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The Effect of Beta Cyclodextrin on the Removal of Cholesterol from Buffalo Milk

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Abstract. This study was conducted to find out the efficiency of beta cyclodextrin (βCD) for the removal of cholesterol from buffalo milk. Standardised (3.5% fat) and homogenised buffalo milk was treated with βCD at three different concentrations i.e., 0.5, 1 and 1.5% (T_1, T_2 and T_3) and compared with a control without βCD treatment. Treatment of milk with βCD at all concentrations did not show any negative impact on pH and acidity of milk. 90% cholesterol was removed when buffalo milk was treated with 1.5% βCD. Treatment of milk with βCD did not reveal significant effect on fatty acid and triglyceride composition of milk as well as physicochemical and sensory characteristics ($P>0.05$). These results depicted that βCD can be used efficiently for the removal of cholesterol from buffalo milk.

Keywords: beta cyclodextrin, cholesterol, fatty acid profile, triglyceride profile, buffalo milk

Introduction

Dietary cholesterol has been implicated in the development of cardiovascular diseases (Hansel *et al*., 2007). Milk contains about 0.25-0.35 g/100 g cholesterol (McSweeney and Fox, 2003). Milk with lower concentration of dietary cholesterol is beneficial for hypercholesterolemic and hypertensive individuals. Good quality yoghurt, ice cream and cheese can be made from low cholesterol milk (Nadeem *et al*., 2015; Lee *et al*., 2007). South Asia has five groups of buffalo breeds (Murrah, Gujrati, Uttar Pradesh, Central Indian and South Indian). Nili- Ravi is regarded as the best performing animal of this group, in terms of milk yield per lactation. Many techniques have been developed for the removal of cholesterol from milk and milk products. The application of beta cyclodextrin (βCD) in milk and milk products is superior to other techniques of cholesterol removal in terms of efficiency and safety of treated stuffs (Lee *et al*., 2007). The insolubility of βCD in milk serum offers a unique benefit of cholesterol removal with negligible amount of residues in the treated stuffs. Scientific studies have evidenced that residues of βCD do not have harmful effects in human body and intestinal micro flora can fully decompose residues of βCD (Loftsson and Brewster, 1996). Removal of cholesterol from cow milk and milk products has been studied in detail by Alonso *et al*. (2009) and Kwak *et al*. (2002). Several studies have revealed that concentration of beneficial unsaturated fatty acids in milk can be enhanced by manipulating the ration of dairy animals. Studies of Brzoska and Sala (2001) have suggested that feeding strategies do not have any significant influence on the concentration of cholesterol in milk. Chemical composition of buffalo is significantly different from cow milk in terms of major and minor constituents (Fox and Mc Sweeney, 1998).

The removal of cholesterol from buffalo milk by βCD has not been studied so far. Therefore, it is important to study the suitability of βCD in the removal of cholesterol from buffalo milk, which is the second largest source of milk on the globe. This study aimed on the removal of cholesterol from buffalo milk by βCD and studies the effect of βCD on chemical and sensory characteristics of treated milk.

Materials and Methods

Raw materials. Buffalo milk was obtained from Livestock Production Research Institute, Bahadar Nagar, Okara, Pakistan. Food grade βCD was purchased from Beta Parma Shanghai, China. The reagents used in this study were GC-grade and purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA).

Method. Standardised (3.5% fat) and homogenised milk (200-bar) was treated with βCD at three different concentrations i.e., 0.5, 1 and 1.5% w/w (T_1, T_2 and T_3) and compared with a control without βCD treatment. Milk was heated to 65 °C, standardised at 3.5% fat, homogenised in a sanitised two stage homogenizer (APV) at 200 and 50 bar pressure in the first and second

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stage, respectively, cooled down to 10 °C, treated with βCD at 0.5, 1 and 1.5% concentrations. Milk was stirred at 800 rpm in a blender (Misung Company, Korea) for twenty minutes then centrifuged at 72 ×g for ten minutes (HMR 220IV, Hanil Industrial Company, Korea). βCD treated milk was pasteurised at 65 °C for 30 min, immediately cooled down, filled into sanitized PET bottles (250 mL) and stored at 4 °C for further analysis.

