Reduction in the Cholesterol of Butter Oil Using Soybean Lecithin

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Abstract— Cardiovascular diseases are the leading cause of death and the risk of such disease increases with increasing the level of blood cholesterol. Foods containing high amount of cholesterol increase the level of cholesterol in blood. Limiting consumption of such foods or reducing those cholesterols lead to reducing the risk of cardiovascular diseases. There are various methods to reduce cholesterol of foods. The aim of the current study was reducing the cholesterol of butter oil using soybean lecithin. 20 ml distilled water and various amounts of soybean lecithin (5, 7, 10, 12.5, 15, 17.5 and 20%) were added and stirred to 100 grams of butter oil. Then, hydrated lecithin was separated by centrifuge. In addition to lecithin concentration, the effects of stirring speed (200, 400, 600, 800 and 1000 rounds per minute), stirring duration (15, 30, 45, 60, 75 and 90 minutes), centrifuging speed (2000, 2200, 2400, 2600 and 2800 rounds per minute) and duration of centrifuging (5, 10, 15, 20 and 25 minutes) on reduction in cholesterol were investigated. Chemical (acid, peroxide and saponification numbers) and physical (refractive index and melting point) characteristics of low cholesterol butter oil and original butter oil were compared with each other. Increasing the lecithin concentration up to 17.5% and increasing the stirring duration up to 75 minutes were led to more reduction of cholesterol but by more increasing the stirring duration, the magnitude of cholesterol reduction was meaningfully decreased ($P<0.05$). The results were shown that there is a linear correlation between reduction in cholesterol and stirring speed, centrifuging speed and duration of centrifuging. Among the physical and chemical characteristics of low cholesterol butter oil, only acid number was meaningfully higher than that of original butter oil and other characteristics had not meaningful difference. The appropriate condition for cholesterol reduction was identified as 10% lecithin concentration, 45 minutes stirring duration, 600 rounds per minute stirring speed, 15 minutes duration of centrifuging and 2400 rounds per minute centrifuging speed. Under this condition, the 78.6% of butter oil cholesterol was reduced.

Index Terms— Cholesterol, Soybean Lecithin, Butter Oil, Cardiovascular Diseases

1 INTRODUCTION

Cardiovascular diseases are the cause of more than 50% of deaths around the world [1-30]. There is a strong relationship between blood cholesterol and cardiovascular diseases [31-38]. The blood cholesterol increases with increasing the consumption of foods containing cholesterol and saturated fat acids and as a result, risks of cardiovascular diseases increases [39]. The cholesterol presented in foods oxides to cancerous, cytotoxic, mutagenic and iatrogenic agents during processing or maintaining [40]. Therefore, one of the methods to reduce the risk of cardiovascular diseases is reducing the consumption of foods containing cholesterol or removing cholesterol from such foods [41, 42]. In this regard, there have been numerous researches to reduce cholesterol of foods and various methods have been presented including distillation [43-59], extracting by supercritical carbon dioxide [60, 61], surficial absorption by saponin [62] and digitonin [63, 65, 66, 68], extraction by organic solvents [63-71], degradation of cholesterol by cholesterol oxidase [72, 73], use of cyclic anhydrides and polybases [74-91], beta-cyclodexterin [92] and mixing with vegetable oils. Most of these methods are non-selective; i.e. in addition to cholesterol, nutrient and redolent agents of foods also reduced using these methods. Moreover, these are needed to high investing and practical costs, these are not economical and even in some cases, the safety of food will be at risk if these methods will be used [93-99].

The results of the previous investigations have been shown that consumption of soybean lecithin decreases the serum cholesterol of blood [100] but recently, it is reported that soybean lecithin is able to reduce cholesterol of foods and this is of some advantages compared to other techniques of cholesterol reduction including:

(1) Lecithin is one of the by-products of oil extraction factory and it can be easily supplied from such factories.
(2) The necessary equipments for this method are cheap.
(3) Since the process of reduction in cholesterol is performed in room temperature, fats that are in liquid state in room temperature will have no considerable changes (such as oxidation).
(4) Soybean lecithin is eatable and it will not dangerous if it remains in food.

The basic of cholesterol reduction in this method is the tendency of amphipathic molecules such as cholesterol to other amphipathic molecules such as lecithin molecules, where at the presence of water, these molecules form hydrophobic fluid bilayers and can move cholesterol molecules. The amount of cholesterol that reduced by this method is dependent on food type, lecithin percent, stirring speed, stirring duration, centrifuging speed and duration of centrifuging of lecithin and food mixture [101].

