Team Members: 12/10/10

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Title:

Nutritional Testing and Compliance Lab

Background:

The U.S. Department of Agriculture is required to promote proper nutrition and prevent inaccurate or fraudulent advertising and claims about products and foods. Therefore, the department has decided to investigate a number of questions about cholesterol in foods. Our group is being asked to design experiments to test current theories and make recommendations for a detailed report to be released shortly to guide consumers (8).

For example, which home or commercially prepared foods are major or minor sources of cholesterol? Does cholesterol content vary in different parts of meats or vegetables? Should we eat or discard the rind, skin, yolk or what? Which fast-food franchises have high (low) cholesterol content in their French fries or other foods (8)?

Our team was asked to select a specific question to study and to clear it with our supervisor and other teams, so that each team is doing a unique project to better cover the problem. We are then asked to develop a proposal with a flow chart of the experiment and procedures we will use. It must include relevant information from at least two sources (8).

Our objective was to determine the cholesterol content in foods or supplements; to analyze excretion of cholesterol and correlate this with diet or supplementation. To

recommended the best methods to prepare foods or to take supplements to maintain good health (8).

Cholesterol is a class of lipids that is characterized by a four-ring structure called the cyclopentanoperhydrophenanthrene, or a sterol. Cholesterol is also only present in animal tissues. Meats, egg yolk, and dairy products are the common dietary sources of cholesterol, and contain fairly large amounts. Phytosterols (also called plant sterols) are a group of steroid alcohols, phytochemicals naturally occurring in plants. Phytosterols occur naturally in small quantities in vegetable oils, especially corn oil and soybean oil. One such phytosterol complex, isolated from vegetable oil, is cholestatin, composed of campesterol, stigmasterol, and brassicasterol, and is marketed as a dietary supplement. They are white powders with mild, characteristic odor, insoluble in water and soluble in alcohols. They have applications in medicine and cosmetics and as a food additive taken to lower cholesterol (7).

Equipment needed:

For this experiment there was a long list of things that we needed, such as (8):

- Spectrophotometer
- 1, 5 and 10 mL pipettes with pipettors
- 100 µL pipettors and tips
- Parafilm squares
- Glass Waring blender and blade cup assembly
- Mortar and pestle
- Glass funnel
- Whitman 1PS filter paper

- 50°C Water bath w/ test tube rack
- 35°C Water bath w/ test tube rack
- Graduated, conical centrifuge tubes
- Clinical centrifuges
- Bent tip 9" Pasteur pipettes

The reagents needed were (8):

- "Liebermann-Buchard reagent
    - Keeps for 1-2 weeks, but may deteriorate if kept for a longer period. To prepare 100 mL of reagent, add slowly, under continuous stirring, 10 mL of concentrated sulfuric acid to 60 mL of acetic anhydride. Since the reaction is strongly exothermic," cool the beaker in an ice bath. "Add 30 ml of acetic acid and 0.6 g of anhydrous sodium sulfate."[10] Make 200 mL/lab sections.
- Chloroform and methanol - 500 mL each
- 10 mM Cholesterol standard - 0.386 g cholesterol in 100 mL of 1:1 CHCl3:MeOH

Safety Requirements:

With the reagents we were using three of them were and are very harmful. Chloroform can cause cancer in laboratory animals, and is also listed as a probable human carcinogen. Inhalation and ingestion of this is not recommended and may become fatal. This is an irritant and exposure to alcohol may increase toxic effects. Prolonged or repeated skin contact can cause

dermatitis. Methanol may be fatal or cause blindness if swallowed. Vapor harmful, and is flammable liquid vapor. May cause central nervous system depression, and cannot be made nonpoisonous. Liebermann-Buchard Reagent can cause irritation to skin, corrosive, carcinogenic affect, and can cause skin to become itchy, redden or even blister(1,2,3,4,5,9,10).

However like all of our other labs we made sure that we were using the safety sheets for these three reagents, to wear our goggles and gloves, to wear no loose clothing or open toed shoes, and to dispose our chemicals in the marked waste dispensers and to not put anything down the sinks.

