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EVALUATION OF PHYSICO-CHEMICAL PROPERTIES OF COLOSTRUM SUPPLEMENTED DAHI

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ABSTRACT

The aim of the present investigation was to evaluate the physico-chemical, sensorial characteristics of dahi prepared by diluting colostrum with skim milk. *In vitro* phagocytic activity and lymphocyte proliferation index was also carried out. The addition of colostrum in dahi, is supposed to improve the nutritional quality of dahi. Bovine colostrum @10% was used to fortify dahi which resulted in a significant change in water-holding capacity, syneresis, viscosity, firmness, work of shear, work of adhesion and stickiness. Colostrum fortified dahi had increased amount of protein, ash, IgG, IgA, Transforming Growth Factor β I (TGF β I), Transforming Growth Factor β II (TGF β II), Insulin like growth factor I and II (IGF I and II) when compared to control. Sensory analysis indicated that the product was acceptable up to one week when stored at 7–8 °C with no significant difference ($P > 0.05$) in the sensory scores and in TGF β I TGF β II, IGF I and IGF II content.

Keywords: Colostrum, dahi, physico-chemical, growth factors, immune factors.

INTRODUCTION

Bovine colostrum is the first mammary secretion immediately after the birth of a calf. It 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 studies have proven that colostrum exerts antimicrobial, antioxidative, immunomodulatory, anti-inflammatory properties in human beings when taken as a supplement. In our day to day life, many undesirable foreign bodies enters the body through Gastrointestinal tract. These antigens activates the body's immune system triggering different pathways which fights in nullifying the effects of these substances. In most of the cases, the immune components produced by the body are insufficient or in capable of fighting with such invasions. This results in the damage of mucosal layer of GI tract or results in atrophied villi. This may lead to increased translocation of pathogens leading to a more complicated situation. To maintain and restore the health of mucosal layer, targeted nutrition is required, particularly growth factors which are helpful in repairing wounds and restoration of atrophied villi. Colostrum has been used from ages as a rich, natural source of immune factors and growth factors. As dahi is a popular fermented dairy product of Indian sub-continent and is known for its refreshing taste, palatability

and therapeutic values, colostrum can be incorporated in dahi so that consumers find an easy way to consume colostrum fractions on daily basis. Dahi resembles leben in Iran, Jugurt in Turkey, Roba in Iraq, Mazun in Armenia, Villi in Finland, Shosim in Nepal (Tamime and Robinson, 2007). In the present investigation, efforts have been made to manufacture colostrum fortified dahi and thereafter physico-chemical, microbiological, *in vitro* immunomodulatory parameters of colostrum supplemented dahi have been evaluated.

MATERIAL AND METHODS

COLLECTION OF RAW MATERIALS

Bovine colostrum upto 36h was collected from National Dairy Research Institute (NDRI) cattle yard. Skim milk was procured 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 NCDC, NDRI, Karnal.

PROCEDURE FOR MANUFACTURING OF DAHI SAMPLES

Control dahi samples were prepared by standardizing milk to 4% fat and 14% total solids and by heating the milk at 90°C/10min. Then the milk was cooled at

28°C, inoculation was done @2% and incubation was carried out at 28°C for 12-13h. After fermentation (titratable acidity reached 0.75% lactic acid), dahi samples were transferred and stored under refrigeration at 6-8°C. Colostrum fortified dahi samples were prepared with certain modifications. Colostrum fortified milk was standardized to 4% fat and 14% total solids and heat treatment was given at 68°C/30min. Cooling was done at 28°C for inoculating the starter culture @3% and incubation was done at 28°C for 20-21h. Set dahi was transferred to storage at 6-8°C when the titratable acidity reached 0.75% lactic acid.

PHYSICO-CHEMICAL, MICROBIOLOGICAL ANALYSIS OF DAHI SAMPLES

Dahi samples were analysed for pH, water holding capacity (Parnell-Cluies *et al.*, 1986), syneresis (Chawla and Balachandran, 1994), and viscosity (measured by Contraves Rheomat RM 108ER). Firmness, work of adhesion, work of shear and stickiness were determined by back extrusion method using a texture analyzer TA-XT2i (M/s Stable Microsystems, UK). The color of dahi samples was measured using a Colorflex colorimeter supplied by Hunterlab (Hunter Associates Laboratory, Inc., Reston, VA, USA). The dahi samples were analysed for titratable acidity, total solids, fat, protein, lactose and ash by using standard procedure as laid down by SP:18, part XI, BIS, 1981. pH was measured by digital pHmeter. 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 Usca Life science Inc., Wuhan). For enumeration of Lactic acid bacteria, Yeast and mold count, coliform count, *Staphylococcus aureus* count, Salmonella count, M17 agar, potato dextrose agar, violet red bile agar, Baird Parker agar, salmonella-shigella agar respectively (Hi media Pvt Ltd., Mumbai, India) were used.

