J Am Oil Chem Soc (2010) 87:109–114
DOI 10.1007/s11746-009-1477-1

ORIGINAL PAPER

Effects of Bleaching and Deodorization Processes of Beef Tallow on Cholesterol Removal by Lecithin Treatment

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Received: 18 April 2009 / Revised: 13 September 2009 / Accepted: 14 September 2009 / Published online: 15 November 2009

Abstract The effects of treatment with commercial lecithin followed by bleaching and deodorization processes on cholesterol reduction (removal) of Iranian beef tallow were studied. Steam-rendering extracted fat was subjected to lecithin treatment and the effects of four variables, i.e., ratio of lecithin to tallow (1:5, 1:10, 1:20 and 1:30 w/w), stirring time (0.5, 1.5, 3, 6 and 12 h), stirring rate (200, 500, 1,000 and 1,250 rpm) and ratio of lecithin to water (1:2, 1:5 and 1:10 w/v) were investigated. The results showed that cholesterol removal is increased with increasing the lecithin to tallow ratio and the stirring rate. Increasing stirring time up to 1.5 h increased cholesterol removal; however, longer times did not have any significant effect on it. The ratio of lecithin to water did not have any significant effect on cholesterol removal, either. It was concluded that up to 43% of the tallow cholesterol content could be removed by lecithin. Treatment with lecithin increased tallow acidity and color but did not have any effect on the peroxide value (PV). It is proposed that since tallow, as a slaughterhouse by-product, is not expensive, by this method we can improve its eating quality for use in dietary or industrial applications.

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Keywords Beef tallow · Bleaching · Cholesterol reduction · Deodorization · Lecithin

Introduction

It has been found from laboratory experiments on animals and humans that a high intake of cholesterol can increase the plasma cholesterol level [1]. Among serum lipids, cholesterol is the most important contributor to cardiovascular and coronary heart diseases (CHDs), a leading cause of death in most of the world.

As the link between high serum cholesterol levels and heart disease has become increasingly apparent, cholesterol-free and cholesterol-reduced food products have become more attractive to consumers, and food products that have no or reduced cholesterol are gaining popularity as well as an increasing share of the market.

Consequently, removal or reduction of cholesterol in high cholesterol foods has the potential to substantially increase marketability and value. This is particularly true for high cholesterol foods such as marine fish oils (500–800 mg cholesterol/100 g), butter (250–300 mg cholesterol/100 g), beef tallow (about 110 mg cholesterol/100 g), lard (about 100 mg cholesterol/100 g) and egg yolks (about 5.2 g cholesterol/100 g (dry weight)).

Moreover, cholesterol is easily oxidized by exposure to air, free radicals, light or a composition of these, either in solution or food [2]. Oxidation products are known to be carcinogenic, mutagenic and cytotoxic [2–5].

Dietary cholesterol along with palmitic acid has a synergistic effect on raising plasma cholesterol [6]. Therefore, a low consumption of dietary cholesterol is recommended as an effective way to reduce plasma cholesterol [7]. Many ways to reduce or remove cholesterol in foodstuffs are cited in the

literature [8]. The removal or reduction of cholesterol is not a trivial matter. Several different techniques to accomplish this task have been developed, each with varying levels of success. These include: short-path molecular distillation, vacuum molecular distillation [9], supercritical carbon dioxide extraction [10–13], extraction by saponin [14], digitonin [15, 16], β-cyclodextrin [7, 17, 18] acid anhydride [19] and cholesterol oxidase enzyme [20]. Other methods include the use of calcium or magnesium bromide salts to precipitate cholesterol, as well as extraction using modified vegetable oils.

Most of these methods are destructive, i.e. they extract other substances in addition to cholesterol, and costly. It has been found that phospholipids (mostly lecithin) can extract (remove) food cholesterol by formation of a hydrophobic fluid bilayer [9, 21].

In this method, the cholesterol-bearing fat/oil product (beef tallow) is brought into contact with a preparation of phospholipid (lecithin) aggregate to form an aqueous separation mixture. The aqueous separation mixture is mixed for a time sufficient to selectively reduce the cholesterol content of the fat/oil product through partitioning of the sterol into the phospholipid aggregate portion of the aqueous separation mixture. Following this, the (Chol) sterol-reduced fat/oil is removed from the aqueous separation mixture [22].

Alternatively, the correspondingly sterol-enriched phospholipids may also be isolated from the aqueous separation mixture and used for a variety of purposes such as those described below.

