Total Cholesterol Levels in the Maltese sausage

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Abstract

An interesting, but relatively unexplored field in the assesment of the Maltese diet, is the evaluation of the nutritional status of certain traditional dishes and delicacies which, if not endemic to our islands, may only be found in the vicinity of the Mediterranean region. Amongst the more important constituents of such foods, one will most certainly dwell on the cholesterol content of these dishes, this being the most abundant sterol in the body and definitely one with the greatest health implications.

With this perspective in mind a study was undertaken to investigate the total cholesterol levels of the Maltese sausage. Using a semi-enzymatic analytical procedure, the total cholesterol concentration of a particular sample was measured, and the value found to be approximately 1mg per gm. This value could have serious consequences on atherosclerotic patients.

Introduction

The Maltese sausage is a common delicacy on the Maltese Islands and is very often an added entry to most local traditional dishes. It is made up primarily of pork offals enclosed in a gut membrane, either raw or smoked, but the exact constitution and relative abundance of the various pork portions depend very much on the tastes of the sausage maker. As these sausages are traditionally made by hand, there is no standard recipe, and as a result, great variations may exist from one sample to another. Consequently, it was not the aim of the experiment to obtain an absolute value for the total cholesterol content of the Maltese sausages, but nevertheless, to obtain a value which is indicative of the cholesterol content present. Furthermore, the use of the Test conbinations as generously supplied by Boehringer Mannheim GMBH provided an opportunity of evaluating this method of analysis.

Principle and Methodology

Measurement of the cholesterol concentrations was based on the formation of a light absorbing dye in the visible region of the spectrum.

Any cholesterol esters present were first hydrolysed to free cholesterol and fatty acids (Fig. 1). In the presence of oxygen and cholesterol oxirase, the free cholesterol was then converted to — cholestone and finally in a reaction catalysed by catalase, the hydrogen peroxide produced in the latter reaction, oxidised methanol to formaldehyde which subsequently reacted with ammonium ions and acetylacetone to form a yellow lutidine-dye. The concentrations of the lutidine-dye (3, 5-diacetyl —1, 4-dihydrolutidine) formed was measured by the increase of absorbance in the visible range at 405 nm and as this concentration was stoichiometric with the amount of cholesterol, it gave a direct indication of the original cholesterol concentration present.

The analytical procedure was divided into four main operations, namely

1. The standardisation of the spectrophotometer in use as specified by the B.P.
2. The preparation of the test solutions.
3. The treatment of the Maltese sausage prior to assay.
4. The assay and spectrophotometric measurement of the increase in absorbance in the visible range at 405 nm.

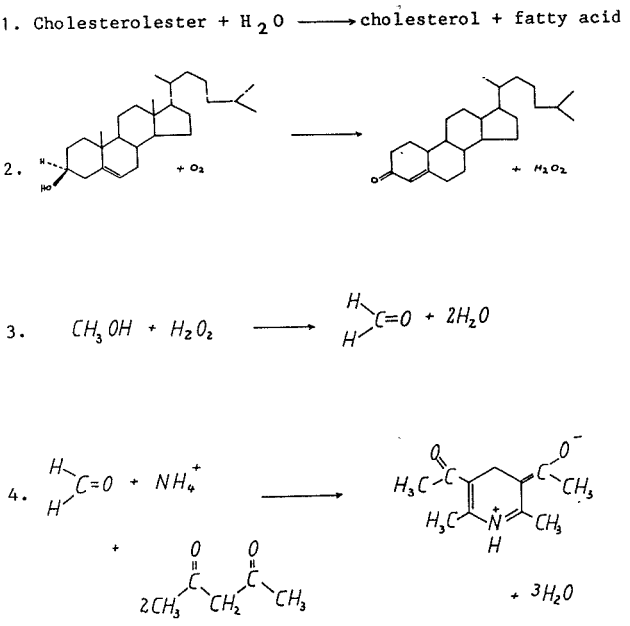

Fig. 1 Hydrolysis of any cholesterol ester present

The British Pharmacopoeia 1980 stipulates various requirements. But only two specifications were considered namely the control of absorbance and the limit of stray light.

In order to achieve the stated stability the test reagents were stored in a refrigerator at +2°C to +8°C prior to use. Furthermore, freshly redistilled water from a glass distilling apparatus was used for dissolving auxilliary reagents. Amongst the test solutions prepared, a cholesterol standard solution was made to give a final standard cholesterol solution of 0.25mg/ml concentration.

However, prior to assay, the sausage sample had to be subjected to a chemical treatment in order to facilitate the extraction of cholesterol present. This involved an initial exposure of a well broken down sample to a volume of 1 Molar methanolic solution of potassium hydroxide in the presence of a quantity of clean sea sand. This sand was previously ashed in an oven to free it from any organic material present. The flask and contents were then heated to 160°C in a waterbath placed on an electric hotplate and refluxed for 25 minutes while stirring with a magnetic stirrer. Repeated reflux and washing with specific volumes of isopropanol resulted in a cholesterol containing supernatant which on filtering gave a clear solution and which could subsequently be assayed.

Each assay run required the addition of further reagents and an incubation period of 1 hour at 37-40°C. This applied to both samples, standard, as well as sample and standard blank solutions respectively. The absorbance at 405nm was monitored using a double-beam spectrophotometer, and by means of a mathematical equation, the cholesterol concentration was determined as a function of the mean value of absorbance. Statistical considerations gave a final value of the cholesterol content per gram of sausage as being equal to 1.315 + 0.579 mg.

Discussion

Although this semi-enzymatic estimation does have its drawbacks, when compared to a fully-enzymatic estimation, it provides, together with the latter, a number of major advantages, namely a high degree of specificity, the absence of the need to deproteinise the sample, and the absence of drug induced interactions.

On the other hand, the major disadvantage of this method are the time consuming hydrolysis which is liable to interference and the tedious but indispensable removal of the reducing substance formed in the course of alkaline hydrolysis. This introduces a wide range of error. In view of this, a fully enzymatic method for the determination of total cholesterol content is suggested in which the conversion of cholesterol is also enzymatic (using cholesterol oxidase) and which occurs under mild and convenient conditions. The accuracy and precision of a fully enzymatic method are thus highly satisfactory and recommended. Furthermore, such a procedure would also offer the opportunity for a differential analysis of the free and esterified cholesterol content in a given sample. This may be achieved by the separate but successive addition of cholesterol oxidase and cholesterol to any assay mixture.

Conclusion

The estimation of cholesterol is of paramount importance both in the food industry, as well as in clinical diagnosis, hypercholesterolaemia being the major risk factor for atherosclerosis and related disorders.

Keeping in mind the methodology involved and the associated range of error one may conclude that the total cholesterol content in this particular sample of Maltese sausage is approximately of 1 mg per gram of sausage. This value has to be seen in the light of the observation that a reasonable 'bite' of sausage by an adult would comprise from 4 to 7 grams of sausage content. One has therefore to evaluate the amounts of total cholesterol taken into the gut on continuous ingestion of this favourite delicacy, especially when the adult eating the sausage may unknowingly be an artherosclerotic subject.

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