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Manufacturing Cholesterol-Reduced Butter by ß-Cyclodextrin and Rosemary and Study Some of its Chemical and Physical Properties

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Abstract: This study was carried out to examine the effect of addition 10% from β-cyclodextrin (β-CD) treatment (T1) or 10 mg from alcoholic extract of rosemary (AER) treatments (T2) and mixture of 5% β-CD and 5 mg from AER/100 gm butter treatment (T3) on reducing cholesterol in manufacturing butter compared with control butter treatment (C) and study some of physical and chemical properties at zero time and during storage for 4 weeks at 6±1°C. Results indicated that 90% of the cholesterol was removed by β-CD and 58% by AER and 80% by the mixture of them. The result reveled that there was a significant differences in the chemical composition between control butter and butter of treatments in % for fat, moisture and SNF also results showed that there was no significant differences in the sensory evaluation which conducted after processing in the characters color, taste and flavor, texture and rancidity between the control and treatments butter, while Butter of treatments stay more acceptable than control butter in all periods of storage, especially treatments T2 and T3. The obtained results showed that T2 and T3 treatments was quit low in development of each peroxide value (POV) and acid degree value (ADV) of fat in some stages of storage, which have retained their validity according to the scale of accepted level for POV and ADV as even after storage at 6±1°C for 4 weeks.

Key words: cholesterol-reduced butter, β-cyclodextrin, rosemary, Chemical physical properties

INTRODUCTION

Butter is one of dairy products it has economic and nutritional importance. The research suggests that there are more than twenty health benefit contributed by butter, including its a good source of vitamin A which is essential to the health of the thyroid and adrenal gland also features boasts a high concentration of lauric acid which is necessary to reduce the inflammation caused by fungi in addition to its rich in lecithin which is necessary for cholesterol metabolism and also a source of vitamins E, D, A and K and contains many substances that play a role as anti oxidant, also butter is source for selenium and linoleic acid which are necessary to boost the immune system in addition to being a rich source of energy and its essential for the growth of children's brains and other beneficial but the factor which limited its used that it was rich in cholesterol (Hettinga, 2005; Butter, 2010a, b). In the past decades 44% of Americans have changed their eating habits to lower their cholesterol level the International Dairy Statistics (2003) reported that world per capita slowly decreased consumption of butter from 1999 to 2002 in most European countries, Asia and the United States. This shift in eating habits has caused a dramatic increase in no-cholesterol, low-cholesterol, reduced-cholesterol and low fat products available in the marketplace (Best, 1989). A manufacturer in New Zealand previously reported trial production of a reduced-cholesterol butter (Wilson, 1990) and consumer and industry demand has led to an interest in manufacturing reduced-cholesterol dairy products. Previously, several studies have indicated that the cholesterol in food, including that in milk, yogurt, cream and cheese, can be reduced effectively by β-cyclodextrin (β-CD), (Kwak *et al*., 2002; Shim *et al*., 2003; Hwang *et al*., 2005; Kim *et al*., 2005; Kwak *et al*., 2005; Lee *et al*., 2006). Because β-CD is nontoxic, edible, non hygroscopic and chemically stable (Nagamoto, 1985), it has positive attributes when used for cholesterol removal from foods. Although using powdered β-CD allows for about 90% cholesterol removal, it may not be adequate for separating cholesterol from food systems and for β-CD reuse, Moreover because of the high cost of β-CD and other operating expenses, β-CD is nearly impossible to apply to related manufacturing processes. Rosemary have recently gained considerable attention as nutraceuticals for their blood cholesterol-lowering efficacy (Milessa *et al*., 2013; Umit *et al*., 2011). This interest has now been translated into a range of health-promoting functional products, Most organizations concerned with cardiovascular health have recommended substantial increases in polyunsaturated fatty acid (PUFA) intake and decreases in saturated fat intake (Expert Panel on Detection, 2001). This sustained

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campaign has led to large increases in PUFA intake in some countries, such as the United States and such increases may in part be responsible for the recent decline in coronary disease mortality (Horrobin and Manku, 1983). Among the PUFA, Rosemary is of special interest because its contains an oil characterized by a high content of-linolenic acid (GLA, all cis-6,9,12-octadeca-trienoic acid (Milessa *et al*., 2013). Despite numerous demonstrations of the cholesterol-lowering properties of PUFA such as GLA, the precise mechanism is still not fully understood (Ihara-Watanabe *et al*., 1999). Large amounts of PUFA are required to produce a substantial and meaningful reduction in plasma cholesterol. Based on this information, we assumed that the addition of Rosemary could enhance the cholesterol-lowering effect of cholesterol-reduced butter. This study was designed to develop a cholesterol-reduced butter with added B-CD alone and Rosemary alone and mixture of them to examine the effects on lowering cholesterol and on the chemical and sensory properties of the produced butter.