Analysis. Fat from milk was separated by the standard method (AOAC, 1997) and stored at 60 °C in the biomedical freezer till further analysis (Sanyo). Effect of three various concentrations of βCD on milk composition was determined by using Lactoscope Julie, Z-7 Slovakia. Cholesterol was determined using spectrophotometric method of Rudel and Morris (1973). Percentage of cholesterol was measured by dividing the residual concentration with total concentration, followed by multiplication with 100. 200 µL of the milk fat sample (dried) was taken in a glass test tube of 15 mL capacity, dissolved in 3 mL iso octane and 2 mL 0.5 N sodium methoxide was added in it. Test tube was capped and vortex for 3 min, given the rest of 5 min to separate the layers. Supernatant was injected into GC through GC syringe (Paquot, 1979). Triglycerides measurement was performed on Agilent GC 6890 equipped with FID and WCOT fused silica capillary column which was 25 m× 0.25 mm, coated with OV-17TRI (J.W. Scientific, Folsom, Canada) (Alonso, 1993). Milk fat was characterised for iodine value, refractive index, unsaponifiable matter, free fatty acids, peroxide value and anisidine value according to the standards methods (AOCS, 1990). Colour of milk fat was measured on Lovibond Tintometer (Tintometer Corporation Salisbury, England). Sensory evaluation of low cholesterol milk was performed by a trained panel, comprising of 10 judges. Judges were asked to rate the product according to 9 point scale (1 the worst; 9 the best) as suggested by Larmond (1987).

Statistical analysis. Experiment was organised in a completely randomised design, each treatment was replicated three times and expressed as Mean ± SD, analysed by one way analysis of variance technique, Duncan Multiple Range Test was applied to investigate the significant difference ($P \leq 0.05$) among the treatments (Steel *et al*., 1997).

Results and Discussion

Removal of cholesterol from milk. About 90% cholesterol was removed when milk was treated with 1.5% βCD (Table 1). The considerable removal of cholesterol from milk by βCD can be attributed to the chemical composition of βCD molecule, which is comprised of seven glucose units linked in the form of a bottom less bucket; the size of the cavity is exactly similar to the size of cholesterol molecule. Studies of Alonso *et al*. (2009) reported that 95% cholesterol was removed, when cow milk was treated with 0.3% βCD at 4 °C for 20 min (Alonso *et al*., 2009). Connors (1997) also reported that chemical structure of βCD can help to remove most of the cholesterol from milk and milk products. Entrapment of cholesterol in the βCD ring is the combined effect of Van der Waal forces, hydrogen bonding and lipophilic characteristics of the cavity (Martin, 1993). Studies of Oh *et al*. (1998) depicted milk treatment with saponin and digitonin at various concentrations and reported that removal of cholesterol from milk was not dependent upon the concentration of cholesterol removing agent. Extent of cholesterol removal from milk was dependent upon the dose of βCD (Lee *et al*., 1999).

Table 1. Effect of βCD on the removal of cholesterol (mg/100 g)

Treatments	Before βCD treatment	After βCD treatment	% Removal
Control	0.28±0.02^{a}	----	----
T_1	0.28±0.02^{a}	0.21±0.01^{a}	25.0
T_2	0.28±0.02^{a}	0.13±0.02^{b}	54.5
T_3	0.28±0.02^{a}	0.03±0.01^{c}	89.3

Within a column means denoted by a different letter are statistically different ($P<0.05$).

Effect of βCD on fatty acid and triglyceride composition. Treatment of milk with βCD at the mentioned concentrations did not show any significant effect ($P>0.05$) on fatty acid and triglyceride profile of the milk (Table 2). Milk fat is unique among all the dietary fats for having appreciable amount of short chain fatty acids, which are responsible for typical flavour of milk and milk products. Removal of cholesterol from milk by βCD does not have any effect on the concentration of short-chain fatty acids. Sensory characteristics of βCD treated bulk pasteurized milk were not different from untreated milk (Alonso *et al*., 2009). Sensory characteristics of ice cream formulated from βCD treated milk was not different from control (Nadeem *et al*., 2015; Bazmi and Relkin, 2009; Ha *et al*., 2009). The non-variation in the sensory characteristics of ice creams

could be correlated to the non-significant effect of βCD on fatty acid and triglyceride profile of treated milk (Table 3). The cholesterol content of some Pakistani buffalo breeds ranged from 8.89-10.24 mg/dl (Talpur *et al*., 2007). Triglyceride composition of milk fat treated with 0.6% βCD was not different from untreated milk (Alonso *et al*., 2009). Other strategies of cholesterol removal has a pronounced effect on triglyceride composition, removal of cholesterol from milk through supercritical fluid extraction significantly affected the triglyceride composition (Chen *et al*., 2003; Gonzalez-Hierro *et al*., 1995; Bhaskar *et al*., 1993).