The preparation of the phospholipid aggregate may comprise a combination of aggregated phospholipids and water. The aggregated phospholipids may comprise one or more phospholipid species, or may comprise just one phospholipid such as lecithin. The steps of the method can be performed at ambient temperature if desired. The procedures can be repeated through two or more cycles to attain a desired level of sterol reduction. Alternatively, the method can be performed under conditions of counter-current extraction.

As described herein, the method provides a number of advantages. For example, since the steps of the method in many cases can be performed at ambient temperatures, costs involved in heating are minimized as is the possibility of thermal degradation of the product. In this study we used commercial Soybean lecithin as the cholesterol remover.

The aim of this study was to investigate a commercial lecithin treatment to reduce beef tallow cholesterol.

Experimental Procedures

Materials

Beef tallow was obtained from a large local slaughterhouse (Karaj, Iran) from the flanks of 30 freshly slaughtered hybrid cows. Preparation was carried out by cleaning, washing, drying and grinding of the tallow samples. Finally, steam rendering was done in a jacketed kettle. Rendered fat was filtered to remove any suspended burnt fat particles and stored at −10 °C till use. Acidity (Cd 3a-63), peroxide value (Cd 8-53) and color (Cc 13e-92) of the samples were determined according to AOCS official methods [23, 24].

Bleaching

The rendered tallow fat was heated up to 95 °C, and then 1% bleaching earth (Iranian Tonsil®, Ion Shimi Co., Tehran, Iran) was added. Finally the mixture was agitated for 30 min under a rotary evaporator at 85 °C [25]. Fat and bleaching earth were filtered through Whatman filter paper No. 1 in a vacuum oven at 60 °C [26].

Deodorization

The bleached tallow fat was deodorized using a pilot deodorizer plant (Oilseeds Planting Development Inc., Tehran, Iran) at 180 °C and 0.8 bar vacuum under a nitrogen stream.

Commercial Lecithin Purification

Commercial soybean lecithin was obtained from a soybean oil refinery. To purify this, 10 g of commercial lecithin (Lucas Meyer, France) was warmed up to 50 °C and 40 ml acetone was added and stirred for 5 min. The insoluble portion was extracted by acetone four times [24]. The purified lecithin was dried at 50 °C under 220 mmHg vacuum and ground.

Tallow Treatment with Lecithin

A lecithin paste was prepared by mixing the purified dry powder commercial soybean lecithin with water at varying ratios (1:2, 1:5 and 1:10 w/v) with heating to about 60 °C prior to homogenization with a mechanical stirrer for 15 min at 500 rpm. As desired, various electrolytes such as acids, bases and/or salts may be added to the water to alter the polar characteristics of the cholesterol. This can alter the distribution coefficient of the amphipathic cholesterol between the phospholipid and fat phases. In addition, various emulsifiers such as monoglycerides may be incorporated in the aqueous separation mixture to improve the sterol extraction efficiencies.

Then, rendered fat was added to the lecithin paste (at ratios 1:5, 1:10, 1:20 and 1:30 w/w) to form an aqueous separation mixture. The separation mixture was thoroughly mixed using a mechanical stirrer. After mixing for a given period of

time (0.5, 1.5, 3, 6 and 12 h), the cholesterol-reduced fat was separated from the cholesterol-enriched lecithin and the remainder of the aqueous separation mixture by filtration through a Whatman filter paper (No. 1) [27].

Beef tallow should be kept at temperatures that maintain it in a liquid state during the procedures. The remaining operations were performed at ambient temperatures as described above.

Determination of Cholesterol

A solution containing 0.4% 5α-cholestane as the internal standard (Fluka, Swiss) and cholesterol solutions with concentrations of 0.2, 0.4, 0.6, 0.8, 1 and 1.2 g in 100 ml of *n*-hexane (Merck, Germany) were prepared. To 5 ml of each cholesterol solution, 100 µl of 5α-cholestane solution was added. A 2-µl aliquot of each solution was injected into a GC column (Varian Chrompack, USA) and the cholesterol/cholestane area ratio was obtained. The calibration curve was drawn by plotting cholesterol content versus cholesterol/cholestane area ratio [28].

The calibration curve equation was obtained as:

$$y = 1.5432x + 0.5296 \quad r^2 = 0.9965 \tag{1}$$

where, y is the cholesterol content (mg) and x, is the cholesterol/cholestane area ratio.

