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The Impact of Lactoferrin Fortification on the Health Benefits and Sensory Properties of Yogurt

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ABSTRACT

The aim of this work was to study the effect of milk supplementation with lactoferrin on the rheological and sensory properties of yogurt and to evaluate the efficiency of lactoferrin-fortified yogurt in the treatment of iron deficiency anemia (IDA), microcytic hypochromic anemia, in children. For this purpose, yogurt was manufactured from milk fortified with commercial lactoferrin and the yogurt sensory characteristics, pH and concentration of lactic acid of supplemented yogurts, were determined throughout the shelf-life period storage (7 days) at 4°C and compared with a control yogurt. Furthermore, the use of lactoferrin-fortified yogurt in treatment of IDA in comparison to commercial lactoferrin alone (lactoferrin dissolved in water) were assessed with 20 children (2–5 years old) suffering from IDA. Children were divided into two groups (A and B), 10 children each. Group A received lactoferrin-fortified yogurt while, group B received commercial lactoferrin dissolved in water. The blood cells indices, Hb, HCT, MCV, TLC, Platelets, Serum iron, Serum ferritin, TIBC and Transferrin were analyzed before and after treatment for each group. The results showed that manufactured fortified yogurt was firm, with acceptable sensory properties and the Hb and red blood cell indices significantly improved in patients received lactoferrin-fortified yogurt even than patients received lactoferrin only. This study concluded that lactoferrin addition had no significant effect on yogurt manufacture and the yogurt produced from milk fortified with lactoferrin had a vital supportive treatment for the management of IDA in children.
Keywords: yogurt; lactoferrin; iron deficiency anemia.

INTRODUCTION

Lactoferrin (LF) considered as an important host defense molecule and has a wide range of physiological functions such as antiviral, antimicrobial, and anticancer activities (Farid et al., 2019). LF is a multifunctional iron-binding glycoprotein, and a member of a transferrin family, thus belonging to those proteins capable of binding and transferring Fe^{3+} ions (Metz-Boutigue *et al.*, 1984). LF is found mainly in bovine milk in concentration ranged from 1.15µg/ml to 485.63µg/ml and has been used in the food industry for more than 20 years and is now a component of certain products in the category of functional foods (Shah, 2001; Franco *et al.*, 2010). The recent worldwide production scale of bovine lactoferrin is assumed to be more than 60 tone/ year (Tomita et al., 2009).

Some researchers suggested that, lactoferrin could play a role in treating iron deficiency anemia (IDA) that constitute a global health issue (Rosa *et al.*, 2017). Iron deficiency (ID) is the most common and widespread nutritional disorder worldwide and is evident in more than 1.5 billion people worldwide especially in developing countries and according to an estimation of World health organization 39% of children aged 0-4 years and 48% of children aged 5-14 years were anemic in those countries (WHO 2001; Grosbois *et al.*, 2005). Children with iron deficiency anemia (IDA) got tired more easily, play less, have poor school achievement and low immunity compared to healthy children, in addition, it may cause negative effects in motor and mental functions, which may be permanent (Lozoff *et al.*, 1998; Lozoff *et al.*, 2000; Edison *et al.*, 2008). Most patients usually respond well to oral iron preparations, but long-term use of oral iron is limited by its side effects that, although not severe, are often worrisome to patients (Gereklioglu *et al.*, 2016).

It was noticed that breastfed infants during the first 6 months of life do not show signs of ID despite low concentration of iron in breast milk, so it has been suggested that the iron-binding protein lactoferrin (LF) facilitates iron absorption as it increase its bioavailability from breast milk (Ke *et al.*, 2015). LF is excreted in colostrum's of human milk in a concentration up to 7g/L and the concentration in mature milk declines around 7-fold throughout lactation to 1g/L (Hennart *et al.*, 1991). Bovine LF is a natural safe product that commonly prescribed as oral preparations to patients with IDA with good efficacy (Rezk *et al.*, 2016).

