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Anamika Das
PhD Scholar, Dairy Chemistry Division, National Dairy Research Institute, Karnal, Haryana, India

Raman Seth
Principal Scientist, Dairy Chemistry Division, National Dairy Research Institute, Karnal, Haryana, India

Correspondence
Anamika Das
PhD Scholar, Dairy Chemistry Division, National Dairy Research Institute, Karnal, Haryana, India

Chemical compositional analysis and physical attributes of curd fortified with bovine colostrum whey powder

Anamika Das and Raman Seth

Abstract
The present study aimed at evaluating the physico-chemical attributes of curd manufactureded by supplementation of bovine colostrum whey powder. The addition of colostrum whey powder to curd is expected to enhance the nutritional quality of curd. Curd is an inevitable part of Indian diet and therefore it can act as a vehicle to deliver the immune and growth factors of colostrum. Bovine colostrum whey powder @2% was added to curd which resulted in a significant changes in water-holding capacity, syneresis, viscosity, firmness, work of shear, work of adhesion and stickiness. As compared to control curd samples, colostrum whey powder supplemented curd had increased amount of protein, ash, Immunoglobulin G and A(IgG and IgA), Transforming growth factor I and II(TGFβ I and II), Insulin like growth factor I and II(IGF I and II).

Keywords: Colostrum whey powder, curd, growth factors, immune factors, immunomodulatory activity

1. Introduction
Curd is a traditional fermented milk product of Indian origin and is consumed by people all over India on daily basis. Bovine colostrum is the first lacteal secretion immediately after parturition which is rich in immune factors like immunoglobulins, lactoferrin, lysozyme, lactoperoxidase, proline rich polypeptides and growth factors like insulin like growth factor I and II, epidermal growth factor, transforming growth factor β I and II, growth hormone. Clinical investigations have revealed that colostrum is taken as a supplement by human beings, it shows antimicrobial, antioxidative, immunomodulatory, anti-inflammatory activity. Colostrum whey powder fortified curd will help in delivering the bioactive components of colostrum whey powder to consumers on regular basis. The major drawback of using colostrum is its lower heat stability which hinders its utilization in processing of different products. Although different processing techniques like high pressure processing can be used in preserving the bioactivity of immune factors and growth factors but this type of processing increases the cost of production. Curd is an excellent source of B vitamins and calcium whose bioavailability is more in the body as compared to milk (Bhuiyan *et al*., 2010) [2]. Curd exhibits therapeutic properties in gastrointestinal disorders like constipation, diarrhea, dysentery (Gandhi and Nambudripad, 1975) [6] and is helpful in lowering blood cholesterol (Mann and Spoerry, 1974) [12]. In the present study, curd has been chosen as a vehicle to deliver the immune factors and growth factors of colostrum and hence efforts have been made to prepare bovine colostrum whey powder fortified curd and thereafter physico-chemical, microbiological attributes have been evaluated.

2. Materials and methods
2.1 Organoleptic analysis
The curd samples were subjected to sensory evaluation using 9-point Hedonic scale according to Amerine *et al*.,(1965) [10] The samples were presented to five trained panellists for evaluation of organoleptic quality such as flavor, colour and appearance, body and texture and overall acceptability.

2.2 Procurement of raw materials
Bovine colostrum upto 36h was collected from National Dairy Research Institute (NDRI) cattleyard, Karnal.

Bovine colostrum whey powder was prepared by freeze drying method according to the procedure of Das and Seth, 2011 [5]. Skim milk was collected from experimental dairy of the institute. NCDC-167 culture (Mixed mesophilic starter culture, comprising of *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *diacetylactis, Lactococcus lactis* subsp. *cremoris* and *Leuconostoc citrovorum*) was procured from National Collection of Dairy Culture, NDRI, Karnal.