Table 2. Effect of βCD on fatty acid profile of buffalo milk

Fatty acid	Control	Treatments		
		T_1	T_2	T_3
C4:0	4.17±0.03	4.15±0.08	4.14±0.03	4.12±0.06
C6:0	2.41±0.05	2.39±0.10	2.38±0.02	2.35±0.02
C8:0	2.15±0.07	2.14±0.05	2.12±0.08	2.11±0.05
C10:0	1.83±0.13	1.80±0.07	1.79±0.01	1.78±0.08
C12:0	2.65±0.02	2.62±0.04	2.60±0.04	2.58±0.07
C14:0	11.75±0.25	11.71±0.29	11.68±0.12	11.58±0.18
C16:0	32.27±0.36	32.16±0.45	32.11±0.29	32.09±0.14
C18:0	11.18±0.19	11.12±0.11	11.06±0.15	11.05±0.19
C18:1	24.06±0.43	24.02±0.16	24.01±0.36	23.97±0.11
C18:2	2.34±0.01	2.33±0.02	2.31±0.02	2.29±0.01

All means values of control and experimental samples for fatty acid composition mentioned in Table 1 are non-significant (P>0.05); T_1: 0.5% βCD; T_2: 1% βCD; T_3: 1.5% βCD.

Table 3. Effect of βCD on triglyceride profile of buffalo milk

Triglyceride	Control	Treatments		
		T_1	T_2	T_3
C26	0.45±0.04	0.47±0.01	0.48±0.01	0.51±0.02
C28	1.38±0.02	1.35±0.12	1.33±0.01	1.31±0.01
C30	2.25±0.11	2.19±0.08	2.14±0.05	2.11±0.08
C32	4.19±0.09	4.15±0.17	4.08±0.014	4.01±0.12
C34	6.41±0.13	6.39±0.22	6.38±0.16	6.35±0.04
C36	11.42±0.22	11.48±0.05	11.58±0.06	11.63±0.03
C38	14.02±0.24	14.09±0.15	14.15±0.11	14.17±0.07
C40	11.67±0.06	11.72±0.04	11.75±0.13	11.79±0.12
C42	6.82±0.14	6.85±0.07	6.87±0.08	6.91±0.19
C44	5.48±0.07	5.51±0.06	5.54±0.04	5.56±0.09
C46	6.42±0.02	5.35±0.04	6.31±0.09	6.29±0.08
C48	7.38±0.01	7.28±0.09	7.22±0.15	7.16±0.09
C50	9.65±0.10	9.62±0.08	9.61±0.19	9.58±0.05
C52	8.83±0.03	8.81±0.14	8.80±0.22	8.79±0.02
C54	3.44±0.08	3.45±0.02	3.47±0.07	3.48±0.01

All means values of control and experimental sample for fatty acid and triglyceride composition mentioned in Table 2 are non-significant (P>0.05).

Effect of βCD on milk composition. The results regarding chemical composition of milk treated with various treatments of βCD are given in Table 4. The compositional attributes, pH and acidity of the treatments were not affected by βCD treatment. The non-significant effect on pH and acidity may be attributed to the neutral nature of βCD. The non-variation in fat, protein lactose and ash content of treated milk and control could be due to the selective absorption, specific size of the internal cavity of βCD. Treatment of milk with βCD did not have a remarkable effect on the removal of major and minor nutrients of milk, although some water soluble vitamins, short-chain fatty acids, fat, protein and lactose were adsorbed on βCD (Ha *et al*., 2009). Treatment of milk with cross-linked βCD did not have a significant effect on the removal of oleic acid, CLA and phospholipids in βCD treated milk (Alonso *et al*., 2009). Lee *et al*. (2007) did not observe significant difference in chemical composition of βCD treated and untreated milk.