Fortification of food with iron is technically more difficult than fortification with other nutrients as iron is a prooxidant, which promotes lipid oxidation. Therefore, the ideal compound for food fortification should be one that stable during food processing, supplies high bioavailability of iron and does not affect the sensory properties or nutritional value of the food (El-Kholy *et al.*, 2011).

LF may be the compound of choice for food fortification as it is added as a component of yogurt, milk, dietary supplements, and is also used in cosmetic products and tablet coats for oral treatment of saliva-deficient patients (Takahashi et al., 2007; Rahman et al., 2008; Tomita et al., 2009).

Yogurt is one of the most popular fermented dairy products that have been traditionally consumed for their numerous potential health benefits. It contains probiotics such as Bifidobacterium and *Lactobacillus spp.* that play an important role for prevention of multiple gastrointestinal diseases, increases intestinal motility and providing health benefits to the host by modulating immunological responses (Bouvier *et al.*, 2001) and fortification of yogurt with LF can add more health benefits (Tsukahara *et al.*, 2020).

This study aimed to evaluate the effect of milk fortification with commercial lactoferrin on yogurt manufacture, by determining the rheological and sensorial properties of manufactured yogurt in addition to evaluating the efficiency of lactoferrin-fortified yogurt in the treatment of iron deficiency anemia (IDA) in children in comparison to commercial lactoferrin alone.

MATERIALS AND METHODS

Lactoferrin

Pravotin®, sachets of pure lactoferrin 100 mg each, was purchased from Hygint pharmaceuticals, Alexandria, Egypt.

Yogurt preparation

Raw buffalo's milk used for yogurt manufacturing was purchased from local markets in Egypt. Lyophilized starter culture containing equal mixtures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* sub spp *bulguricus* (YoFlex® Express 2.0 Chr-Hansen, Denmark), that are in regular use in dairy plants, were used.

Raw buffalo's milk was pasteurized at 85 °C for 5 min in a stainless-steel double jacket container before being cooled to inoculation temperature (42°C). After cooling, pure LF was mixed with milk samples at a concentration of 100 mg LF/ 100 ml milk. Then the starter culture was inoculated at a concentration of 1:1000 and the mixtures were poured into Polyethylene yogurt cartons (200 gm capacity) and kept at 42 °C in an incubator. pH and curd formation were recorded after 2.5 and 4.0 h. Then kept at the refrigerator at 4^{o} C. A control made up from buffalo milk samples without addition of LF was made in parallel and the pH and curd formation was recorded after 2.5 and 4.0 h. pH value of yogurt samples was determined electrometrically by using a pH-meter (OAKTON 35611-series, USA) and titratable acidity were measured according to AOAC (1990).

Rheological properties and sensory evaluation of manufactured yoghurt

Coagulation time of each sample was calculated till curd formation and pH dropped to 4.6. Syneresis was measured according to Hassan *et al.* (1996). Briefly, 30 g of the curd was put on a funnel lined with filter paper and cut in a single action by clean ladle. The drained whey was collected in a graduated cylinder for 2 h at 5°C. Syneresis degree was expressed by the amount of the drained whey in ml.

Sensory evaluation, appearance, odor, flavor and consistency were evaluated according to ISO 22935-2:2009/IDF 99-2:2009 criteria. In addition, consumer acceptance testing was done in the form of questions asked to the patient's parents/guardians about the texture, flavor and taste of the manufactured yogurt.

Patients

The current work was conducted on 20 children suffered from IDA, microcytic hypochromic anemia, aged 2–5 years. The patient children were admitted to Quesna central hospital outpatient clinic at Menoufia governorate, Egypt. Patients were divided into two groups A and B with 10 children each. Group A received yogurt fortified with LF while group B received commercial LF with concentration of 50 mg/g dissolved in water. The patients did not receive any other medical treatment.

The parents/guardians of the children were informed about the aim and procedures of the study and were provided a written informed consent in accordance with the Research Ethics Committee of Menofia University Hospital.

