COMPARISON OF CHOLESTEROL DETERMINATION IN MEAT BY COLORIMETRIC AND HPLC METHODS

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Background

Determination of cholesterol in foods is important because of consumer health concerns and in terms of some regulatory aspects of labelling. The cholesterol content of meats pointed out in the literature shows a high degree of variation (Bohac & Rhee, 1988), which is attributable to the variety on the composition of samples and, in a greater extent, to the differences in the analytical procedures in use. When compared to the HPLC methodology, the colorimetric procedures are often referred as to overestimating the cholesterol content due to their minor specificity for interfering autoxidative substances. However, they are considered less expensive and easier to approach.

Objectives

The aim of this study was to compare the reliability of two colorimetric methods against HPLC on cholesterol quantification of meat. The influence on the performance of methods due to the sample intramuscular fat content and storage time at –18°C was also evaluated.

Material and methods

After 24 h chilling, fresh meat samples were removed of the calve carcasses from *Longissimus dorsi, Biceps femoris* and *Supraspinatus* muscles. Once excised of the visible fat, samples were then stored during about 12 and 18 months at –18° C before the cholesterol analysis.
Muscle total lipids were extracted by the procedure of Folch *et al* (1957) and dissolved in 25 ml of hexane. Then an aliquot of 1 ml was saponified at 80°C for 15 min. according to Bohac & Rhee (1988). Unsaponifiable material was extracted with 5x25 ml hexane, freed of solvent and the residue dissolved again in 15 ml of hexane HPLC grade.
Cholesterol quantification was measured by two colorimetric methods and through high performance liquid chromatography (HPLC). HPLC was performed on a Spectra-Physics Model Spectra 100, equipped with variable wavelength UV detector set at 206 nm and a Spherisorb S 5W silica, 5µm, 4.0x125 mm cartridge (Waters PSS 845549). The mobile phase was hexane/isopropanol (97:3) at a flow rate of 1.0ml min^{-1}.
In relation to the colorimetric methods, the procedures referred by Bragagnolo & Rodriguez-Amaya (2001) and Tietz (1976) were applied. The former method uses acetic acid saturated with ferrosus sulfate and concentrated sulfuric acid as coloring reagent whereas the second, based in Liebermann-Burchard reaction, utilises for that purpose an medium containing acetic acid, acetic anhydride and concentrated sulfuric acid.

Results and discussion

The cholesterol mean values and standard deviations of veal samples with two levels of intramuscular fat (IMF) obtained through the analytical methods under study, as well as the percentual variation representing the differences between HPLC and colorimetric methods measurements, are shown on Tables 1 and 2. Irrespective of the sample condition, the results from the colorimetric assays were significantly higher than those obtained by HPLC. This trend agrees with the findings of Jiang *et al.* (1991) and Beyer & Jensen (1989) but is in opposition with the conclusions stated by Bragagnolo & Rodriguez-Amaya (2001). No important practical divergences were noted between the colorimetric methods. However, that using the reagent containing acetic acid saturated with ferrosus sulfate presented, in relation to the HPLC assay, less variation on the mean of the difference obtained on samples with IMF higher than 2% (23.9% vs 31.5%) and lower degree of dispersion on the results (R = 0.801 vs R = 0.607) (Figures 1 and 2). All the three methods showed equivalent recovery performances, presenting, in average, 99.4%, 97.2% and 106.1% for HPLC, Liebermann-Burchard and $FeSO_4/H_2SO_4$, respectively (Table 3).
Differences in cholesterol quantification between HPLC and colorimetric methods were not affected by storage time of samples from 12 up to 18 months (Figures 3 and 4). This could be due to the minor development of the cholesterol autoxidative process in samples.

Conclusions

The colorimetric methods overestimate the cholesterol content of meat when compared to HPLC irrespective of the sample IMF contents and storage time at –18°C.

References

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Table 1. Comparison of cholesterol content (mg/g muscle) of Barrosã veal measured by HPLC and Liebermann-Burchard methods.

	HPLC	Liebermann -Burchard	Significance	HPLC - Liebermann-Burchard [1]		
				Mean	Maximum value	Minimum value
Veal (n=51)	0,56±0.09[b]	0,72±0.15[a]	***	33,7%	73,8%	1,6%
Veal with IMF<2% (n=23)	0.59±0.08[b]	0.74±0.14[a]	***	28.0%	66.0%	1.4%
Veal with IMF >2% (n=28)	0.55±0.08[b]	0.70±0.16[a]	***	31.5%	73.0%	1.5%

1 Difference on cholesterol results between HPLC and Liebermann-Burchard colorimetric method.
In the same row, means with identical letters are not significantly different (test LSD, P<0.05).
***p<0.001

Table 2. Comparison of cholesterol content (mg/g muscle) of Barrosã veal measured by HPLC and $FeSO_4/H_2SO_4$ methods.