IN VITRO IMMUNOMODULATORY ACTIVITY

In vitro immunomodulatory potential of colostrum supplemented dahi was conducted by taking spleen and peritoneal fluid of swiss albino mice. Phagocytic activity of peritoneal macrophages and proliferation index of splenocytes from spleen was observed in presence of concanavalin A and lipopolysaccharide (Sigma Chemical Company, Louis, USA).

SENSORY ANALYSIS

The samples of dahi were subjected to sensory evaluation using 9-point Hedonic scale. The samples were presented to a panel of five semi trained judges for evaluation of organoleptic quality such as flavor, colour and appearance, body and texture and Overall acceptability.

STORAGE STUDY

Colostrum fortified dahi samples were subjected to a storage period of 12 days. Physico-Chemical,

microbiological analysis were carried out at an interval of three days

STATISTICAL ANALYSIS

All statistical analyses were performed using SYSTAT (version 6.0.1) software and MS Excel (Microsoft, USA). Results are presented in means ± standard error (SE) and statistical significance was set at P<0.05 and was determined using PostHoc Test (Bonferroni adjustment).

RESULT AND DISCUSSION

SENSORY EVALUATION OF COLOSTRUM FORTIFIED DAHI

Colostrum fortified dahi was subjected to sensory evaluation based on 9-point hedonic scale by a semi trained panel of judges. The sensorial scores of control dahi sample and colostrum fortified dahi samples are presented in table 1. Sensory scores of different parameters of colostrum fortified dahi reveals that colour and appearance, body and texture and overall acceptability had significantly higher score than control dahi whereas the score for flavor did not differ significantly with control dahi.

Table No. 1: Sensory scores for colostrum fortified dahi prepared using NCDC167 culture

Sensory parameters	Control	Colostrum fortified dahi
Flavour	8.19 ^a ±0.94	8.17 ^a ±0.79
Colour and appearance	8.0 ^a ±0.71	8.13 ^b ±0.61
Body and texture	7.23 ^a ±0.69	7.95 ^b ±0.51
Overall acceptability	8.13 ^a ±0.66	8.39 ^b ±0.21
n=8, mean± SE, Values followed by different alphabets(a-c) differ significantly (P<0.05)		

PHYSICO-CHEMICAL, MICROBIAL EVALUATION OF COLOSTRUM FORTIFIED DAHI

The physical, chemical and microbiological characteristic of colostrum fortified dahi was carried out and the observations are presented in table 2 and 3. Compared to control dahi samples, colostrum fortified dahi samples had lesser syneresis, more water holding capacity, higher a* and b* values, increased firmness, work of shear, work of adhesion and stickiness values. The proximate composition analysis revealed that the colostrum fortified dahi had significantly higher values for protein, ash, IgG, IgA, IGF I, IGF II, TGF β I, TGF β II as compared to control dahi but had significantly lower values for lactose content. Colostrum addition resulted in increased protein concentration which might have resulted in a firmer body with less syneresis and more water holding capacity. Similar reports were given by Herrero and Requena, 2005, wherein they reported that increased whey protein content might improve the body structure of dahi due to interaction of whey proteins with

casein micelles. Yeast and mold count, coliform count, *Staphylococcus aureus* count, Salmonella counts were found to be absent in control as well as in colostrum fortified dahi samples. Lactic acid bacteria count was significantly lower in

case of colostrum fortified dahi which might be attributed to the fact that the immune factors of colostrum hindered the rapid growth of microorganisms.

Table No. 2: Physico-chemical analysis of control dahi and Colostrum fortified dahi

