AMETHOD FOR REDUCING CHOLESTEROL LEVEL IN SOME ANIMAL PRODUCTS.

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ABSTRACT

Animal products such as cream, Butter, fats and eggs continued to suffer from being non preferable and decreasing the rate of consumption due to the cholesterol problem. Animal's products are one of natural foods containing high quality protein, essential fatty acids, phospholipids, minerals and vitamins. In this study, β – Cyclodextrin (β – CD) were used to remove cholesterol from cream 70% fat under different conditions. The results inducted that,T12:15% β – CD, pH value 5, sample: water 1:4 and stirring at 150 rpm for 30 min. gave the highest reduction in cholesterol 67.72%.While, T16:15% β – CD, pH value 10, sample: water 1:4 and stirring for 30 min. at 150 rpm would be preferred where cholesterol and total saturated fatty acids were reduced by 67.29% and 49.64%, while total unsaturated and total polyunsaturated fatty acids increased to 50.36 and 13.82% respectively.

INTRODUCTION

A continuing trend among many consumers towards healthy eating, whether by limiting the intake of butter , fats, sugars , and / or salts in the consumed foods or by increasing the utilization of functional foods containing natural or enhanced levels of photochemical and other nutraceutical ingredients.

Cholesterol is an unsaturated lipid which is readily susceptible to oxidation leading to chemically labile hydro peroxides which are decomposed to form secondary cholesterol oxidation products (CPOs). Various food processing and storage treatments can lead to oxidation of cholesterol in the presence of oxygen, heat, light or

radiation to yield COPs. Although more than 70% of these oxidation products have been identified (Smith, 1981) .

The major COPs in foodstuffs are 25- hydroxycholesterol, cholestanetriol, hydroxycholesterol derivatives, ά and β– peroxides and 7 – ketocholesterol and their presence in the human diet may have potentially negative health implications, (Emanuel, *et al.*, 1991). Furthermore, some cholesterol oxidation products have been reported as cytotoxic, atherogenic, mutagenic and carcinogenic (Maerker, 1987). Therefore, a lower intake of high cholesterol foods has been suggested as an effective method for lowering the level of serum cholesterol.

Various physical, chemical and biological methods have been proposed for reducing cholesterol in foods. These include blending with vegetable oils, extraction with organic solvent, adsorption with saponin to form cholesterol complexes.

However, most of these physical and chemical methods tend to be relatively nonselective, for removing flavor and nutritional components with cholesterol. Moreover, some methods are high in the operation cost, (Yen and Tsai, 1995).

The β – Cyclodextrin (β – CD) is a cyclic oligosaccharide composed of ά - (1-4) – linkages of seven glucose unit members. β – CD has a cavity in the center of the molecule and has the capability of forming an inclusion complex with various compounds, including cholesterol. β. – CD has high selectivity on cholesterol in its removal from egg yolk. β – CD also, has advantages of non – toxicity, edibility, non – hygroscopicity, chemical stability and easy separation (Negation, 1985). Thus,it may be a suitable for cholesterol removal substance from foods.

Several studies have been reported on removal of cholesterol from animal fats with β – CD, (Courregelongue and Maffrand, 1989; Davidson, 1990; Oakenfull and Sidhu, 1990 and Makoto, *et al*., 1992).

β – Cyclodextrin are approved for food used in Japan and several European countries and allowed to be used as processing aids in the U.S., (Anonymous, 1989). Aconcern regarding the cholesterol content of the human diet is growing, because of the probable importance of cholesterol and cholesteroloxide products in the development of atherosclerosis (Taylor, *et al*., 1979; Anonymous 1988 and Blankenship, *et al*., 1991). Butter fat has been fractionated using supercritical carbon dioxide, with evidence that cholesterol can be

concentrated into selected fractions (Kaufmann, *et al*., 1982 and Arul, *et al*., 1987).

Countries with a seasonal milk production pattern such as Ireland, New Zealand and Australia, have shown a seasonal variation in milk processability due primarily to seasonal changes in animal diet and stage of lactation, (Phelan, *et al*., 1982). Variations in milk processability lead to a consistent quality of dairy products. The absolute criterion for suitability of milk for processing is its stability during manufacturing and storage often over extended periods. Antioxidents can deal with milk fat oxidation during storage (Touchy , 1987 and Atwal, *et al*., 1991) and seasonal variations in animal feed quality have been found to influence off – flavor development during storage of whole milk powders (Steen, 1977) . Our objective was to determine the influence of animal feed quality on lipid and cholesterol oxidation in stored whole milk powder. In particular, the effects of some treatments on butter fat and cholesterol removal from a butter fats (animals products). However, reductions in fats animal cholesterol levels have unsuccessfally met the demands of health consumers. The degeneration of vegetable and animal fats detected in the incipient stage by alterations in organoleptic properties (I – e . , rancid odor and flavor) , is due to enzymatic and / or chemical processes . Therefor, the aim of study was to remove or reduce cholesterol from cream containing 70% fats.

