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Determining the effect of Garlic, Pepper, Onion, Fenugreek and Curry-Leaves on Egg-Cholesterol using RP-HPLC method

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ABSTRACT

Cholesterol is a wax like substance found in every living cell in the body. A typical egg contains 2:1 ratio of albumen (white) to yolk. Included in the fat of the yolk are 1.075% cholesterol and 7.31% lecithin. Our project aims at reducing the cholesterol present in egg, by the use of food materials which we regularly consume, so that it could be of some use to all those persons having sedentary lifestyles or with busy schedule and has no time for regular exercise. Hence fenugreek (*Trigonella foenum graecum*), Garlic (*Allium sativum*), Ginger (*Zingiber officinale*), Pepper (*Piper nigrum*), and curry leaves (*Murraya koenigii Spreng*) were selected to check whether they have any effect in-vitro, on egg cholesterol using RP-HPLC method. The HPLC apparatus consisted of Model LC 10 AT pumps (SHIMADZU®) and cholesterol was quantified by an isocratic mixture of acetonitrile-2-propanol (1:3). It was concluded that pepper was the most effective cholesterol fighter, followed by curry-leaves. Hence it's advisable to include pepper in our daily diet, along with egg, either in raw or cooked form, and curry-leaves in its cooked form, to reduce cholesterol levels.

Keywords: Cholestrol, egg, pepper, curry-leaves, RP-HPLC.

INTRODUCTION

Cholesterol^{1,2}, is a wax like substance found in every living cell in the body. Cholesterol is required for the structure of cell walls, must be available for the body to produce vitamin D, is essential for the production of digestive juices; insulates nerve fibres and is the basic building block for many hormones. Elevated cholesterol is associated with a greater-than-normal risk of atherosclerosis, high BP, and excessive clotting. Any of these problems may lead to coronary heart disease. Keeping cholesterol levels in the safest range (between 180

and 200 mg/dL³) is one way of statistically reducing the risk of suffering a heart attack or stroke. The bulk of heart attacks occur when the reading is between 200-250 mg/dL.

Cholesterol is elevated¹ in diabetes mellitus, liver disease, kidney disease, hypothyroidism and during pregnancy. Decreased values of cholesterol will appear with cachexia, malabsorption, hyperthyroidism, pernicious anaemia, during estrogen therapy, or drug therapy such as treatment with antibiotics.

A typical egg³ contains a 2:1 ratio of albumen (white) to yolk. Egg white consists of 88.50 % moisture, 9.80 % protein and 0.00 % fat, while the yolk has 57.00 % moisture, 15.50 % protein and 25.60 % fat, most of it unsaturated. Included in the fat of the yolk are 1.075 % cholesterol and 7.31 % lecithin. Cooking does not affect the cholesterol content of eggs. The high concentration of cholesterol in egg yolk has been highlighted in the last decades and has caused restrictions to its consumption. Thus, any feasible means of reducing Cholesterol content of eggs would be of interest to egg consumers.

Thus our project aims at reducing the Cholesterol present in egg, (outside the body) by use of various other food materials which we regularly consume, so that it could be of some use to all those persons having sedentary lifestyles or with busy schedule and has no time for regular exercise. Hence fenugreek (*Trigonella foenum graecum*), Garlic (*Allium sativum*), Ginger (*Zingiber officinale*), Pepper (*Piper nigrum*), and Curry leaves (*Murraya koenigii* Spreng) were selected to check whether they have any effect, *in-vitro*, on Egg-cholesterol using RP-HPLC method.

MATERIALS AND METHODS

Reagents and chemicals

All the chemicals and reagents used were of analytical grade. Acetonitrile and diethyl ether was obtained from SD fine chem. Ltd. Mumbai. Acetone was obtained from Fischer Chemic Ltd. Chennai, Propan-2-ol and Methanol from Qualigens Fine Chemicals, Mumbai and Loba Chemie. Pvt. Ltd. Mumbai respectively.

Chromatographic conditions.

The HPLC apparatus consisted of Model LC 10 AT pumps (SHIMADZU[®]) equipped with a model SPD 10A UV detector (SHIMADZU[®]) set at 215 nm. A 5 mm ODS (250 x 4.6 mm) column was used and the yellow crystals of cholesterol obtained were analyzed, by an isocratic mixture of acetonitrile – 2 propanol (1:3), which flowed at a rate of 0.6 ml/min. The retention time obtained for extracted egg cholesterol was 8.604 min. Similarly the effect of various food materials in raw and cooked form on egg cholesterol, were studied to check if it had any effect on Egg-cholesterol.