2.3 Preparation of Curd samples

Control curd was standardized to 14% total solids. The milk was heated at 90ºC/10min. and then cooled to 28ºC. Culture was inoculated @2% and incubation was carried out at 28ºC for 12-13h. During fermentation, when titratable acidity reached 0.75%lactic acid, the curd samples were transferred to refrigerated storage at 6-8ºC. Colostrum whey powder fortified curd samples were prepared with certain modifications. Colostrum whey powder was added @ 2% and 4% to skim milk and the total solids, fat was standardized to 14% and 4%respectively with the help of cream and skim milk powder. During the preliminary studies it was found that at temperature above 68ºC, there was coagulation of curd mix containing 4% colostrum whey powder. Therefore, curd was prepared using 2% colostrum whey powder and the temperature of heat treatment for curd mix was fixed at 68ºC/30min. Cooling was done at 28ºC for inoculating the starter culture @3% and incubation was done at 28ºC for 20-21h. Set curd was stored at 6-8ºC.

2.4 Physico-Chemical, rheological and microbiological characteristics of curd samples

Curd were evaluated for pH using a digital pH meter, water holding capacity was analysed as per Parnell *et al*., 1986, syneresis was evaluated according to the procedure of Chawla and Balachandran, 1994 [3] viscosity (measured by Contraves Rheomat RM 108ER). The color of curd samples was observed using a Colorflex colorimeter supplied by Hunterlab (Hunter Associates Laboratory, Inc., Reston, VA, USA). Textural parameters like firmness, work of adhesion, work of shear and stickiness were determined by using a texture analyzer TA-XT2i (M/s Stable Microsystems, UK). Titratable acidity, total solids, fat, protein, lactose and ash content of curd samples were analysed by using standard procedure as laid down by SP:18, part XI, 1981 [16]. Analysis of IgG, IgA, IGF I, IGF II, TGFβ I, TGFβ II was done by ELISA (as per the procedure given in the kit supplied by Uscn Life science Inc., Wuhan). Lactic acid bacteria, Yeast and mold count, coliform count, *Staphylococcus aureus* count, Salmonella count, were enumerated using M17 agar, potato dextrose agar, violet red bile agar, Baird Parker agar, Salmonella-Shigella agar respectively(Hi media Pvt Ltd., Mumbai, India).

2.5 Storage study

Colostrum whey powder supplemented curd samples were evaluated during a storage period of 12 days at a temperature of 7-8ºC. Physico-chemical, rheological and microbiological characteristics were analysed at an interval of three days.

2.6 Statistical Analysis

All statistical analyses were performed using SYSTAT (6.0.1Version) software and MS Excel. Results are presented in means ± standard error (SE) and statistical significance was set at $P<0.05$ and was determined using Post Hoc Test (Bonferroni adjustment).

3. Results

3.1 Effect of addition of colostrum whey powder on Sensory scores

Colostrum whey powder fortified curd was subjected to sensory evaluation based on 9-point hedonic scale by a panel of trained judges. The organoleptic scores of control curd sample and colostrum whey powder supplemented curd samples are presented in Table 1. Except flavour, all other parameters of colostrum whey powder fortified curd viz., colour and appearance, body and texture and overall acceptability had significantly increased score than control curd. Colostrum whey powder fortification resulted in increased whey protein content which might have improved the body, appearance of curd due to interaction of whey proteins with casein micelles as reported by Herrero and Requena, 2005 [8].

3.2 Effect of addition of colostrum whey powder on physico-chemical, rheological and microbial quality of curd

The physic-chemical, rheological and microbiological characteristics of colostrum whey powder supplemented curd was carried out and is presented in Table 2 and 3. As compared to control curd samples, colostrum whey powder fortified curd samples had lower syneresis, increased water holding capacity, higher a* and b* values, significantly increased firmness, work of shear, work of adhesion and stickiness values. Similar observations were reported by Mangino, 1984 [11], who reported that decrease syneresis might be related to increase in protein concentration which increases water holding due to the formation of protein matrix.The firmness of yoghurt-a dahi like product is dependent on total solids level, on the protein content and on the type of protein (Gastaldi *et al*., 1997, Penna *et al*., 1997, Kristo *et al*., 2003, Trachoo *et al*., 1998, Abu Jdayil, 2003; Cho *et al*., 1999) [7, 14, 9, 17, 1, 4].