MATERIALS AND METHODS

A – Materials:

Cream containing 70% fat was obtained from Food Tec. Res. Ins., Agric. Res. Center. β - Cyclodextrin 99% produced by Fluke Comp. was used in this work. Cholesterol was purchased from Sigma Chemical Co.

B – Cholesterol removal from cream containing 70% fat (on wet weight).

Various operating conditions for cholesterol removal from cream containing 70% fat with β - Cyclodextrin 99% were investigated. Removal of cholesterol from cream 70% fat occurred under various operating conditions as pH value, time of stirring, sample: water ratio and β - Cyclodextrin 99% level according to (Table 1). For each treatment stirring was accomplished at 50C°, and centrifuging at 7000 rpm for 30 min, then the water layer was discarded and the cholesterol

in samples was determined. All treatments were run in duplicate, and analyses of all samples were carried out in duplicate and averaged..

C -Determination of fat in samples: Fat was determined according to the method described in the, Phelan, *et al*; (1982)

D-Determination of cholesterol:

Cholesterol was determined by spectrophotometer at 550 UV according to the method of, Bachman, *et al.,* (1976) .

Table (1): Treatments conditions used in removing cholesterol from cream containing 70% fats (on wet weight).

Treatments	%β-CD	pH value	Sample: Water ratio	Time of stirring (min.)
T1	10	5	1:2	15
T2	10	5	1:2	30
T3	10	5	1:4	15
T4	10	5	1:4	30
T5	10	10	1:2	15
T6	10	10	1:2	30
T7	10	10	1:4	15
T8	10	10	1:4	30
T9	15	5	1:2	15
T10	15	5	1:2	30
T11	15	5	1:4	15
T12	15	5	1:4	30
T13	15	10	1:2	15
T14	15	10	1:2	30
T15	15	10	1:4	15
T16	15	10	1:4	30

E-Determination of fatty acids:
Separation and identification of fatty acids:
Separation of fatty acids:

The lipid extracted from fresh and treated cream: Cream containing 70% fat, was saponifed with methanolic KOH (20 % w/v) for 24hr. at room temperature. The unsaponifiable matters were extracted three times with diethyl ether. The aqueous layer (soap) was acidified with HCl (1: 1 v/v) and the liberated fatty acids were extracted with petroleum ether (40 / 60 C°). The fatty acids were washed several times with distilled water, then dried over anhydrous sodium sulphate .

Methylation of fatty acids:

The fatty acids were converted into methyl esters as follows: the solvent was distilled off, the residue was dissolved in anhydrous dimethyl ether (0.5 – 1 ml) and methylated by addition of drop of diazomethane solution prepared as reported by Vogel (1975) until the yellow color persists. The mixture was then left at room temperature for 15 min. and the solvent was evaporated on water bath. Finally, the fatty acid methyl esters were dissolved in chloroform. A liquor of this solution was subjected to gas – liquid chromatography for the identification of the methylated fatty acids.

*** Identification and Determination of fatty acids methyl Esters by Gas – liquid chromatography:**

The fractionation of fatty acids methyl esters was conducted using silver column 10 % on gas chromatograph Q11 80/100. The separation conditions were: The column temperature was programmed at 3 C°/ min., initial temperature was 190 C°and final temperature was 220 C° . Chart speed was 5 mm / min., detector temperature was 270 C° and injection temperature was 270 C° . Flow rate of gases were: nitrogen 30 ml / min ., hydrogen 1 ml / min ., air 0. 5oml / min. and sensitivity 16×10^2 . The peak identification was performed by comparing the relative retention times of each peak with those of standard materials fatty acid calculated as percent age of the total identified acids after measuring the peak areas by triangulation.

F-Ks value:

Ks value (indicating the rate at lipids oxidation) was calculated according to Semyonov , *et at*; (1979) as follow :

$$Ks = \frac{\% \text{ Total unsaturated fatty acids}}{\% \text{Total saturated fatty acids}}$$

G- pH value :.