The cholesterol was determined as follows. Firstly, a given amount of fat (5 ± 0.01 g) was weighed and 100 µl 5α-cholestane was added to it, then unsaponifiable matters were extracted by petroleum ether according to AOCS method Ca 6b-5 [24]. Finally desolventization was carried out in rotary evaporator. Flask contents were transferred to a dark bottle by washing with 1.5 ml petroleum ether and after double washing with the same amount of solvent, effluents were added to the bottle. The bottles were kept at −16 °C till analysis. During analysis after mixing in the dark bottles completely, 2 µl of their contents were injected without derivatization into a GC column [29].

The cholesterol/cholestane area ratio was calculated and entered in Eq. 1; thereby the amount of cholesterol could be determined.

The fat cholesterol content (as mg/100 g fat) was calculated using the following formula:

$$z = y/w \times 100 \tag{2}$$

where z, is the cholesterol content (mg/100 g fat), y, the sample cholesterol amount (mg), w, the sample weight (g)

GC Analysis

For analyzing cholesterol, a capillary GC instrument (Varian Chrompack, USA) with an FID detector using a Cpsil8cb capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) was used. The splitless injector and detector were both set at 300 °C. The column oven temperature was 300 °C (isothermal). Helium carrier gas at 25 psi and hydrogen and air at 30 and 300 ml/min, respectively were used.

Statistical Analysis

All the experiments and treatments were triplicated. Data were analyzed using an SAS statistical software package (SAS for Windows, 15.1, 2008). Each value obtained is the mean ± SD of three samples. Significant differences between means were determined by Duncan's Multiple Range tests.

Results and Discussion

Effects of Bleaching and Deodorization

Bleaching and deodorization, respectively reduced cholesterol content by 1.5 and 4.8% (Table 1). Zahir Aghdam et al. [17] reported that bleaching and deodorization can reduce tallow cholesterol by 1.99 and 5.7%, respectively. These two processes can totally reduce squid fish oil cholesterol to 26.5–25.5% [30]. The effects of the operations on acidity, P.V., and the color of the beef tallow are illustrated in Figs. 1 and 2.

Effect of Lecithin Treatment

Effect of Lecithin (Paste) to Tallow Ratio

The effect of the lecithin paste/tallow ratio in cholesterol removal was significant ($P < 0.01$). By increasing this ratio, cholesterol removal was increased to a maximum amount of 40.06% (Table 1). Kodali [27] in his studies using a liquid fraction of tallow observed 50.99% reduction in cholesterol content in the ratio of 1:5 lecithin to tallow. In a representative process, treating 20 g of liquid beef tallow with 4 g of a 1:1 lecithin-water paste for 5 cycles resulted in about a 95% reduction in the cholesterol level.

Table 1 Percentage of cholesterol removal followed by bleaching and deodorization processes

Process	Cholesterol content (mg/100 g)	% removal
Bleaching	93.4 ± 0.3	1.5 ± 0.25
Deodorization	89.9 ± 0.57	4.8 ± 0.56

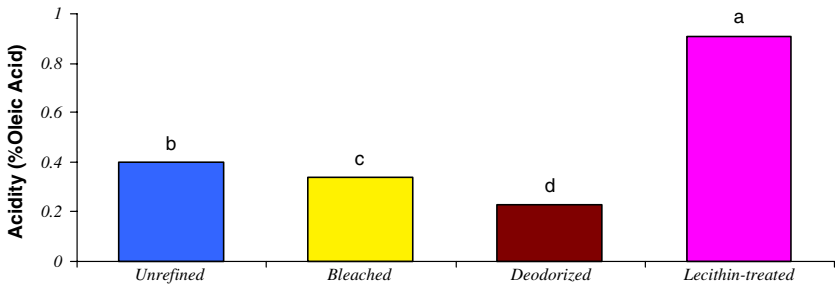

Fig. 1 Changes in acid value of bleached, deodorized and lecithin treated tallow (lecithin treatment: lecithin: tallow = 1:5; stirring time, 1.5 h; stirring rate, 1,250 rpm; lecithin: water = 1:2); *bar values with different letters* are significantly different