Each patient of group A was given 200 gm of yogurt fortified with LF with concentration of 100 mg/100g at morning before breakfast for 4 weeks. While each patient from group B was given 2 sachets of commercial LF, (each sachet contained 100 mg LF) dissolved in 50 ml water in the morning before breakfast for 4 weeks.

The full history, thorough clinical examination and analysis of complete blood count (CBC) were done to all patients before, to fairly assess the influence of each treatment regimen., and after treatment. Analysis were done at Quesna Central Hospital laboratory.

Statistical analysis

Data were collected and analyzed using SPSS (Statistical Package for Social Science) program for statistical analysis, version 18 (Chicago,Inc, Illinose). Data were entered as categorical or numerical, as appropriate. Quantitative data were shown as mean and SD. Student t-test was selected to compare means of 2 sets of quantitative data. Chi-square and Fisher exact test were used to measure the frequency of quantitative data. P (probability) value considered to be of statistical significance if it is less than 0.05.

RESULTS AND DISCUSSION

Lactoferrin is glycoprotein that can bind and transfer iron, it is found in many secretions in the body as saliva, serum, tears, and highly present in milk and colostrum (Chantaysakorn and Richter 2000). Lactic fermentation of foods, such as yogurt, increases the availability of iron (Lisko *et al.*, 2017).

The present study performed on 20 children with IDA, 10 of them received LF with yogurt and the rest received LF alone for 1 month in each group.

The rheological properties, sensory evaluation and acceptability of manufactured yogurt were studied before its use in the treatment protocol, and the result revealed that LF delayed coagulation time of manufactured yogurt with less syneresis and the manufactured yogurt was firm and acceptable for patients (Table 1).

Table 1. Rheological properties of yoghurt samples

Sample	Coagulation time minutes	Consistency	Syneresis *Ml/2h*
Control samples (without LF)	180±10	Firm	6.13 ± 0.95
Buffalo milk samples (with LF)	210±10	Firm	1.97 ± 0.25
P-value	<0.0001		<0.0001

Values are expressed as mean ± standard deviation.

Delayed coagulation time after addition of LF may be attributed to the partial inhibition of lactic acid producing microorganisms (Franco *et al.*, 2010). However, the manufactured yogurt was firm with less syneresis and acceptable for patients, which may be attributed to the addition of LF to milk in a powder form that increased the total solids contents of manufactured yogurt and absorption of excess whey by LF powder.

Consumer acceptance testing recorded in (Table 2) revealed that sensory properties of manufactured yogurt including appearance, odor, flavor and consistency were acceptable for patients which might be attributed to that addition of LF to milk did not interfere with yogurt manufacture and also did not affect taste or odor negatively.

pH and titratable acidity values of lactoferrin fortified yogurt compared to control plain yogurt during storage period were highlighted in figure (A) and (B) and the results revealed that decrease of pH and increase of titratable acidity were slower in lactoferrin fortified yogurt than that of plain yogurt. This may be due to the partial inhibition of lactic acid producing microorganisms by LF (Franco *et al.*, 2010).

The baseline characteristics of the patients included in this study were of average socioeconomic state and living in rural areas with no significant statistical difference. Both groups (A and B) were sex and age matched. Pallor was the main presentation in both groups followed by easy fatigability. The CBC parameters before treatment were ascertained to be comparable in both groups and no significant (P>0.05) complications were recorded in both groups (Table 3).