The pH value in cream– water mixture slurry was measured at room temperature, using digital pH-meter model HANNA 213 microprocessor according to the method of Woye Wodo , *et at* ., (1986).

RESULTS AND DISCUSSION

Cholesterol and fat content:

The data in table (2) show the effect of β - Cyclodextrin concentration being 10 and 15% on the removal of cholesterol from cream containing 70% fat (on wet weight basis). It could be observed that cholesterol content of cream was 59.96 mg/g sample, cream treated with 10% β - Cyclodextrin under different conditions decreased the cholesterol content, while cream treated with 15 % β - Cyclodextrin, showed the highest efficiency for cholesterol removal, but T12 gave the highest reduction in cholesterol 67.72% followed by T16 were the cholesterol content was reduced by 67.29% .While T4, T14 gave the lower reduction 27.77, 27.31%. From these data it could be observed that the high level of 15% β – Cyclodextrin was more effective than the low level (10%) at the pH value 5, sample: water ratio 1:4 and stirring for 30 min. (T4 , T12).While pH value 5 was more effective than pH value 10 in present of 15% β - Cyclodextrin, sample: water ratio(1:4) and stirring for 30 min. (T12, T16).Also ratio of sample :water 1:4 showed more effect than 1:2 in present of 15%, at the pH value 10 for 30 min.(T14 ,T16).These results are in agreement with those of Yen and Tsal (1995) who reported that 26 and 33% cholesterol were removed from dehydrated butter by 5 and 10% β - Cyclodextrin respectively . Also, cholesterol removal increased with increasing the ratio of cream : water (1:4) and stirring for 30 min. compared with the cream : water (1:2) and stirring for 15 min. , This may be due to the fact that higher water content would increase the inclusion between β - Cyclodextrin and cholesterol when stirred for a long time (30 min.). However, too much water would be due to the increase of the interaction between β - Cyclodextrin and cholesterol for removing cholesterol from cream. Also, temperature was important for removing cholesterol from cream Oaken full and

Sidhu (1990) indicated that decreasing the cholesterol from milk with ß – CD was markedly influenced by the temperature. The higher removal was found at a lower temperature. I.e. , 77 , 64 and 62 % cholesterol removed in milk were obtained when treated with β - Cyclodextrin at 4, 8 and 40 c‹ ẗespectively. Concerning the fat content (Table 2), the results indicate that cream treated with β - Cyclodextrin (10 & 15%) caused the increase of fat (on dry weight) and a decrease of cholesterol for all treatments compared with the control. This may be due to the removal of moisture from cream and the concentration of fat due to centrifuging at 7000 rpm for 30 min.

Table (2): Cholesterol content in sample after treating with β – Cyclodextrin under different conditions compared to the control (cream on dry weight basis).

Treatments	Cholesterol content*	Percentage of reduction	% Fat (on dry weight)
Control(cream)	59.96	_	96.34
T1	23.70	60.47	95.21
T2	41.01	31.60	98.83
T3	22.11	63.12	98.47
T4	43.31	27.77	96.10
T5	31.74	47.06	99.45
T6	37.82	36.92	100
T7	35.10	41.46	99.40
T8	39.19	34.64	96.83
T9	24.53	59.09	100
T10	32.48	45.83	99.38
T11	34.05	43.21	100
T12	19.35	67.72	99.41
T13	28.94	51.74	96.97
T14	43.59	27.31	99.70
T15	23.23	61.26	98.67
T16	19.61	67.29	99.47

*(mg/g sample)

B- Fatty acids composition and its fractionation:

Fatty acid composition (as % of total fatty acids) of raw cream containing 70 % fats(on wet weight basis) as affected by the addition of 10 & 15 % β - Cyclodextrin and stirring at 150 rpm for(15& 30 min.) under different conditions (including) pH value of 5 and 10, cream : water (1 : 2 and 1 : 4) are presented in table (3 & 4) .From these results , it could be noticed that the predominant saturated fatty acids were the palm tic ($C_{16:0}$) acid which was 30.61% followed by the myristic ($C_{14:0}$) acids and $C_{10:0}$ which were 14.21 and 10.61 % of the total fatty acids, respectively. At the same time, the lowest percent (6.31 %) was recorded for the $C_{12:0}$. Arachidic acid ($C_{20:0}$) was absent in cream (70 % fat, on wet weight). The abundant monounsaturated fatty acids were the oleic ($C_{18:1}$, 20.12 %) and palmitoleic ($C_{16:1}$, 4.16%) while, the predominant polyunsaturated fatty acids were descendingly arranged as follow : linoleic acid ($C_{18:2}$,3.41%) and linolenic ($C_{18:3}$, 1.26%) respectively. It is evident that the abundant polyunsaturated fatty acids represented the essential fatty acids. Moreover, also concerning the fractionation of fatty acids cream (70 % fat, on wet weight) ,it could be observed in Table (3 and 4) that the total polyunsaturated fatty acids including di and tri – unsaturated fatty acids recorded (4.67%) if total fatty acids by adding the percent of the total monounsaturated fatty acids (24.28%) the total unsaturated fatty acids will be recorded (28.95), at the same time , the total saturated fatty acids were recorded (71.05%) accordingly the Ks – which was obtained by dividing the total unsaturated fatty acids / the total saturated fatty acids of the raw cream 70% fat was (0.41) indicating the highly percent of the total saturated fatty acids versus the low percent of unsaturated fatty acids. Concerning the change in fatty acids composition of cream after stirring(150 rpm) for 15 & 30 min. with 10 and 15% β - Cyclodextrin at 50C° and various conditions including pH value (5 and 10), cream : water (1: 2 and 1 : 4) then centrifugation at 7000 rpm 30 min. From the results, it could be observed that all the various conditions effect on the fatty acids composition and fractions, some fatty acids increased and other decreased, besides that some fatty acids were absent while, the same fatty acids were found. This may be due to various effective conditions such as pH value and stirring. Anyway, the results of fractions in fatty acids were more illustrated and confirmed for the results of fatty acids composition in Table (3).

Table (3): Fatty acids composition of samples after treating with β – Cyclodextrin under different conditions compared to the control (cream containing 70% fat, on wet weight).

*F.A. / Treatment	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$
Control	10.61	6.31	14.21	30.61	4.16	9.31	20.12	3.41	1.26	___
T1	0.09	3.60	11.81	28.69	6.31	18.62	25.22	2.03	2.16	1.47
T2	0.46	3.90	15.64	47.44	2.49	20.50	4.38	3.37	0.31	1.51
T3	1.19	2.35	8.75	26.64	7.11	18.86	27.90	3.28	3.84	0.08
T4	___	1.48	19.20	37.55	1.49	23.44	15.53	0.53	0.88	___
T5	21.07	3.57	0.01	17.13	0.21	21.68	29.51	4.16	2.67	___
T6	2.61	7.41	26.61	17.31	0.14	23.46	17.32	3.21	1.93	___
T7	0.56	8.66	30.30	14.65	0.14	21.57	20.00	4.12	___	___
T8	3.41	14.61	17.81	9.81	3.61	26.71	18.41	3.67	1.47	0.49
T9	0.13	5.38	11.61	34.82	2.28	14.94	22.28	4.84	3.12	0.60
T10	0.57	4.74	15.63	18.54	___	23.02	30.00	4.10	3.10	0.30
T11	0.11	8.26	37.29	14.03	___	17.76	21.95	0.30	0.90	___
T12	0.57	4.74	15.63	21.54	3.03	23.02	25.50	4.47	1.50	___
T13	4.21	7.60	8.91	20.31	10.41	18.21	22.61	4.67	2.78	0.43
T14	14.61	9.61	9.97	34.61	___	11.22	19.31	0.67	____	____
T15	1.75	13.77	19.71	11.61	2.71	20.30	17.81	6.31	4.71	1.32
T16	4.61	3.21	7.21	14.61	11.93	20.00	24.61	5.61	8.21	___

*Fatty acids

Table (4): Fatty acids fractions of samples after treating with β – Cyclodextrin under different conditions compared to the control (cream containing 70% fat on wet weight).

*F.A.F. / Treatment	T.sat.	T.unsat.	T.mono.	T.di.	T.tri.	T.ploy.	Ks
Control	71.05	28.95	24.28	3.41	1.26	4.67	0.41
T1	64.28	35.72	31.53	2.03	2.16	4.19	0.56
T2	89.45	10.55	6.87	3.37	0.31	3.68	0.12
T3	57.87	42.13	35.01	3.28	3.84	7.12	0.73
T4	81.57	18.43	17.02	0.53	0.88	1.41	0.22
T5	63.45	36.55	29.72	4.16	2.67	6.83	0.58
T6	77.40	22.60	17.46	3.21	1.93	5.14	0.29
T7	75.74	24.26	20.14	4.12	___	4.12	0.32
T8	72.84	27.16	22.02	3.67	1.47	5.14	0.37
T9	67.48	32.52	24.56	4.84	3.12	7.96	0.48
T10	62.80	37.20	30.00	4.10	3.10	7.20	0.59
T11	76.85	23.15	21.95	0.30	0.90	1.20	0.30
T12	65.50	34.50	28.53	4.47	1.50	5.97	0.53
T13	59.53	40.47	33.02	4.67	2.78	7.45	0.68
T14	80.02	19.98	19.31	0.67	____	0.67	0.25
T15	68.46	31.54	20.52	6.1	4.71	11.02	0.46
T16	49.64	50.36	36.54	5.61	8.21	13.82	1.01