Parameters	Control dahi	Colostrum fortified dahi
pH	4.62 ^a ±0.63	4.65 ^a ±0.67
Titratable Acidity(%lactic acid)	0.77 ^a ±0.48	0.75 ^a ±0.53
Syneresis(ml/100gm)	28.90 ^a ±0.31	28.78 ^b ±0.67
Viscosity(Pa.s)	1.21 ^a ±0.47	1.25 ^b ±0.53
Water holding capacity (gm/100gm)	38.1 ^a ±0.43	40.1 ^b ±0.21
Color		
L*	83.73 ^a ±0.44	83.62 ^a ±0.33
a*	-0.30 ^a ±0.32	-4.21 ^b ±0.44
b*	32.17 ^a ±0.24	32.83 ^b ±0.31
Firmness(N)	1.24 ^a ±0.87	1.91 ^b ±0.42
Work of Shear(N.s)	-2.13 ^a ±0.57	32.78 ^b ±0.64
Work of Adhesion(N.s)	30.71 ^a ±0.26	-2.54 ^b ±0.32
Stickiness(N)	-0.307 ^a ±0.23	-0.324 ^b ±0.31
TS%	14.12 ^a ±0.84	14.10 ^a ±0.79
Fat %	4.3 ^a ±0.21	4.12 ^a ±0.83
Protein%	3.75 ^a ±0.77	4.88 ^b ±0.49
Lactose%	5.2 ^a ±0.51	4.0 ^b ±0.63
Ash%	0.81 ^a ±0.57	0.89 ^b ±0.55
IgG(g/100gm)	0.09 ^a ±0.91	0.59 ^b ±0.85
IgA(g/100gm)	0.03 ^a ±0.67	0.04 ^a ±0.94
IGF I(µg/100g)	1.21 ^a ±0.98	2.14 ^b ±0.73
IGF II(µg/100g)	0.74 ^a ±1.26	1.15 ^b ±0.81
TGF β1(µg/100g)	0.19 ^a ±0.78	0.20 ^b ±0.71
TGF β2(µg/100g)	0.89 ^a ±0.67	1.21 ^b ±0.92

n=10, mean±SE, Values followed by different alphabets coloumn wise differ significantly(P<0.05)

Table No. 3: Microbial analysis of control dahi and Colostrum fortified dahi

Type of count	Control	Colostrum fortified dahi
Lactic acid bacteria count (log cfu/gm)	7.36 ^a ±1.47	6.95 ^b ±1.28
Yeast and mold count	Nil	Nil
Coliform count	Nil	Nil
<i>S.aureus</i> count	Nil	Nil
Salmonella count	Nil	Nil

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

IN VITRO IMMUNOMODULATORY STUDIES

In vitro phagocytic activity and lymphocyte proliferation activity was assessed in control dahi samples and colostrum fortified dahi samples. Dahi supplemented with colostrum had significantly higher values which may be attributed to the presence of immune factors and growth factors of colostrum. In presence of lipopolysaccharide, lymphocyte proliferation index was observed to be 0.59 for control dahi whereas it was found to be 0.87 for colostrum fortified dahi. Similarly, in presence of Concanavalin A, lymphocyte proliferation index was observed to be 0.55 for control dahi whereas it was found to be 0.93 for colostrum

fortified dahi. Phagocytic activity was found to be 21% for colostrum fortified dahi whereas it was observed to be 16% for control dahi.

STORAGE STUDIES

Sensory scores of storage period are presented in table 4. Sensory scores were significantly higher on 4th and 8th day of storage as compared to 1st day. Scores decreased significantly from 12th day of storage. A change in physical properties of colostrum fortified dahi 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 fortified dahi. All the constituents i.e. total solid, fat, protein, ash, IgG, IgA, IGF I, IGF II, TGF β I, TGF β II except lactose 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, *S.aureus* count, Salmonella count were found to be absent in colostrum fortified dahi samples.

Till 4th day, lactic acid bacteria count increased significantly and thereafter till 12th day it decreased.

CONCLUSION

Dahi exerts therapeutic properties in curing gastrointestinal disorders like constipation, diarrhea, dysentery and also helps in lowering blood cholesterol. Although colostrum contains a plethora of antimicrobial compounds which may hinder the growth of starter culture in dahi, yet dahi fortified with colostrum at lower level do not significantly affect the physico-chemical properties.

Table No. 4: Sensory scores of Colostrum fortified dahi during 12days of storage period

Parameter	Days of storage			
	1 st day	4 th day	8 th day	12 th day
Flavor	7.17 ^a ±0.79	7.79 ^b ±0.12	8.1 ^c ±0.23	7.0 ^d ±0.44
Color and appearance	7.83 ^a ±0.61	8.10 ^b ±0.14	8.30 ^c ±0.29	7.11 ^d ±0.14
Body and texture	7.55 ^a ±0.51	8.38 ^b ±0.18	8.2 ^c ±0.24	7.13 ^d ±0.34
Overall acceptability	7.89 ^a ±0.21	8.1 ^b ±0.23	8.2 ^c ±0.32	6.78 ^d ±0.21

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

Table No. 5: Evaluation of Physical properties of Colostrum fortified dahi during 12days of storage period