	HPLC	$FeSO_4/H_2SO_4$	Significance	HPLC - $FeSO_4/H_2SO_4$ [1]		
				Mean	Maximum value	Minimum value
Veal (n=51)	0,56±0.09 [b]	0,72±0.16[a]	***	32.0%	51.0%	0.0%
Veal with IMF < 2% (n=23)	0.59±0.08 [b]	0.78±0.15[a]	***	32.1%	70.8%	2.6%
Veal with IMF > 2% (n=28)	0.55±0.08 [b]	0.68±0.15 [a]	***	23.9%	55.8%	0.0%

1 Difference on cholesterol results between HPLC and $FeSO_4/H_2SO_4$ colorimetric method.
In the same row, means with identical letters are not significantly different (test LSD, P<0.05).
***p<0.001

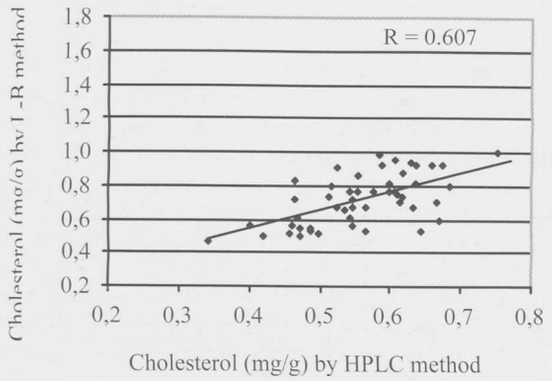

Figure 1 Relation of cholesterol measured by HPLC and Liebermann-Burchard colorimetric method.

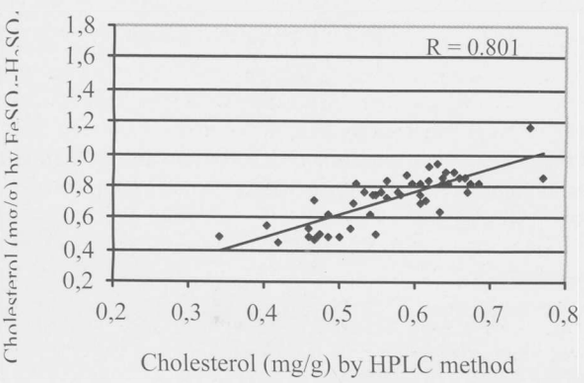

Figure 2. Relation of cholesterol measured by HPLC and $FeSO_4/H_2SO_4$ colorimetric method.

Table 3. Recovery index of cholesterol content.

Experiment	HPLC	Liebermann-Burchard	$FeSO_4/H_2SO_4$
1	99.8%	92.9%	-
2	107.0%	107.7%	107.5%
3	90.8%	90.9%	104.7%
4	99.8%	-	-
Mean value	99.3%	97.2%	106.1%

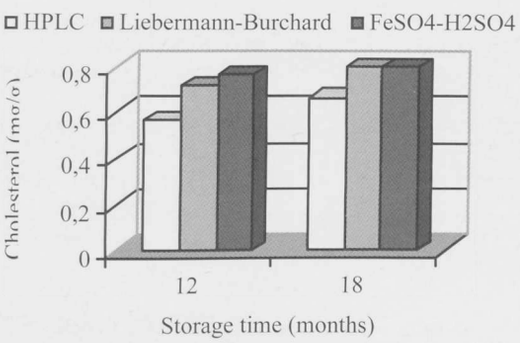

Figure 3. Influence of storage time on cholesterol mean values of muscles with IMF < 2%.

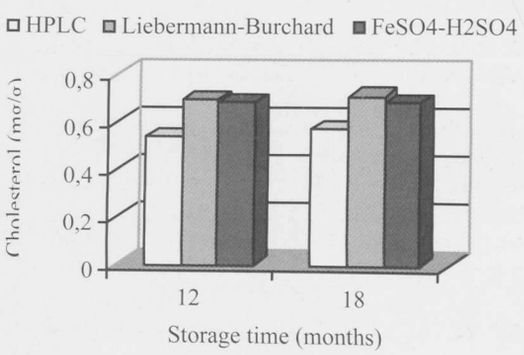

Figure 4. Influence of storage time on cholesterol mean values of muscles with IMF > 2%.