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Estimation of Sterols in Edible Fats and Oils

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Abstract: The present investigation was carried out for the estimation of sterol in edible fats and oils. Ten samples of vanaspati ghee which were most commonly available in the market were subjected to sterol estimation. The samples included Dalda, Tullo, Habib, Fauji, Kissan, ACP, Latif, Family, Zaiqa and Handi vanaspati. Dalda, Tullo and Habib vanaspati contained relatively less amount of cholesterol (10, 14 and 15 mg/g respectively) While, Fauji, Kissan, ACP and Latif vanaspati had the intermediate values of cholesterol (24, 25, 29 and 30 mg/g respectively). Family, Zaiqa and Handi vanaspati contained maximum amount of cholesterol (33, 37 and 40 mg/g respectively). Dalda vanaspati had the least (10 mg/g) while, Handi vanaspati had the highest (40 mg/g) amount of cholesterol. Among other samples butter and Haleeb Desi ghee contained the high amount of cholesterol (27, 7 mg/g respectively), while milk fat, beef tallow and fish fat were low in cholesterol content (3.5, 1 and 4 mg/g respectively). Bear fat had the immense quantity of cholesterol (80 mg/g). The sterol content of edible oils i.e. Corn (23 mg/g), Soybean (9 mg/g), Rapeseed (5 mg/g) and Coconut (0.8 mg/g) were reported less as compared to the sterol content of mustard oil (64 mg/g).

Key words: Atherosclerosis, cholesterol, phytosterol, bear fat, edible fats and oils

Introduction

The heart diseases are increasing day by day and the heart attack is a major cause of death world over. In Pakistan the heart disease has become one of the most fatal diseases to cause death and its percentage below 40 years population is 20% (1 in 5 people). There are several reasons for heart diseases but one of the most important reasons is Hypercholesterolemia i.e. the increased concentration of cholesterol in blood. Cholesterol belongs to the sterol group of fats. It is present in egg yolk, dairy products, fatty meals and organs meat (Khan, 1988). Cholesterol has a good side because; it performs a number of vital functions in the body, such as providing an essential component of membranes and serving as a precursor of bile acids, steroid hormones and vitamin D (Harvey and Champe, 1994). The blood stream carries cholesterol in particles called lipoproteins. But too much of circulating cholesterol can injure arteries especially coronary arteries that supply to the heart. This leads to the accumulation of cholesterol-laden "plaque" in vessel linings, a condition called atherosclerosis. When blood flow to the heart is impeded, the heart muscle becomes starved of oxygen, causing chest pain (angina). If a blood clot completely obstructs a coronary artery affected by atherosclerosis a heart attack (myocardial infarction) or death can occur (Lippy, 1998). Heart disease is a number one killer of both men and women in America. More than 90 million American adults have elevated blood cholesterol level. Heart disease deaths have declined steadily over the last 30 years. Indeed between 1990 and 1994 heart disease deaths have decreased by 10.3%. A key factor in this drop is that the public, patients and doctors are better informed about the risk associated with elevated cholesterol, benefits of life style changes and medical measures aimed at lowering cholesterol (Henkel, 1998). The results from all clinical trials show that a one percent reduction in total cholesterol causes a two percent reduction in heart attack (NIH report, 1988). Reduction of blood cholesterol by the administration of the drugs or by the modification of diet decreases the risk of coronary artery disease. Thus, it is useful to identify and limit foods that are rich in cholesterol. The present investigation was carried out to identity and limits those samples of edible fats that are rich in cholesterol. Normally the intake of cholesterol in diet is not very high. It is necessary to limit the intake to less than 300 mgs, in a day (Sharma, 2000). But what is often not realized is that a much bigger source of cholesterol is body itself. About 1g of cholesterol is daily synthesized in body and all the 27 carbon atoms of cholesterol are synthesized from acetyl-COA (Mehta *et al*., 1983). Intake of certain fats such as saturated fats, which are present in animal fat and which solidify in winter, such as coconut oil, palm oil and hydrogenated oils (vanaspati) raise the cholesterol level. On the other hand, taking vegetable oils known as poly-unsaturated fats like safflower oil, mustard oil, sunflower oil lower the cholesterol. This is also done by taking monounsaturated fats like olive oil (Sharma, 2000). As the plant sterols lower down the level of cholesterol in blood thus, the level of phytosterol was also estimated in Mustard oil, coconut oil, Corn oil, Rapeseed (Canola) oil and Soybean.

Materials and Methods

Sample collection and preparation: Different samples of edible fats and oils were purchased from local

market. Samples of fat/oil were taken and weighed them to 1 gm. Chloroform was used as solvent to make the volume 10 ml. Samples were stirred to dissolve completely and diluted to 10 times. 3 ml of diluted sample solutions were taken and their absorbance was determined on Spectrophotometer after adding the reagents. Liberman-Burchard Reagent reacts with the Sterol to produce a characteristic green colour whose absorbance is determined on Spectrophotometer at 640 nm.

Instrumentation: The Spectronic 20-D (Milton Roy Company) was employed in the analysis of samples. Sterol estimation was carried out by Liberman-Burchard method.