Approach:

To begin this lab, each we we cleaned the test tubes and any other pieces of equipment that we would be using so that we knew it was cleaned. We then obtained our samples. Our first week we tested an egg and a walnut. The second week we tested a blueberry muffin, peanut, vanilla yogurt, and mashed potatoes.

Liquid Substance (8)

To test the liquid substances, such as the egg yolk, mashed potatoes, and yogurt, we first weight out approximately 1g to the nearest 0.0001g. We then added 10mL of chloroform:methanol (2:1) and vortexed the solution. Then using Whitman 1PS filter paper, we filtered the solution into a test tube. Afterwards we added .2 times the amout of filtrate or 0.02% $CaCl_2$ solution and vortexed and then allowed it to separate out into two phases. We then removed the upper phase, and doubled the total volume of the lower phase by the addition of chloroform:methanol:water (2:4:3) and vortexed and allowed to sit again untill it seperated into two levels. We then removed the upper layer again and recorded the volume of the lower phase,

and transferred .05ml of the total lipid extract to another test tube for the quantification of cholesterol test.

Solid Substance (8)

For this test we used the walnut, peanut, and blueberry muffin. To begin we weighed out approximitaly 1g of the substance and grounded it with a mortar with 5ml of chloroform:methanol(2:1). This was then transferred into a test tube, and the mortar was washed with 5ml of chloroform:methanot (2:1) and added to this test tube. This was then placed in a water bath at 50ºC for 10 min. After the ten minutes, using Whitman 1PS filter paper, we then filtered the sample into a graduated test tube. We measured the volume of filtrate, and then added 0.2 times that volume of a 0.02% $CaCl_2$ solution. We then vortexed the solution and allowed it to separate into two separate phases. We removed the upper phase with a Pasteur pipette when this happed. To double to total volume of the lower phase, we added chloroform:methanol:water (2:4:3). We once again vortexed an allowed it to sit and have two phases form, and removed the upper lever. We then transferred 0.5mL of the lipid extract to another test tube for the quantification of cholesterol test.

Quantification of Cholesterol (8)

To perform our final test on our samples we prepared test tubes with .5ml of each, along with chloroform:methanol(2:1), 2.5mM cholesterol, 5.0mM cholesterol, and 10mM cholesterol. Then slowly we added 5ml of Liebermann-Buchard reagent to each test tube, covered and vortexed. We then placed each tube in a water bate at 35^{0c} for 10 minutes. During this time we zeroed the spectrophotometer by setting it to 550mm and then zeroing it with a dilution of 1ml

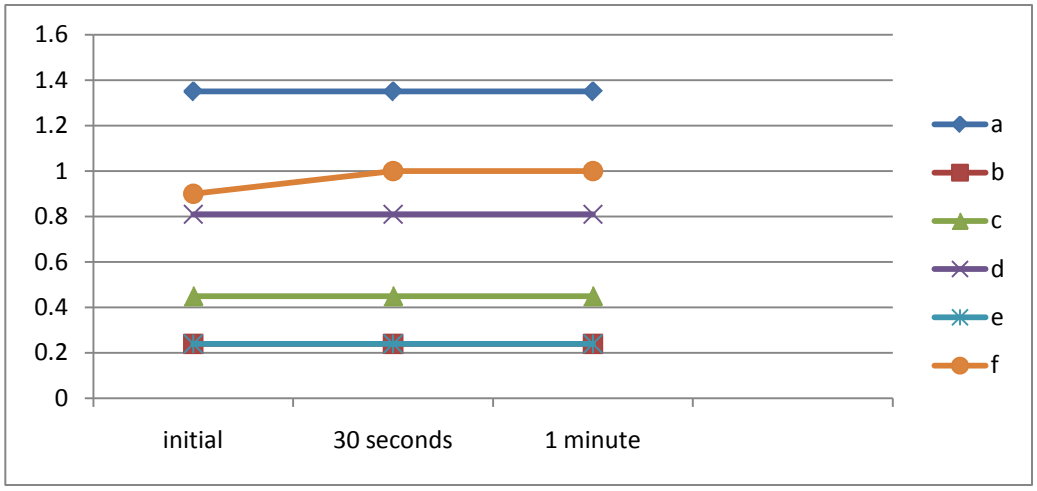
chloroform and 1ml of methanol. After the 10 minutes, we placed each of the test tubes into the spectrophotometer and took the readings at the initial time, 30 seconds, and 1 minute.