Parameters	1 st day	4 th day	8 th day	12 th day
pH	4.65 ^a ±0.67	4.60 ^b ±0.45	4.53 ^b ±0.51	4.48 ^c ±0.43
Titrateable Acidity (%lactic acid)	0.75 ^a ±0.53	0.78 ^b ±0.46	0.83 ^c ±0.51	0.87 ^d ±0.31
Syneresis(ml/100gm)	31.80 ^a ±0.67	30.82 ^b ±0.41	30.62 ^b ±0.39	32.31 ^c ±0.48
Viscosity(Pa.s)	1.25 ^a ±0.53	1.39 ^b ±0.53	1.38 ^b ±0.21	1.26 ^a ±0.39
Water holding capacity (gm/100gm)	40.1 ^a ±0.21	40.86 ^b ±0.48	40.95 ^b ±0.31	40.08 ^a ±0.39
Color				
L*	81.62 ^a ±0.33	81.50 ^a ±0.41	81.48 ^a ±0.49	81.49 ^a ±0.39
a*	-4.21 ^a ±0.44	-4.18 ^a ±0.29	-4.19 ^a ±0.64	-4.20 ^a ±0.53
b*	32.83 ^a ±0.31	32.82 ^a ±0.32	32.89 ^a ±0.41	32.87 ^a ±0.48
Firmness(N)	1.91 ^a ±0.42	1.92 ^a ±0.53	1.91 ^a ±0.39	1.86 ^b ±0.43
Work of Shear(N.s)	-2.54 ^a ±0.64	-2.55 ^a ±0.51	-2.57 ^a ±0.43	-2.47 ^b ±0.38
Work of Adhesion(N.s)	32.78 ^a ±0.32	32.76 ^a ±0.38	32.77 ^a ±0.41	32.68 ^b ±0.31
Stickiness(N)	-0.324 ^a ±0.31	-0.326 ^a ±0.41	-0.325 ^a ±0.34	-0.318 ^b ±0.47

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

Table No. 6: Evaluation of proximate chemical composition of Colostrum fortified dahi during 12days of storage period

Parameters	1 st day	4 th day	8 th day	12 th day
TS%	14.10 ^a ±0.79	14.13 ^a ±0.41	14.10 ^a ±0.52	14.03 ^b ±0.63
Fat %	4.12 ^a ±0.83	4.10 ^a ±0.31	4.11 ^a ±0.36	4.10 ^a ±0.41
Protein%	4.88 ^a ±0.49	4.89 ^a ±0.42	4.87 ^a ±0.49	4.81 ^b ±0.51
Lactose%	4.01 ^a ±0.63	3.95 ^b ±0.72	3.90 ^c ±0.51	3.81 ^d ±0.43
Ash%	0.89 ^a ±0.55	0.90 ^a ±0.41	0.89 ^a ±0.32	0.90 ^a ±0.43
IgG(g/100gm)	0.59 ^a ±0.85	0.58 ^a ±0.71	0.57 ^a ±0.54	0.51 ^a ±0.68
IgA(g/100gm)	0.04 ^a ±0.94	0.04 ^a ±0.68	0.04 ^a ±0.52	0.03 ^b ±0.71
IGF I(μg/100g)	2.14 ^a ±0.73	2.11 ^a ±0.71	2.12 ^a ±0.63	2.0 ^b ±0.54
IGFII(μg/100g)	1.15 ^a ±0.81	1.15 ^a ±0.72	1.11 ^a ±0.68	1.05 ^b ±0.53
TGF β1(μg/100g)	0.20 ^a ±0.71	0.20 ^a ±0.33	0.21 ^a ±0.42	0.13 ^b ±0.38
TGF β2(μg/100g)	1.21 ^a ±0.92	1.22 ^a ±0.41	1.22 ^a ±0.53	1.11 ^b ±0.61

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

Table No. 7: Effect of 12 days of storage period on microbial growth of colostrum fortified dahi

Type of count	1 st day	4 th day	8 th day	12 th day
Lactic acid bacteria count (log cfu/gm)	6.96 ^a	7.09 ^b	7.01 ^c	6.94 ^a
Yeast and mold count	nil	nil	nil	nil
Coliform count	nil	nil	nil	nil
<i>S.aureus</i> count	nil	nil	nil	nil
Salmonella count	nil	nil	nil	nil

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

Colostrum supplemented dahi can be prepared by carrying out certain modifications during processing such as increasing the incubation time, inoculation level, lower heat treatment temperature and using lower level of colostrum. These modifications can be attributed to the addition of colostrum. Colostrum fortified dahi is rich in immunoglobulins and growth factors as compared to control dahi and therefore is nutritionally more beneficial than plain dahi.

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