Standard cholesterol solution: 10 mg of standard cholesterol dissolved in 10 ml chloroform, shaked well.

Liberman-Burchard reagent: 0.5 ml of sulfuric acid dissolved in 10 ml of acetic anhydride. Covered and kept in ice bucket.

Procedure: Pipette out standard cholesterol solution in 6 test tubes and marked them as S_1, S_2, S_3, S_4, S_5 and S_6. Added standard cholesterol solution as 0.5, 1, 1.5, 2, 2.5 ml in five test tubes whereas, tube 6 was kept blank. Then, 2 ml of the Liberman-Burchard reagent was added to all six tubes and the final volume was made equal in each tube by adding chloroform as shown in Table 1. The tubes were covered with black carbon paper and kept in dark for 15 minutes. Then, set zero of spectrophotometer with blank (S_6) at 640 nm. The absorbance of all standards (six tubes) were determined on Spectrophotometer and standard graph was plotted (Fig. 1). Finally, 3 ml of sample solutions were taken and their absorbance was determined on Spectrophotometer after adding the Liberman-Burchard reagent and chloroform.

Results and discussion

Almost every adult living in an industrialized nation develops some degree of atherosclerosis, commonly known as "hardening of the arteries". Atherosclerosis leads to strokes, heart attacks and other serious health problems. The heart disease is linked to risk factors. The principal risk factors are high cholesterol, smoking and high blood pressure. Other risk factors include: diabetes, obesity, family history of heart disease and stress. The present investigation is an overview of how food choices can effect one's cholesterol level. Every animal cell, both human and nonhuman, contains cholesterol. Cholesterol is important in some cell functions and liver produces all of the cholesterol of the body needs. There is no need to consume cholesterol in diet. Cholesterol and fats move thorough the body in protein packages called lipoproteins. Low density lipoproteins (LDLs) carry cholesterol to the organs through the arteries. The LDLs deposit their load through the inner walls of the arteries and promote atherosclerosis. Since LDLs promote atherosclerosis, they are known as bad cholesterol. The higher the LDL level greater the risk of heart problems."Good cholesterol" found in high density lipoproteins (HDLs) moves back to the liver where it is disposed of. People who exercise, do not smoke and stay their ideal weight tend to have higher level of HDLs. Blood cholesterol levels are effected by many factors. Population groups with an average cholesterol level of 150 mg/dL or less are largely free of atherosclerosis. For cholesterol level above 150 mg, the risk of heart disease increases (Annonymous, 1978). Cholesterol in food increases the level of LDLs (Johnson and Greenland, 1990). Meat, fish and eggs all contain cholesterol, while plant products do not. Choosing lean cuts of meat is not enough; the cholesterol is mainly in the lean portion. Many people are surprised to learn that chicken contain as much cholesterol as beef. Every four ounce serving of beef or chicken contains 100 mg of cholesterol. Basing diet on plant foods i.e. grains, beans, vegetables and fruits is the best way to keep saturated fat intake low and to avoid cholesterol completely. One study showed that people who adopt a vegetarian diet reduce their fat intake by 26% and achieve a significant drop in cholesterol levels in just six weeks (Maseri *et al.,* 1984). Besides the very low level of fat eaten in a typical vegetarian diet, vegetable proteins also helps decrease risk of heart attack. Studies have shown that replacing animal protein with Soy protein reduce blood cholesterol levels even when the total amount of fat and saturated fat remain the same (Carrol *et al.,* 1984).

The present investigation was carried out to estimate the amount of sterol in edible fats and oils. Ten samples of vanaspati ghee which were most commonly available in the market were tested. The samples included Dalda, Tullo, Habib, Fauji, Kissan, ACP, Latif, Family, Zaiqa and Handi vanaspati. The cholesterol content of these fats ranged from 10-40 mg/g (Table 2). Dalda, Tullo and Habib vanaspati contained relatively less amount of cholesterol (10, 14 and 15 mg/g respectively) While, Fauji, Kissan, ACP and Latif vanaspati had the intermediate value of cholesterol (24, 25, 29 and 30 mg/g respectively). Family, Zaiqa and Handi vanaspati contained maximum amount of cholesterol (33, 37 and 40 mg/g respectively). Dalda vanaspati had the least (10 mg/g) while, Handi vanaspati had the highest (40 mg/g) amount of cholesterol (Table 2). Other samples of edible fats which were subjected to sterol estimation were butter (Desi), Haleeb Desi ghee, beef tallow, Milk fat and fish fat (Table 3). Among these samples butter and Haleeb Desi ghee contained the high amount of cholesterol (27, 7 mg/g respectively), while milk fat, beef

Table 1: Liberman-Burchard method for cholesterol estimation

Reagents(ml)	S_1	S_2	S_3	S_4	S_5	S_6
Standard cholesterol solution	0.5	1	1.5	2	2.5	-
Liberman-Burchard reagent	2	2	2	2	2	2
Chloroform	4.5	4	3.5	3	2.5	5