Results:

For our first week of lab, our results were as followed:

	A: cholorfomrm: Methanol	B: 2.5mM cholesterol	C: 5.0mM cholesterol	D: 10mM cholesterol	E: egg	F: Walnut
Initial	1.35	.24	.45	.81	.24	.9
30 sec	1.35	.24	.45	.81	.24	1.0
1 min	1.35	.24	.45	.81	.24	1.0

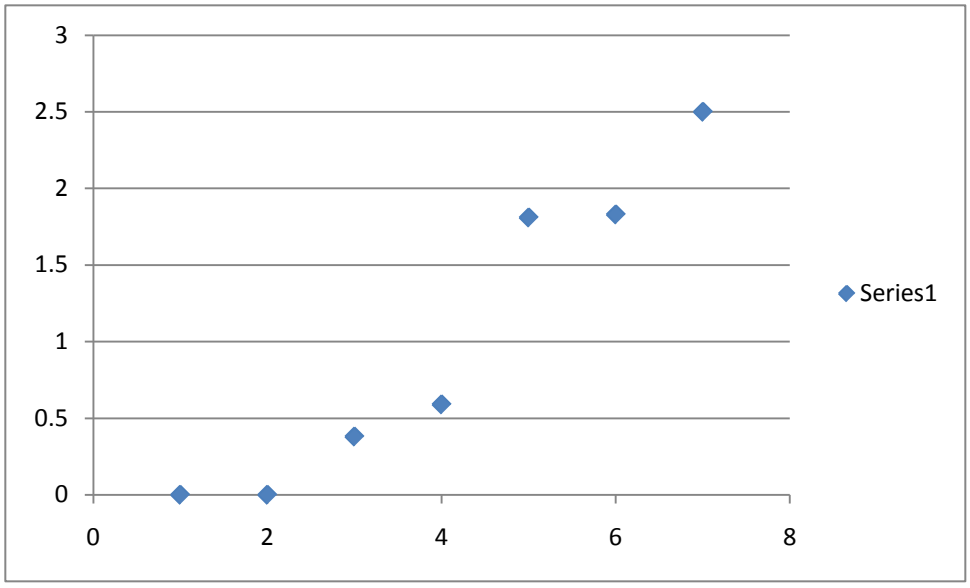
And our graph is as follows:



Because of some complications with the lab, we have lost our results for the second week. Since this happened we had to find an equation to find the mM/l numbers of the food samples and the cholesterol mg numbers. The equation that I found to be most helpful was to divide the mg readings found in a nutrition book for each of the food samples by 38.67 to give us mmole. From this we were able to determine the values for all of the food samples:

Food Substance	Cholesterol in mg	Millimole/l
Blueberry muffin	70mg	1.81mM/l
Hamburger meat	71mg	1.83mM/l
Peanut	0mg	0mM/l
Walnut	0mg	0mM/l
Egg	96.67mg	2.5mM/l
Mashed potatoes	23mg	.59mM/l
Vanilla yogurt	15mg	.38mM/l

The final graph looks like this:



From our results we can conclude that the food we tested that contained the highest level of cholesterol would be the egg, followed by hamburger meat, a blueberry muffin, mashed potatoes, yogurt, and then the walnut and peanut. Because of the way the muffin, meat, and yogurt is made and prepared is why there is cholesterol found in it. Because of the butter, and milk, and eggs that are added to the recipes the food then has cholesterol added. If the mashed potatoes had not been made with milk or butter there is no cholesterol found in the food product because it is a plant, like the peanut and walnut.

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