Fatty acids fractions:
T. sat.: Total saturated fatty acids
T. unsat.: Total unsaturated fatty acids
T. mono.: Total mono-unsaturated fatty acids
T. di.: Total di-unsaturated fatty acids
T. tri.: Total tri-unsaturated fatty acids
T. ploy.: Total polyunsaturated fatty acids
Ks: Total unsaturated fatty acids/ Total saturated fatty acids

Data of fatty acids fractions in Table (4) indicate the, T3 (10% β – CD, pH value 5, sample: water 1:4 and 15 min. for stirring at 150 rpm) gave the highest Ks value compared with control. But, T16(15% β – CD, pH value 10, sample: water 1:4 and 30 min. for stirring at 150 rpm), gave the highest value of decreasing total saturated fatty acids and increasing the total unsaturated fatty acid ,moreover T16 gave the highest value Ks and polyunsaturated fatty acid compared with the control and other treatments either (10 or 15% β – CD). Generally, according to cholesterol content and fatty acids fractions, the cream treated with 15 % β – CD, pH value 10, ratio of cream : water (1 : 4) and stirring at 150 rpm for 30 min.(T16) would be preferred where cholesterol and total saturated fatty acids were reduced by 67.29% and 49.64%, while total unsaturated and total polyunsaturated fatty acid increased to 50.36 and 13.82%, respectively.

CONCLUSION

Removal of cholesterol from cream increased with increasing β - Cyclodextrin (β – CD) concentration. About 67.29% of cholesterol could be removad from cream by 15% β - Cyclodextrin with 1:4 ratio cream : water, pH value 10, stirring at 150 rpm and at 50C° for 30 min. then centrifugation(7000 rpm for 30 min.) and increasing of polyunsaturated fatty acid and Ks value. Too much water is important to increase the efficiency of removing cholesterol from cream.

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طريقة لخفض نسبة الكولسترول فى بعض المنتجات الحيوانية

***على محمد بيومي ، **على أحمد عبد الحليم .**

*قسم بحوث الأغذية الخاصة والتغذية ،** قسم بحوث تكنولوجيا اللحوم والأسماك ، معهد بحوث تكنولوجيا الأغذية ، مركز البحوث الزراعية

المنتجات الحيوانية مثل القشدة والزبد والدهون والبيض انخفض مستواها حاليا في الاستهلاك وذلك بسبب محتواها العالي من الكولسترول الذي يسبب كثير من المشاكل الصحية لكن في المقابل المنتجات الحيوانية واحدة من المكملات الطبيعية للغذاء وذلك لمحتواها العالي من البروتين الحيواني عالي القيمة الحيوية والأحماض الدهنية الأساسية والفوسفوليبيدات والمعادن والفيتامينات ولذلك كان الهدف من هذة الدراسة استنباط طريقة لإزالة أو إنقاص محتوى الكولسترول من بعض المنتجات الحيوانية (منتج القشدة المركزة 70%دهن) باستخدام (10&15%) β- Cyclodextrin والماء المقطر بنسبة 1:4 و 1:2 ماء: قشدة والتقليب لمدة 15دقيقة و30دقيقة على 50 درجة مئوي على (5&10)pH value ثم الطرد المركزي على 7000 rpm لمدة 30 دقيقة لكل المعاملات مع استخدام عينة كنترول .

تبين من الدراسة : أن إزالة أو خفض الكولسترول من القشدة المعاملة يزداد بزيادة تركيز β-Cyclodextrin ونسبة الماء حيث أثبتت النتائج أن حوالي 67% تقريبا من محتوى الكولسترول في القشدة يزال بواسطة 15% β- Cyclodextrin واستخدام 1:4 ماء : قشدة والتقليب لمدة 30 دقيقة حيث كانت هذة أفضل المعاملات(المعاملة رقم 16) . أيضا أدت إلى زيادة الأحماض الدهنية الأساسية والأحماض الدهنية عديدة عدم التشبع ومعامل Ks.