Table 2: Concentration of Cholesterol in different samples of vanaspati ghee

Name of fat	Absorbance	Cholesterol (mg/g)
Dalda vanaspati	0.1	10
Tullo vanaspati	0.14	14
Habib vanaspati	0.15	15
Fauji vanaspati	0.24	24
Kissan vanaspati	0.25	25
ACP vanaspati	0.29	29
Latif vanaspati	0.30	30
Family vanaspati	0.33	33
Zaiqa vanaspati	0.37	37
Handi vanaspati	0.4	40

Table 3: Cholesterol content of fats

Name of fat	Absorbance	Cholesterol (mg/g)
Bear fat	0.8	80
Butter (Desi)	0.27	27
Habib Desi Ghee	0.07	7
Fish fat	0.04	4
Milk fat	0.035	3.5
Beef tallow	0.01	1

Table 4: Phytosterol content of plant oils

Name of oil	Absorbance	Sterol (mg/g)
Mustard	0.64	64
Corn	0.23	23
Soybean	0.09	9
Rapeseed (Canola)	0.05	5
Coconut	0.008	0.8

tallow and fish fat were low in cholesterol content (3.5, 1 and 4 mg/g respectively). Butter had the maximum (27 mg/g) while, the beef tallow had the minimum (1 mg/g) cholesterol (Table 3). Beef fat is poor source of cholesterol but it contains Myristic acid which leads to cholesterol in blood. Bear fat was also analyzed for the sterol content which contained the greatest amount of cholesterol (80 mg/g) as shown in Table 3.

As the plants oil are the concentrated source of phytosterols which compete with the absorption of cholesterol in the body and thus has the ability to lower down the body cholesterol; the level of phytosterol was also estimated in the plant oils by Liberman-Burchard method. This method is not only specific for cholesterol but for any sterol having the C_3 OH group. Corn oil, Soybean oil, coconut oil, Rapeseed oil and mustard oil

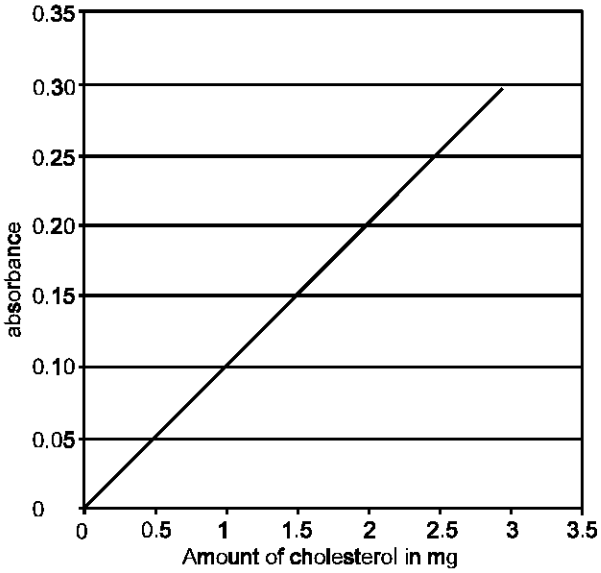

Fig. 1: Standard graph

were analyzed for their sterol content. The sterol content of Corn oil (23 mg/g), Soybean oil (9 mg/g), Rapeseed oil (5 mg/g) and Coconut oil (0.8 mg/g) were reported less as compared to the sterol content of mustard oil (64 mg/g). Thus, the mustard oil is rich source of sterol whereas, coconut oil is the poor source. The principal phytosterol of Corn oil, Soybean and peanut oil is Beta-sitosterol. Between 500 mg and 10 gram of beta-sitosterol per day has been used in clinical research to reduce elevated blood cholesterol (Berges *et al.*, 1995). Some recent studies have suggested that not all the monounsaturated fats are the same in their ability to lower cholesterol. Scientists in Germany fed hamsters a moderate amount of canola, olive, sunflower, corn oil and monounsaturated palm oil. All the fats did better than the palm oil in lowering cholesterol and were not significantly different from each other. Corn oil had significantly lowered LDL and VLDL concentration. Unfortunately, HDL cholesterol (good cholesterol) also dropped (Rafael, 1999).

Following recommendations may be useful in reducing the cholesterol level.

1 Cholesterol level can be decreased by cholesterol-lowering drugs, but these are expensive and can be avoided in many cases. Life long dependence on drugs is not desirable and there may be side effects for individuals.

2 Have your cholesterol level tested before drugs are necessary. Monitor regularly.

3 Reduce intake of food containing saturated fat and cholesterol. Eat low fat milk (exceptions for infants). Trim meat fat.

4 Use plant foods i.e. grains, beans, vegetables, fruits and berries. Vegetables oils should be used for cooking instead of vanaspati ghee.

5 Mental stress causes an increased release of adrenaline which may elevate cholesterol levels. Relaxation techniques such as stretching, deep breathing or meditation help in lowering blood cholesterol.

6 Stop smoking and exercise more. Aerobic exercise raises level of HDL cholesterol and may also reduce level of LDL.

7 Use of dietary fiber may reduce cholesterol.

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