MATERIALS AND METHODS

Separated and pasteurized cream 30% fat was obtained from the Dairy Plant of Agriculture college, Baghdad University. Commercial β-CD (purity 99.1%) was purchased from Nihon Shokuhin Cako Co. Ltd. (Osaka, Japan). Rosemary were obtained from unit of medicine and aromatic plant in Agriculture College-Baghdad university.

Treatment Cream with β-CD and AER: Bulk pasteurized cream (3 kg) was stirred with 10% β-CD at 1,400 rpm with a blender in a temperature-controlled water bath at 40°C for 30 min. The cream was then centrifuged at 166×g for β-CD removal. In the same way 10 mg from AER was added/100 gm cream all treatments were run in triplicate.

Butter Manufacturing: Four different experimental butters were manufactured: control Butter (C) made with cream wasn't treated with β-CD or Rosemary; treatments (T1) made from cream treated with 10% β-CD (wt/wt); (T2) made from cream treated with alcoholic extract of Rosemary (10 mg/100 gm); (T3) made from cream treated with mixture of (5% B-CD and 5 mg from alcoholic extract of rosemary). The treated cream was churned at 13 to 14°C by using a mechanical butter churn until butter granules were visible. The buttermilk was drained off and the butter was washed in 10°C tap water. Salt (2%) was then added and the butter was molded. After manufacture, the molded butter was weighed and filled into 150 mL sterilized glass cups covered with aluminum foil, then rabidly cooled and stored 4 weeks at 6±1°C. The Butter samples were analyzed for fat, ash content and moisture as described by Ling (2008).also peroxide value (POV) of fat was assessment directly and during storage according to AOAC (2004) and acid degree value (ADV) of fat determined according to Deeth and Fitz-gerald (2006).

Sensory evaluation: The samples were served in small cups and evaluated by 10 trained panelists from among the staff of Food science department, college of Agriculture, Baghdad University. The samples were evaluated for color, flavor, texture and rancidity at zero time and during storage for 4 weeks at 6±1°C according to forma mentioned by Nelson and Trout (1964).

Extraction and determination of cholesterol: For the extraction of cholesterol, 1 g of butter sample was placed in a screw-capped glass tube (15×180 mm), The sample was saponified at 60°C for 30 min with 5 mL of 2 m ethanolic potassium hydroxide solution (Adams *et al*., 1986). After cooling to room temperature, the cholesterol was extracted with 5 mL of hexane. The hexane layers were transferred to a round-bottomed flask and dried under vacuum. The extract was re dissolved in 1 mL of hexane and stored at -20°C until analysis. Total cholesterol was determined by kits the percentage of cholesterol reduction was calculated as follows:

- Cholesterol reduction (%) = 100-(amount of cholesterol in β-CD treated butter x100/amount of cholesterol in the control)

Statistical analysis: Data from determination of the optimum conditions were analyzed by one-way ANOVA (SAS, 2004). The significance of the results was analyzed by the least significant differences test. A difference of $p<0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Chemical composition of butter: The chemical compositions of treatments butter C, T1, T2 and T3 are presented in Table 1. It illustrated that there was a significant differences in% of fat, moisture and SNF between control butter and butter of treatments after manufacturing, the fat% were 83,82,81,81 in C, T1, T2 and T3, respectively while the moisture% were 9.40, 6.00, 10.0, 7.30 for previous treatments, respectively and SNF% were 7.6, 12.0, 9 0, 11.7, respectively. The result which obtained in this study was in the limited mentioned by other researcher, Ling (2008) report that butter should content Fat from 70-85% and SNF 6-12% dependent upon the quality of raw material which used in manufacture butter. The result of this study was also similar to moisture and fat% found by Doosh (2011) which was 12 and 80%. The low % of moisture in butter of T1 may be due to β-CD its carbohydrate substance which has ability to absorb large quantity of water and it removed with β-CD during manufacturing process.