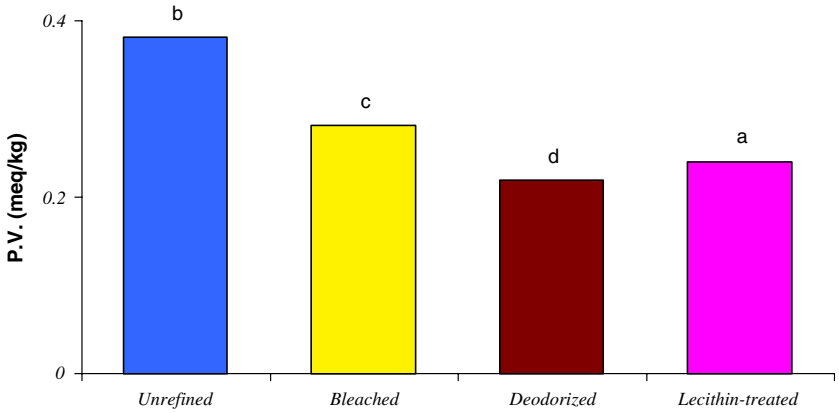

Fig. 2 Change of PV after bleaching, deodorization and lecithin treatment (lecithin: tallow = 1:5; stirring time, 1.5 h; stirring rate, 1,250 rpm; lecithin: water = 1:2); *bar values with different letters* are significantly different

The amounts of fat/oil and lecithin may be proportionately scaled up or down as desired.

Raising this ratio results in an increase in the area of the hydrophobic fluid bilayer, hence because of the high affinity of this bilayer to cholesterol, more cholesterol is stirred toward it and increased removal occursand a more enriched-cholesterol lecithin is achieved.

Effect of Stirring (Mixing) Time

Increasing the stirring time up to 1.5 h increased cholesterol removal (32.6%). Comparison of the means showed no significant differences between 1.5-, 3-, 6- and 12-h stirring times. Therefore it was concluded that a stirring time 1.5 h is adequate (Table 2).

Table 2 Percentage of cholesterol removal in different ratios of lecithin to tallow

Lecithin/tallow	% removal
1:5	40.06 ± 1.72^{a}
1:10	32.06 ± 2.15^{b}
1:20	18.87 ± 1.91^{c}
1:30	13.83 ± 1.46^{d}

Results are expressed as means ± SD. For each column, means with different letters are significantly different ($P < 0.05$)

Stirring time, 1.5 h; stirring rate, 500 rpm; lecithin: water = 1:2 (w/w)

Effect of Stirring Rate

Stirring rate had a significant and direct effect on cholesterol removal. Cholesterol removal increased with the stirring rate. Maximum cholesterol removal (42.77%) was achieved with a stirring rate of 1,250 rpm (Table 3).

Since lecithin/fat mixtures are rheologically viscous, increasing the stirring rate decreases viscosity and increases the contact between lecithin and cholesterol which leads to higher removal. A mechanical stirrer can lead to better results.

Effect of Lecithin to Water Ratio

Changing this ratio had no significant effect on cholesterol removal (Table 4). It is suggested that 1:2 w/v lecithin to

Table 3 Percentage of cholesterol removal at different stirring times

Stirring time (h)	% removal
0.5	23.1 ± 1.91^{b}
1.5	32.6 ± 1.80^{a}
3	33.93 ± 2.15^{a}
6	31.73 ± 2.04^{a}
12	32.23 ± 1.51^{a}

For each column, means with different letters are significantly different ($P < 0.05$)

Lecithin: tallow = 1:10; stirring rate, 500 rpm; lecithin: water = 1:2

water is appropriate for preparing an effective paste (Table 5).

The Effect of Lecithin Treatment on Tallow Acidity, PV, and Color

Treatment with lecithin of rendered tallow fat significantly increased its acidity from 0.23 to 0.91 (Fig. 1). This increase is mainly associated with the high acidity of commercial lecithin (1.3%).

Lecithin treatment increased PV but not significantly (Fig. 2). It had a significant increasing effect on yellow and red colors, respectively, from 1.63 and 0.4 to 71.33 and 4.1 (Lovibond unit) which seems to be due to pigment migration from commercial lecithin to the tallow (Fig. 3).