Rattray and Jelen, 1997 [15] reported that α-lactalbumin and β-lactoglobulin plays a major role as a gelatininzing agent because of the presence of free sulphhydryl groups. Increased whey proteins in colostrum might have improved the rheological characteristics of colostrum fortified curd as these parameters are associated with the forces involved in internal bonds of the product. Similar results were observed by Mangino *et al*., 1984 [11], where whey retentate was added during yogurt manufacturing.

The chemical composition analysis revealed that the colostrum whey powder supplemented curd had significantly higher values for protein, ash, IgG, IgA, IGF I, IGF II, TGF β I, TGF β II as compared to control curd but had significantly lower values for lactose content. This might be due to the addition of colostrum whey powder which is rich in immune factors and growth factors but has lower amount of lactose. Lactic acid bacteria count was significantly decreased in case of colostrum whey powder fortified curd which may be due to the fact that the immune factors might have slowed down the growth of lactic acid bacteria. Yeast and mold count, coliform count, *Staphylococcus aureus* count, Salmonella count were found to be absent in control as well as in colostrum whey powder fortified curd samples.

3.3 Effect of addition of colostrum whey powder on Sensory scores, physic-chemical, microbial parameters during storage

Organoleptic scores during storage period are presented in Table 4. Sensory scores were significantly higher on 4th and

8th day of storage in comparison to 1st day. Organoleptic scores decreased significantly from 12th day of storage. Changes in physical properties of colostrum whey powder fortified curd is presented in table 5. It was observed that throughout the storage period, pH decreased and acidity increased significantly. Syneresis decreased significantly till 8th day and then increased on 12th day. Viscosity and water holding capacity increased significantly till 8th day. There was no significant change in l*, a*, b* values till 12th day. Firmness, work of shear, work of adhesion and stickiness did not change significantly upto 8th day of storage. Table 8 reveals the changes in chemical composition of colostrum whey powder fortified curd. All the constituents i.e. total solid, fat, protein, ash, IgG, IgA, IGF I, IGF II, TGF β I, TGF β II did not show any significant decrease till 8th day of storage whereas lactose decreased significantly throughout the storage period. Table 7 reveals that throughout the storage period of 12 days, yeast and mold count, coliform count, *Staphylococcus aureus* count, Salmonella count were found to be absent in colostrum whey powder fortified curd samples. Till 4th day, lactic acid bacteria count increased significantly and thereafter till 12th day, it showed a decreasing trend.

Table 1: Sensory scores for colostrum whey powder fortified curd prepared using NCDC167 culture

Sensory parameters	Control	Colostrum whey powder fortified curd
Flavour	$8.19^{a}\pm0.94$	$8.17^{a}\pm0.74$
Colour and appearance	$8.0^{a}\pm0.71$	$8.19^{b}\pm0.67$
Body and texture	$7.23^{a}\pm0.69$	$7.85^{b}\pm0.55$
Overall acceptability	$8.13^{a}\pm0.66$	$8.49^{b}\pm0.23$

Mean± SE, Values followed by different alphabets (a-b) coloumnwise differ significantly ($P<0.05$)

Table 2: Physico-chemical analysis of control curd and Colostrum whey powder fortified curd