Physicochemical properties of fat. The effect of βCD on the chemical characteristics of milk fat has been stated in Table 5, that shows that melting point, iodine value and refractive index of all the treatments were non-significantly affected by different concentrations of βCD. Melting point of the fats depends upon fatty

Table 4. Effect of various concentrations of βCD on composition of buffalo milk

Parameter	Control	Treatments		
		T_1	T_2	T_3
Fat%	3.51±0.08	3.49±0.04	3.48±0.02	3.45±0.15
Protein%	3.22±0.11	3.22±0.05	3.21±0.11	3.21±0.04
Lactose%	4.78±0.05	4.78±0.07	4.76±0.21	4.79±0.10
Ash%	0.81±0.03	0.82±0.04	0.82±0.01	0.81±0.01
SNF%	8.92±0.22	8.95±0.11	8.99±0.17	9.04±0.15
TS%	12.58±0.07	12.62±0.06	12.72±0.13	12.80±0.0
pH	6.75±0.08	6.73±0.05	6.69±0.01	6.70±0.03
Acidity%	0.15±0.01	0.16±0.02	0.16±0.03	0.17±0.01

All the parameters of milk composition mentioned in Table 4 are non-significantly (P>0.05) different from each other; SNF = solids not fat; TS = total solids.

Table 5. Effect of βCD on physicochemical characteristics of buffalo milk fat

Treatment	MP	IV	FFA	SV	USM	AV	PV	RI
Control	34.8±0.10	39.15±0.24	0.08±0.01	118.49±0.49	0.68±0.01a	6.16±0.09	0.25±0.03	1.452±0.01d
T_1	34.6±0.20	39.48±0.34	0.08±0.02	117.62±0.64	0.62±0.03a	6.05±0.12	0.22±0.01	1.455±0.01c
T_2	34.5±0.1	39.66±0.22	0.09±0.02	117.78±0.85	0.52±0.01b	5.91±0.05	0.21±0.02	1.458±0.02b
T_3	34.4±0.10	39.88±0.19	0.08±0.01	117.92±0.55	0.41±0.01c	5.72±0.09	0.21±0.01	1.459±0.01a

The mean value of all the parameters given in Table 3 are statistically non-significant (P>0.05); MP = melting point (°C); IV = iodine value (wijs); FFA = free fatty acids%; SV = saponification value; USM = unsaponifiable matter%; AV = anisidine value; PV = peroxide value (meqO_2/kg); RI = refractive index@ 40 °C.

acid composition and solid fat content (Kaylegian and Linsday, 1995). Cholesterol belongs to the unsaponifiable fraction of the lipids of animal origin and possesses high melting point 149.5 °C. Iodine value and refractive index of all the treatment was not different from the control (P>0.05). Free fatty acids are related to the keeping quality of the fats, higher values are associated with lower keeping quality (Fereidoon, 2005). Anisidine value determines the secondary and tertiary stages of autoxidation (Erickson, 1995). Peroxide value measures the primary stages of oxidative breakdown caused by the free radical mechanism. Treatment of buffalo milk with βCD did not reveal any problem of fat oxidation.

Sensory evaluation. Treatment of buffalo milk with βCD did not have any negative effect on sensory characteristics of low cholesterol milk (Table 6). A panel of ten trained judges was unable to distinguish low cholesterol milk from normal milk for colour, flavour and aroma. Sensory characteristics of low cholesterol cheddar cheese were not different from standard cheese (Kwak *et al*., 2002; 1999). Sensory characteristics of dairy products derived from low cholesterol milk was similar to the standard dairy products (Alonso *et al*., 2009), butter (Kim *et al*., 2006) and cream (Han *et al*., 2007; Ortega *et al*., 2006).

Table 6. Effect of various concentrations of βCD on sensory characteristics of buffalo milk

Treatment	Colour	Taste	Smell	Overall acceptability
Control	8.5±0.21	8.4±0.05	8.2±0.05	8.1±0.09
T_1	8.3±0.06	8.2±0.15	8.1±0.23	8.1±0.08
T_2	8.2±0.05	8.0±0.04	8.1±0.18	8.0±0.11
T_3	8.2±0.10	8.1±0.08	7.9±0.02	7.9±0.12

All the parameters of sensory evaluation mentioned in Table 5 are no significantly (P>0.05) different from each other.

Conclusion

Ninty percent cholesterol was removed when milk was treated with 1.5% βCD. Treatment of buffalo milk with various concentrations of βCD (0.5-1.5%) did not have any significant effect on fatty acid composition, triglyceride profile, milk composition and physicochemical characteristics of milk fat. Sensory characteristics of low cholesterol milk were almost similar to the control. The overall acceptability score of T_3 was 7.9 out 9 (total score) which was more than 87% of the total score. Cholesterol from buffalo milk can be efficiently removed by βCD.

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