol in *longissimus thoracis* muscle, respectively. Values below the results observed in this study.

Table1: Means ± standard error of total cholesterol (mg/100g) in samples of raw and freeze-dried bovine meat on the longissimus thoracis e semitendinosus muscles.

Total Cholesterol mg/100g					p-value (p=0.01)
Sample	longissimus thoracis	C.V.[1]	semitendinosus	C.V.[2]	
Freeze-dried	104.66 ± 2.1 A	3.1	103.44 ± 2.7 A	2.9	
Raw	92.21 ± 4.7 B	5.9	89.7 ± 4.5 B	4.3	
C.V.[3]	3.26		4.59		

C.V(%)1- coefficient of variation of longissimus thoracis for each methodology. C.V(%)2- coefficient of variation of semitendinosus for each methodology. C.V(%)3- Total coefficient of variation for each muscle.

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[15] worked with Podolia young bulls and they observed means for total cholesterol about 49.69, 47.99 and 46.76 in *longissimus dorsi*, *semimembranosus* and *semitendinosus*, respectively, using chromatography. [16] used three different cattle breeds (Piemontese, Limousin and Friesian) observed means of total cholesterol around 50.98, 50.86 and 50.99, respectively, results around 54% and 50% lower than the present study. The methodology purposed in our research (using freeze-dried meat), obtained better measures for cholesterol levels in both muscles comparing to those observed by [4]. The total cholesterol measures for *longissimus thoracis* and *semitendinosus* with freeze-dried meat were 12% and 13% higher comparing to raw meat, respectively. This probably occurred due to the absence of water, which may be the factor that interfered on the measure of cholesterol. It is known that water and cholesterol is polar and a polar, respectively, as a result, there is the possibility of micelles forming that hinders the extraction of cholesterol from the sample by n-hexane (a polar). Thus, carrying out the analysis in the raw sample may produce the above described fact, thus impairing the total quantification of cholesterol in the sample compared to the freeze-dried sample. This is the explicit case for the article of [3], which worked on the lipid extraction in cod, with the same tests of varied volumes of water, chloroform and methanol and quantified the lipid extraction for each proposed method. The authors observed that, with the increase of the volume of water during the process of lipid extraction and maintaining the same volume of methanol and chloroform, the lipid extraction was smaller when the water volume was higher. Thus, it is evident that water has an important influence on the quantification of lipids in general. Another factor that may have influenced the results was the spraying of the freeze-dried sample, thereby increasing the contact area of the cholesterol molecule with the n-hexane solvent. Thus, increasing the quantification of cholesterol in the freeze-dried sample compared to the raw sample. The freezing-dried is a process in which the sample is freeze and submitted to a vacuum chamber where occurs the sublimation of water contained in the feed. Due to this process utilizes low temperatures, there is no possibility of oxidation of fat and cholesterol, so it does not affect the precise measurement of cholesterol. The lyophilization decreases the water activity, what makes harder or impossible the microbial growth, so the samples can be stored for longer periods excluding the risk of rancidification (oxidation). The process takes about 3-4 days, until the samples are completely dry, which may be considered as a negative aspect. Whereas, after the end of the process, there are only advantages, like easier storage, since the samples take up less space, faster dissolution than the raw meat, what makes the analyses faster, and it is not necessary to defrost previously.

We may consider the standard error for each muscle and meat (raw or freeze-dried), as well as the total coefficient of variation (C.V.^{3}) and for freeze-dried and raw meat for *longissimus thoracis* (C.V.^{1}) and *semitendinosus* (C.V.^{2}). As we can observe, the coefficient of variation for freeze-dried meat was lower comparing to raw meat, what ensure more reliable measurement results. The lower coefficient of variation for freeze-dried meat can be due to the homogeneity of samples, which results in lower variation of the values found. So, the methodology adapted to freeze-dried bovine meat can be utilized to measure the total cholesterol with reliability.

Conclusion

The utilization of freeze-dried meat was more effective on the extraction and measurement of total cholesterol in both muscles. The total cholesterol measures for *longissimus thoracis* and *semitendinosus* with freeze-dried meat were 12% and 13% higher comparing to raw meat, respectively.

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Conflicts of Interest

The authors declare no conflicts of interest

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