Parameters	Control curd	Colostrum whey powder fortified curd
pH	$4.62^{a}\pm0.63$	$4.63^{a}\pm0.61$
Titratable Acidity (expressed as % lactic acid)	$0.77^{a}\pm0.48$	$0.78^{a}\pm0.59$
Syneresis (ml/100gm)	$28.90^{a}\pm0.31$	$28.69^{b}\pm0.61$
Viscosity(Pa.s)	$1.21^{a}\pm0.47$	$1.26^{b}\pm0.58$
Water holding capacity (gm/100gm)	$38.1^{a}\pm0.43$	$40.19^{b}\pm0.26$
Color		
L*	$83.73^{a}\pm0.44$	$83.62^{a}\pm0.36$
a*	$-0.30^{a}\pm0.32$	$-4.21^{b}\pm0.48$
b*	$32.17^{a}\pm0.24$	$32.85^{b}\pm0.34$
Firmness(N)	$1.24^{a}\pm0.87$	$1.90^{b}\pm0.49$
Work of Shear(N.s)	$-2.13^{a}\pm0.57$	$32.79^{b}\pm0.69$
Work of Adhesion(N.s)	$30.71^{a}\pm0.26$	$-2.58^{b}\pm0.32$
Stickiness(N)	$-0.307^{a}\pm0.23$	$-0.326^{b}\pm0.39$
TS%	$14.12^{a}\pm0.84$	$14.10^{a}\pm0.73$
Fat %	$4.3^{a}\pm0.21$	$4.28^{a}\pm0.88$
Protein%	$3.75^{a}\pm0.77$	$4.83^{b}\pm0.42$
Lactose%	$5.2^{a}\pm0.51$	$4.1^{b}\pm0.69$
Ash%	$0.81^{a}\pm0.57$	$0.88^{b}\pm0.59$
IgG(g/100gm)	$0.09^{a}\pm0.91$	$0.57^{b}\pm0.88$
IgA(g/100gm)	$0.03^{a}\pm0.67$	$0.04^{a}\pm0.96$
IGF I(µg/100g)	$1.21^{a}\pm0.98$	$2.11^{b}\pm0.75$
IGF II(µg/100g)	$0.74^{a}\pm1.26$	$1.11^{b}\pm0.83$
TGF β1(µg/100g)	$0.19^{a}\pm0.78$	$0.20^{b}\pm0.73$
TGF β2(µg/100g)	$0.89^{a}\pm0.67$	$1.21^{b}\pm0.94$

mean±SE, Values followed by different alphabets (a-b) coloumn wise differ significantly ($P<0.05$)

Table 3: Microbial analysis of control curd and Colostrum whey powder fortified curd

Type of count	Control	Colostrum whey powder fortified curd
Lactic acid bacteria count (log cfu/gm)	$7.36^{a}\pm1.47$	$6.98^{b}\pm1.32$
Yeast and mold count	Nil	Nil
Coliform count	Nil	Nil
S.aureus count	Nil	Nil
Salmonella count	Nil	Nil

n=6, mean± SE, Values in same row with similar superscript letter (a-b) do not differ significantly ($P>0.05$) and vice versa.

Table 4: Sensory scores of Colostrum whey powder fortified curd during 12days of storage period

	Days of storage			
Parameter	**1st day**	**4th day**	**8th day**	**12th day**
Flavor	$7.19^{a}\pm0.79$	$7.78^{b}\pm0.12$	$8.11^{c}\pm0.23$	$7.09^{d}\pm0.44$
Color and appearance	$7.83^{a}\pm0.61$	$8.11^{b}\pm0.14$	$8.32^{c}\pm0.29$	$7.21^{d}\pm0.14$
Body and texture	$7.55^{a}\pm0.51$	$8.36^{b}\pm0.18$	$8.23^{c}\pm0.24$	$7.33^{d}\pm0.34$
Overall acceptability	$7.89^{a}\pm0.21$	$8.11^{b}\pm0.23$	$8.25^{c}\pm0.32$	$6.88^{d}\pm0.21$

n=6, mean± SE, Values in same row with similar superscript letter (a-d) do not differ significantly ($P>0.05$) and vice versa

Table 5: Evaluation of Physical properties of Colostrum whey powder fortified curd during 12days of storage period