Extraction of cholesterol from egg-yolk

Egg yolk was isolated and 10 ml of methanol⁴ and 50 mL diethyl ether⁵ was added. The suspension was mixed for 5 min and then filtered. The filtrate was washed with additional 10 ml ether. The solution was evaporated to dryness. 23 ml diethyl ether was added to the above dry residue and 150 ml Acetone was added slowly with continuous stirring. Lecithin was precipitated as gummy bulk. This solution was filtered and the lecithin's was washed with 10 ml acetone, and thus isolated from cholesterol. The acetone-ether solution was distilled to obtain a liquid oil residue which was saponified with 25 ml of 15 % KOH in ethanol. 70 ml

water was added and the solution was extracted twice with 60 ml ether. Ether phase was washed and dried with MgSO₄ and was evaporated to dryness.

Preparation of Standard solutions

A stock solutions of 1 mg/ml cholesterol was prepared using a mixture of Acetonitrile and 2-propanol (1:3v/v). The standard solutions were prepared by dilutions of the stock solutions to give solutions containing cholesterol in concentration range of 100 µg/mL to 400 µg/mL.

Assay method

With the optimized chromatographic conditions, a steady baseline was recorded. The standard solution was injected and the chromatogram was recorded. The retention time of cholesterol was 8.604 min. The amount of cholesterol extracted from egg-yolk was calculated.

2 g of each samples like Ginger, Garlic, Onion, Fenugreek and Pepper were accurately weighed and powdered and was added to the egg-yolk. It was mixed well for about 10 min and the egg-cholesterol was isolated and analyzed. The effect of various cholesterol fighters in its raw form and cooked form on egg-cholesterol were estimated and reported.

Table 1 : Effects of food materials on egg-cholesterol level

Food materials	Egg-cholesterol level *		Percentage reduction *	
	Raw (mg)	Cooked (mg)	Raw(mg)	Cooked (mg)
CURRY LEAVES	51.68±0.05	30.12±0.05	9.34±0.05	47.00±0.05
FENUGREEK	32.19±0.05	47.04±0.05	43.52±0.05	17.47±0.05
GARLIC	19.46±0.05	30.42±0.05	65.85±0.05	46.63±0.05
ONION	24.48±0.05	34.44±0.05	57.05±0.05	39.57±0.05
PEPPER	19.84±0.05	21.84±0.05	65.19±0.05	61.68±0.05

* mean of three values

RESULTS AND DISCUSSION

The linearity of the method was ranging from 100 µg/mL to 400 µg/mL for pure cholesterol. The calibration curve was constructed by plotting response factor or against concentration of pure cholesterol. The slope, intercepts and co-efficient values were found to be A=73839.0835, B=222661.4536, C=0.996810 respectively. The results show that an excellent correlation exists between response factor and concentration of cholesterol within the concentration range.

The cholesterol content estimated in Egg-yolk weighing 18 g (on an average), was found to be 57.00 mg/yolk. All the food materials been selected for the purpose of the study, was found to reduce egg-cholesterol, in-vitro, under laboratory conditions. The food materials like Garlic, onion, fenugreek, were found to reduce the egg-cholesterol level in its RAW form, better compared to be more effective, which reduces cholesterol by 65.85 % and 65.19 % respectively. Onion and fenugreek were found to be effective as Garlic but Onion and Garlic has got some negative characteristics like causing bad odour, when used in its raw form. Similarly fenugreek in its raw form produces mild diarrhoea for the first few days and then which may fade as the person taking it adapts. Curry-leaves are the only food material which significantly reduces the cholesterol level in its COOKED form compared to its RAW form.

From the above result it can be concluded that PEPPER is the most effective Cholesterol fighter, followed by curry-leaves, when compared to the other food materials that have been selected in our study. Hence it's advisable to include PEPPER in our daily diet, along with egg, either in RAW or COOKED form, and curry-leaves in its cooked form so as to reduce cholesterol levels, especially for the persons with lack of regular exercise and is at risk of developing high Blood Cholesterol level thus avoiding the use of various cholesterol lowering drugs which produce various side-effects⁶ that include digestive complaints, dizziness, headaches, mental disorders including brain fog, loss of memory, and signs of dementia or Alzheimer's.

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