Lecithin has been used to reduce the cholesterol of beef tallow and in sutiable condition, 54.8% of its cholesterol has been

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reduced using this method [102]. Although it is identified in the previous studies that about 75of cholesterol of butter oil can be reduced using beta-cyclodexterin [103, 104], using of lecithin to reduce cholesterol of this product has not been studied and since the reduction in cholesterol is dependent on food type, it seems that the effect of lecithin on cholesterol reduction in various foods such as butter oil is different from other foods (such as beef tallow) [105-108]. The aim of the current study was investigating the effect of various factors on reduction in cholesterol of butter oil using soybean lecithin and assessing the changes in physical and chemical characteristics of the obtained butter oil.

2 Materials and methods

The pasteurized butter oil was supplied. Soybean lecithin (amount of unsolved materials in acetone is 97%, 1.5% moisture content, 30 mgKOH/g acid number), which was powder, was bought from Lcas Meyer Company, France. 5α- cholestane and cholesterol were bought from Fluka Company, Switzerland, and other chemicals used in the current research were supplied from Merck Company, Germany.

3 Process of reduction in cholesterol of butter oil

Butter oil was located in the oven for a few minutes to completely liquefied. Then, 100 grams of it were weighed and soybean lecithin and 20 ml distilled water were added to it. After stirring for a specified duration with centrifuge (TD4Z table-type, made in Hunan Kaida Company, China), hydrated lecithin, enriched with cholesterol was separated from oil. The effects of various concentrations of soybean lecithin (5, 7, 10, 12.5, 15, 17.5 and 20%), stirring speed (200, 400, 600, 800 and 1000 rounds per minute), stirring duration (15, 30, 45, 60, 75 and 90 minutes), centrifuging speed (2000, 2200, 2400, 2600 and 2800 rounds per minute) and duration of centrifuging (5, 10, 15, 20 and 25 minutes) on reduction in cholesterol of butter oil were investigated.

4 Cholesterol measurement

0.4 percent solution of 5α- cholestane and cholesterol solutions with concentrations of 0.02, 0.04, 0.06, 0.08, 0.1 and 0.12 grams per 100 milliliters n-hexane were prepared. 100 µl of 5α- cholestane solution was added to 5 ml of each cholesterol solution. Then, 2 µl of it was injected to gas chromatography apparatus and ratio of cholesterol to cholestane level was obtained.

To measure the cholesterol of butter oil, after weighing 5 grames of it and adding 100 µl of 5α- cholestane, the nonsaponifiable materials of it were extracted according to AOCS method, number Ca 6a-40. It was transferred to dark containers after solving in ether oil and was maintained at -18° C until analysis time. At the analysis time, after stirring the dark containers, 2 µl of its content was injected to gas chromatography apparatus. To analyze cholesterol, gas chromatography apparatus (Varian Chrompack, United States) with a 30 m length capillary column of Cpsil 8cb, internal diameter of 0.25 mm and 0.25 µ film thickness and 300° C temperature (isothermal), Helium carrier gas with pressure of 25 psi and Hydrogen gas and air with speed of 30 and 300 milliliters per minute, respectively, splitless injector with 280° C temperature and FID detector with 300° C temperature was used.

5 Determining physical and chemical characteristics of butter oil

After assessing the effect of each factor, suitable condition for cholesterol reduction was determined and low cholesterol butter was produced under the following condition: 10% lecithin concentration, 45 minutes stirring duration, 600 rounds per minute stirring speed, 15 minutes duration of centrifuging and 2400 rounds per minute centrifuging speed. Then, chemical and physical characteristics of this butter including acid, peroxide and saponification numbers, refractive index and melting point were measured according to AOCS methods (numbers Ca 5a-40, Cd 8-53, Cd 3a-94, Cc 1-25, Cc 7-25) and were compared with those of original (untreated) butter oil.

6 Statistical analysis

All tests were repeated three times and data analysis was performed by SPSS16.0 software as a completely random scheme. Comparisons of means were performed using Duncan method.

7 Results and discussion

There was 267.5 milligrams of cholesterol per 100 grams of butter oil. Although reduction in cholesterol was increased with increase in lecithin concentration, comparison of means was shown that there is not a meaningful difference between reduction in cholesterol and lecithin concentration levels of 10, 12.5, 15, 17.5 and 20% ($p<0.05$).