Table 1: Chemical composition of butter after manufacturing

Treat.	Moisture (%)	Fat (%)	SNF (%)	pH
C	9.40	83	7.6	5.71
T1	6.00	82	12	5.70
T2	10.00	81	9	5.70
T3	7.30	81	11.7	5.65
L.S.D	2.10*	1.00*	2.4*	ns

*p<0.05, Treat: Treatment

Table 2: Cholesterol content and rate of cholesterol removal in treatments butter

Treatments	Cholesterol mg/100 g	Removal (%)
C	240	zero
T1	25	90
T2	100	58
T3	48	80

Cholesterol content and rate of cholesterol removed in butter: The cholesterol content of the control butter was 240 mg/100 g, while in butter of treatments T1, T2 and T3 were 25, 100, 48 mg/100 g, respectively. The cholesterol reduction reached 90.0% with the 10% β-CD treatment T1 and 58% with alcoholic extract for rosemary treatment T2 and 80% with the mixture from β-CD and alcoholic extract of rosemary treatment T3 which concentration both of them was reduced to half to conduct the economic and health benefit from using small amount from β-CD. This result was in accordance with our previous study which used powdered β-CD, the reduced cholesterol reached 92.1% (Jung *et al*., 2005) and more than 90% has been found in other dairy products (Kim *et al*., 2004-2006; Han *et al*., 2005).

Sensory evaluation of butter: Table 3 shown the Results of sensory evaluation for treatments and control butter directly after manufacturing and during storage for 4 weeks at 6 ± 1°C, the results reveled that there was no significant differences in sensory evaluation studied represented to the color, flavor, textures and rancidity between the control butter and treatments butter at zero time this confirms that non β-CD neither Rosemary extract with Butter shows undesirable sensory impact of the consumer. After 1 week of storage butter of treatments got the highest scores special treatments T2 and T3 in terms of flavor, texture and rancidity, while control butter get lower scores the reason for this belong to the chemical changes that occur on fat like hydrolysis of fats (fat rancidity) which gets by action of bacterial lipases which is produced by psychrophilic bacteria which enzymes were resistance to the heat treatment that's utilized in butter processing and this enzyme will work to break down triglyceride and released short chain fatty acid in concentrate enough to give the rancid flavor, which is undesirable by the consumer as well as undesirable products which is accompanied by the accumulation of oxidative stress (Cogan, 1980).

While these changes was not shown in butter of treatments T2 and T3 this was due to the action of rosemary which contain different biologically active compounds work as antioxidants and antimicrobial in the same time, such as phenolic compounds and flavonoids which inhibit the growth of microorganisms, especially psychrophilic bacteria which is responsible for butter lipolysis during storage (Cogan, 1980). A similar study Kifah and Sajid (2013) indicated that the addition of alcoholic extract of rosemary to processed cream improved the overall quality and did not result in any adverse effect on the sensory. But in subsequent periods of storage notes that the all type of butter trod the same behavior of the previous period of time until 4 weeks of storage control butter became unacceptable sensory, meanwhile butter of treatments T2 and T3 still acceptance. From these results we can say that the treatment butter with rosemary prolong the period of validity of butter special treatment T3 in addition to reduced cholesterol.

Peroxide value (POV) in butter: Table 4 illustrated the changes in peroxide value POV of control butter (C) and butter of treatments T1, T2 and T3 during storage for 4 weeks at 6±1°C. The results reveled that there was differences in the primary values of POV between the different treatments of butter, it was 1.42, 1.43, 0.99, 1.30 meq/1 kg fat for treatments C, T1, T2 and T3, respectively the low value was in T2 this may be due to action of alcoholic extract of rosemary which used in manufacturing this butter, the POV values were comply with Iraqi standard specification for butter, which required POV less than 10 meq/1 kg fat. But after 1 week of storage the values for all butter treatments were increased but it was slight in treatments T2 and T3. The reason belong to the presence of natural antioxidant compounds in rosemary which worked to reduce or prevent the development in the POV to reach to the final products of oxidation. While after 4 weeks of storage, the evolution in POV values for treatment C and T1 reached to 10 meq/1 kg fat, this treatments become unacceptable while treatments T2 and T3 still in acceptance limits, this results refers to that there was clear effect for alcoholic extraction of rosemary in reducing the development of POV during storage.