Parameters	1st day	4thday	8th day	12th day
pH	4.69a±0.67	4.60b±0.45	4.53b±0.51	4.47c±0.43
Titratable Acidity (% lactic acid)	0.75a±0.53	0.78b±0.46	0.83c±0.51	0.88d±0.31
Syneresis (ml/100gm)	31.80a±0.67	30.82b±0.41	30.62b±0.39	32.31c±0.48
Viscosity (Pa.s)	1.25a±0.53	1.39b±0.53	1.38b±0.21	1.23a±0.39
Water holding capacity (gm/100gm)	40.1a±0.21	40.86b±0.48	40.95b±0.31	40.10a±0.39
Color				
L*	81.62a±0.33	81.50a±0.41	81.48a±0.49	81.49a±0.39
a*	-4.21a±0.44	-4.18a±0.29	-4.19a±0.64	-4.20a±0.53
b*	32.83a±0.31	32.82a±0.32	32.89a±0.41	32.87a±0.48
Firmness(N)	1.91a±0.42	1.92a±0.53	1.91a±0.39	1.86b±0.43
Work of Shear(N.s)	-2.54a ±0.64	-2.55a±0.51	-2.57a±0.43	-2.47b±0.38
Work of Adhesion(N.s)	32.78a-±0.32	32.76a±0.38	32.77a±0.41	32.68b±0.31
Stickiness(N)	-0.324a±0.31	-0.326a±0.41	-0.325a±0.34	-0.318b±0.47

n=6, mean± SE, Values in same row with similar superscript letter (a-d) do not significantly differ (p>0.05) and vice versa

Table 6: Evaluation of proximate chemical composition of Colostrum whey powder fortified curd during 12days of storage period

Parameters	1st day	4thday	8th day	12th day
TS%	14.11a±0.79	14.13a±0.41	14.10a±0.52	14.03b±0.63
Fat %	4.12a±0.83	4.10a±0.31	4.11a±0.36	4.10a±0.41
Protein%	4.88a±0.49	4.89a±0.42	4.87a±0.49	4.81b±0.51
Lactose%	4.01a±0.63	3.93b±0.72	3.90c±0.51	3.82d±0.43
Ash%	0.89a±0.55	0.90a±0.41	0.89a±0.32	0.90a±0.43
IgG(g/100gm)	0.51a±0.85	0.50a±0.71	0.52a±0.54	0.51a±0.68
IgA(g/100gm)	0.04a±0.94	0.04a±0.68	0.04a±0.52	0.03b±0.71
IGF I(µg/100g)	2.14a±0.73	2.11a±0.71	2.12a±0.63	2.0b±0.54
IGFII(µg/100g)	1.15a±0.81	1.15a±0.72	1.11a±0.68	1.05b±0.53
TGF β1(µg/100g)	0.20a±0.71	0.20a±0.33	0.21a±0.42	0.13b±0.38
TGF β2(µg/100g)	1.21a±0.92	1.22a±0.41	1.22a±0.53	1.11b±0.61

n=6, mean± SE, Values in same row with similar superscript letter (a-d) do not significantly differ (P>0.05) and vice versa

Table 7: Effect of 12 days of storage period on microbial growth of colostrum whey powder fortified curd

Type of count	1st day	4thday	8th day	12th day
Lactic acid bacteria count (log cfu/gm)	6.98a ±1.21	7.07b±0.91	7.02c±1.08	6.94a±0.85
Yeast and mold count	Nil	nil	nil	nil
Coliform count	Nil	nil	nil	nil
S. aureus count	Nil	nil	nil	nil
Salmonella count	Nil	nil	nil	nil

n=6, mean±SE, Values in same row with similar superscript letter (a-c) do not significantly differ (P>0.05) and vice versa

4. Conclusion

Although colostrum whey powder contains antimicrobial compounds which may prevent the growth of starter culture in curd, yet curd supplemented with colostrum whey powder at lower level do not significantly affect the physico-chemical properties. Colostrum whey powder supplemented curd can be manufactured using lower level of colostrum whey powder as higher level of colostrum will hinder the development of desirable characteristics of set curd. Colostrum whey powder fortified curd is rich in immunoglobulins and growth factors as compared to control curd and therefore is nutritionally more beneficial than plain curd.

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