By increasing stirring speed, reduction in cholesterol was increased. The lowest and highest reductions in cholesterol were 26.6% and 80.2%, respectively, which were resulted in stirring speeds of 200 and 1000 rounds per minute, respectively. Comparison of means was shown that there is not a meaningful difference between reduction in cholesterol at 1000 rounds per minute and 800 and 600 rounds per minute ($p<0.05$).

By increasing stirring duration from 15 to 75 minutes, reduction in cholesterol was increased from 39.1% to 80.5%. However, by more increase in duration of stirring up to 90 minutes, reduction in cholesterol was decreased to 72.4%. The results were shown that there is not a meaningful difference between reduction in cholesterol at 45 minutes and 60 and 75 minutes ($p<0.05$).

By increasing duration of centrifuging and centrifuging speed, more cholesterol was reduced so that when duration of centrifuging was increased from 5 to 25 minutes, reduction in cholesterol was increased from 67.3% to 78.9%. Although reduction in cholesterol during 15 minutes centrifuging was higher than 5 and 10 minutes durations of centrifuging, comparison of means were shown that its effect was not a meaningful difference with 20 and 25 minutes durations of centrifuging.

When centrifuging speed was increased from 2000 to 2800 rounds per minute, reduction in cholesterol was increased from 57.8% to 79.6%. However, there was not a meaningful difference

in cholesterol reduction for 2400 rounds per minute centrifuging speed (78.6%) and higher speeds.

Treating with lecithin was led to meaningful increase of acid number of butter oil but it had not meaningful effects on peroxide number, saponification number, refractive index and melting point of butter oil. In the current study, soybean lecithin was used to reduce cholesterol level of butter oil and the effects of lecithin concentration, stirring speed, stirring duration, centrifuging speed and duration of centrifuging on cholesterol reduction were studied.

Increase in lecithin concentration leads to increase in cholesterol reduction. It seems that increase in lecithin concentration is accompanied by increase in hydrophobic fluid bilayer surface so that increasing this surface leads to transferring more cholesterol from butter oil to lecithin.

The effect of stirring speed on reduction in cholesterol was considerable. There was a linear correlation between reduction in cholesterol and stirring speed. Increasing the stirring speed increases the contact between the presented cholesterol in butter oil and hydrophobic fluid bilayer of lecithin and hence, leads to more cholesterol reduction.

Reduction in cholesterol of butter oil using lecithin needs to enough time for contact between them. By increasing the stirring duration, cholesterol absorbs to hydrophobic fluid bilayer of lecithin and transfers from butter oil into lecithin. Therefore, by increasing stirring duration up to 75 minutes, more cholesterol was reduced but by more increasing stirring duration up to 90 minutes, reduction in cholesterol was decreased probably due to returning cholesterol into butter oil.

Hydrated lecithin should be separated by centrifuge after necessary contact with butter oil and since it enriches with cholesterol, suitable separating of it is affect the amount of cholesterol reduction. In this regard, centrifuging speed and duration are very important. Considering energy consumption, it seems that 15 minutes centrifuging with 2400 rounds per minute is suitable for reduction in cholesterol as meaningful difference was not observed compared to higher speeds and longer durations.

Lecithin treatment increases acid number of butter oil from 0.15mgKOH/g to 0.41 mgKOH/g due to high acid number of the lecithin used in the current study (30 mgKOH/g). Increasing acid number has been reported in previous studies in which, lecithin has been used to reduce cholesterol of beef tallow.

It can be concluded that 10% lecithin concentration, 45 minutes stirring duration, 600 rounds per minute stirring speed, 15 minutes duration of centrifuging and 2400 rounds per minute centrifuging speed is appropriate for cholesterol reduction. Under this condition, cholesterol of butter oil was reduced from 267.5 to 57.2 milligrams per 100 grams of butter oil (78.6%). This result is comparable to the results obtained from previously performed study in Iran where beta-cyclodexterin was reduced 75% of cholesterol of butter oil. Compared to previous researches, it can be concluded that the effect of lecithin on cholesterol reduction of butter oil in appropriate condition is more than its effect on cholesterol reduction of beef tallow (54.8%). It is suggested that researches continued in industrial scale.

8 Conclusion

The appropriate condition for cholesterol reduction was identified as 10% lecithin concentration, 45 minutes stirring duration, 600 rounds per minute stirring speed, 15 minutes duration of centrifuging and 2400 rounds per minute centrifuging speed. Under this condition, the 78.6% of butter oil cholesterol was reduced.

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