Acid degree value (Fat rancidity): Table 5 shows the ADV values for control butter and butter of treatments T1, T2 and T3 at zero time and during storage for 4 weeks at 6±1°C, the results showed that there was significant differences in primary values of ADV, for all treatments it was 1.70,1.60,0.88,0.82 meq/100 gm fat, respectively. The values were with the acceptable limits for ADV scale. After 1 week of storage there was a development in ADV but the least was in treatment T2 and the highest in treatment C, this was due to the role of antibacterial

Table 3: Sensory evaluation of butter during storage at 6±1°C for 4 weeks

ST (weeks)	Treat	Color	Texture	Flavor	Rancidity	Total 40%
0	C	9.8	9.6	9.8	9.8	
	T1	9	9.2	8.8	9.8	39
	T2	8.8	10	8.6	9.8	36.8
	T3	8.8	10	8.6	9.8	37.2
1	C	9	9.2	8.4	9.8	37.2
	T1	9	9.2	8.4	9.8	36.4
	T2	8.7	9	9	9.8	36.4
	T3	8.8	9	8.6	9.8	36.5
2	C	9	8.8	8.4	9.6	36.2
	T1	8.6	9.3	8.5	9.8	35.8
	T2	8.7	8.8	8.8	9.8	36.2
	T3	8.8	8.6	8.6	9.6	36.1
3	C	8.8	8.7	8	8	35.6
	T1	8.5	8.8	8	8.5	33.5
	T2	8.5	8.7	8.8	9.6	33.8
	T3	8.6	8.7	8.5	9.5	35.6
4	C	8.5	8.1	7.5	8	35.3
	T1	8.5	8.2	7.8	8	32.1
	T2	8.5	8.5	8.5	9.5	32.5
	T3	8.4	8.6	8.4	9.5	35
LSD		0.78*	1.12*	2.00*	2.50*	34.9

ST : Storage time, Treat : Treatment, *p<0.05

Table 4: Peroxide value for processed chesses during storage for 4 weeks at 6±1°C

	Peroxide number (meq/1 Kg fat)				
	Period of storage (weeks)				
Treat	0	1	2	3	4
C	1.42	2.5	5.11	8.11	10.71
T1	1.43	2.1	5.23	8.5	10.66
T2	0.99	1.26	2.49	2.55	2.69
T3	1.3	1.67	2.79	2.9	3
LSD	*0.20	*0.25	*0.95	*1.21	*2.4

Treat. : Treatment

Table 5: Acid Degree value for processed chesses during storage at 6±1°C for 4 weeks

	ADV (meq/100 g fat)				
	Period of storage (weeks)				
Treat.	0	1	2	3	4
C	1.7	1. 81	2	2.8	2.25
T1	1.6	1.74	2	3	2
T2	0.88	0.913	1.16	1.28	1.5
T3	0.82	0.94	1.26	1.75	1.8
LSD	ns	0.14*	0.10*	0.09*	0.14*

Treat. : Treatment, *p<0.05

compound obtained in rosemary extract, which helped in preventing the development of ADV induced by bacterial lipase which produced by psychrophilic bacteria which was resistant to high temperature, while natural lipase in milk (lipoprotein lipase) destroyed by pasteurized temperature (Cogan, 1980). From this follows the psychrophilic bacteria was main responsible for the development of ADV in butter during storage. Rosemary had property to work as anti bacterial so worked to prevent the growth of psychrophilic bacteria during storage. Thus led to low development in ADV of butter of treatments T2 and T3 and evolution in some stages of storage is almost a small and insignificant compared with the control and T1 butter. After 4 weeks of storage ADV has trod the same behavior of the previous period the values of all treatments were rising and the values were 2.25, 2.0, 1.50, 1.80meq/100 gm fat for C, T1, T2 and T3, respectively, butter of treatments T2 and T3 still in the acceptable standard for ADV which refers that the values of ADV should not exceed 2.0 meq/100 g fat, while a control butter became refused by the scale values of ADV and butter of T1 at the broader of acceptance, the butter became unacceptable by consumers and the rancid flavor distinguish when ADV arrival higher than 2.0 meq/100 g fat, which is due to release of short chain free fatty acids like Butyric and Caproic in concentrations enough to appeared rancid flavor (Deeth and Fitz-gerard, 2006). The result which obtained encourages to use alcoholic extract from rosemary in prolonged the shelf life of butter.

The present study designed to develop a cholesterol-reduced butter with β-CD and AER and to examine the effect of the different treatments on the chemical and sensory evaluation of the product. This study indicates that using β-CD and AER could be an effective way to remove cholesterol from butter.

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