Table 4 Percentage of cholesterol removal at different rates of stirring of lecithin to tallow mixture

Stirring rate (rpm)	% removal
200	22.47 ± 1.92^{d}
500	32.31 ± 1.15^{c}
1,000	37.33 ± 1.65^{b}
1,250	42.77 ± 1.82^{a}

For each column, means with different letters are significantly different ($P < 0.05$)

Lecithin: tallow = 1:10; stirring time, 1.5 h; lecithin: water = 1:2

Table 5 Mean comparison for cholesterol removal at different lecithin to water ratios

Lecithin/water	% removal
1:5	23.32 ± 2.12^{a}
1:10	30.60 ± 1.06^{a}
1:20	28.13 ± 2.36^{a}

For each column, means with the same letters are not significantly different ($P < 0.05$)

Lecithin: tallow = 1:10; stirring time, 1.5 hr; stirring rate, 500 rpm

Conclusions

This research features a novel method of reducing the cholesterol content of a fat product, i.e., beef tallow. However, the method is applicable to a variety of sterols, including, without any limitation, natural or synthetic plant sterols (phytosterols, e.g., *p*-sitosterol, campestrol and stigmasterol), mycosterols and animal terols including cholesterol. Since the steps of the method can be performed at ambient temperature if desired, costs involved in heating are minimized as is the possibility of thermal degradation of the product.

The procedures can be repeated through two or more cycles to attain a desired level of cholesterol reduction. Alternatively, the method can be performed under conditions of countercurrent extraction.

Soybean lecithin, which was used in the preparation of the paste, is a by-product of plant oil refining and is both abundant and relatively inexpensive. Various lecithin powders enriched for phospholipid content are available commercially and may also be used in the present method; such lecithin powders are also within the scope of the term "lecithin" as used herein.

Additionally, a minimal amount of equipment is required, and since all required materials are food grade, the method requires no special precautions regarding handling, waste disposal, or contamination of the final product(s).

The extent of cholesterol removal can be varied depending upon the lecithin to water ratio, the ratio of lecithin paste to fat/oil product, the contact surface area, time of contact (mixing) and, as mentioned above, the number of cycles through which a fat/oil product is taken.

The combination of vegetable oils and cholesterol-free animal fats may provide a variety of high stability fats suitable for solid applications such as *trans* fatty acid-free margarines, spreads and shortenings.

It is to be noted that practice of the method of the present study yields not only a fat product with reduced

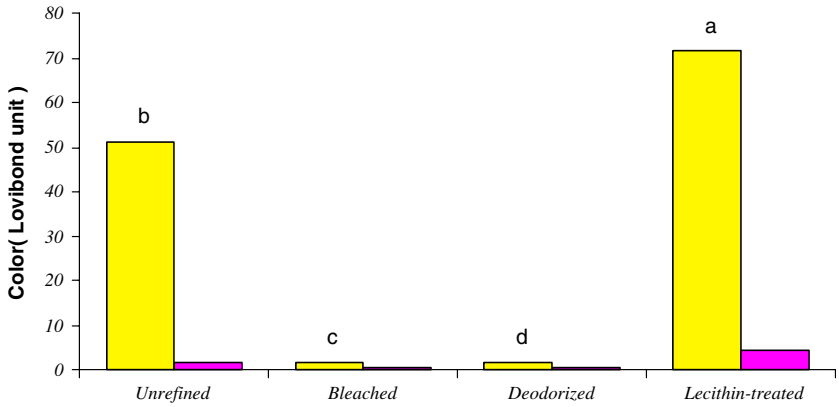

Fig. 3 Changes in red and yellow colors as a result of bleaching, deodorization and lecithin treatment of tallow (lecithin: tallow = 1:5; stirring time, 1.5 h; stirring rate, 1,250 rpm; lecithin: water = 1:2); *bar values with different letters* are significantly different (color figure online)

levels of cholesterol, but also yields a preparation of lecithin aggregate that is correspondingly enriched in such molecules. The cholesterols may be isolated from the lecithin using any of several known methods. For example, a cholesterol-enriched lecithin of the present research may be dehydrated, then applied to a silica gel column and extracted with a non-polar solvent to remove and recover the cholesterol.

Cholesterol-enriched lecithin is valuable in its own right. For example, the cholesterol-enriched lecithin may be marketed as a feed supplement used, for example, to enhance the growth of young animals. To this end, it is possible to run the extraction methods through multiple cycles or through a counter current exchange process, and to recycle the resulting cholesterol-enriched lecithin back into the process until it is maximally loaded with the desired sterols. This provides a particularly rich source of cholesterol that can be further processed as described above.

Acknowledgments We would like to express our sincere appreciation to Mr. Eng. K. Tabarhaydar, Chemistry Department Head of the Iranian Scientific and Industrial Research Center of Excellence, for his continuous guidance, scientific support and insight through the course of this work. Special gratitude goes to Agricultural Graduate Pardis of Tehran University for their funds supported the present research.

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