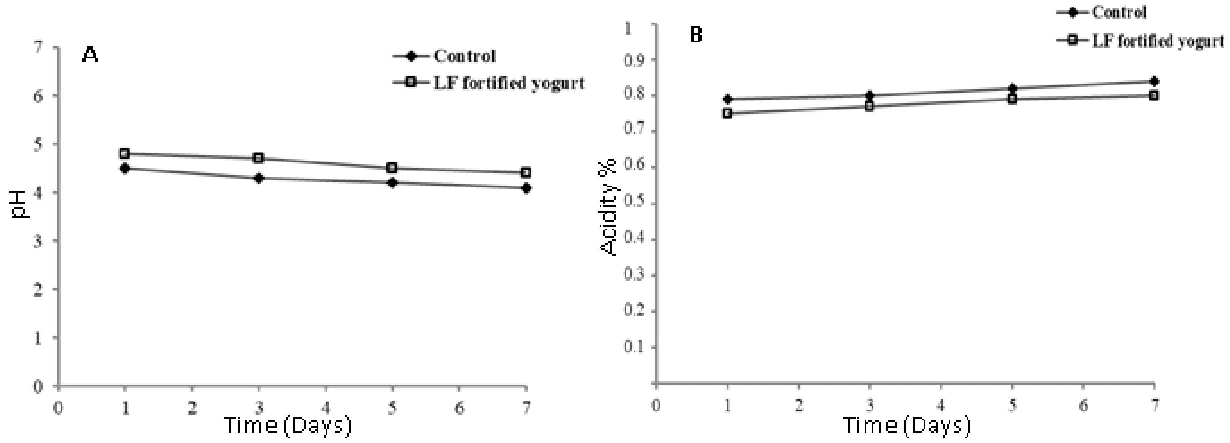

Figure. pH (A) and titratable acidity (B) values of lactoferrin fortified yogurt compared to control plain yogurt during storage period

Table 2. Sensory properties of synthesized yoghurt

Examined sample	1st day	3rd day	5th day	7th day
		Appearance		
Plain yogurt	7.85 ±1.10	7.46±1.3	7.21±0.94	7.14±1.22
LF fortified yogurt	7.46±0.98	7.30±1.2	7.27±1.20	7.21±1.13
P-value	0.243	0.688	0.861	0.852
		Odor		
Plain yogurt	8.11±1.12	7.75±1.54	7.45±1.25	7.22±1.41
LF fortified yogurt	7.85±1.31	7.67±1.12	7.46±1.34	7.34±1.62
P-value	0.504	0.852	0.980	0.804
		Flavor		
Plain yogurt	8.62±1.3	8.21±0.9	7.90±0.85	7.82±1.1
LF fortified yogurt	8.10±1.2	7.95±0.95	7.68±1.40	7.52±1.3
P-value	0.196	0.379	0.551	0.435
		Consistency		
Plain yogurt	8.15±1.20	7.75±0.95	7.45±0.85	7.33±1.10
LF fortified yogurt	7.95±0.90	7.79±1.41	7.67±0.97	7.58±1.22
P-value	0.554	0.916	0.450	0.500

The results of CBC parameters before and after treatment in both groups indicated that, treatment with yogurt fortified with LF and only LF improved the Hb and red blood cell indices significantly in both groups (P<0.05), while total leucocytes count (TLC) and platelets showed no significant changes (Tables 4, 5).

This improvement may be attributed to the hematogenic role of LF and the physiological modulation of LF receptors in anemic patients.

Table 3. Baseline characteristics of the studied groups (n=20)

		Yogurt fortified by LF	Commercial LF only	P-value
General Characteristics				
Gender	Male	5	5	------
	Female	5	5	
Age (years)	Mean ± SD	4.2±1.1	4.5±1.3	0.739
Socioeconomic state	Average	6 (60%)	7(70%)	0.462
	Low	4(40%)	3(30%)	0.462
Residence	Rural	8 (80%)	7 (70%)	0. 0.803
	Urban	2 (20%)	3 (30%)	0.0803
Clinical manifestations	Pallor	10 (100%)	10 (100%)	-----
	Easy fatigability	6 (60%)	5 (50%)	0.935
	Palpitations	4 (40%)	5 (50%)	0.917
	Headache	2 (20%)	3 (30%)	0.872
Laboratory investigations				
Hb(gm/dL)	Mean ± SD	9.34±0.63	9.29±0.65	0.834
HCT (%)	Mean ± SD	26.96±2.4	26.38±2.7	0.532
MCV (fl)	Mean ± SD	63.59±6.4	58.36±5.1	0.064
TLC ($\times10^3$/mcL)	Mean ± SD	6.7±1.64	6.5±1.39	0.739
Platelets ($\times10^3$/mcL)	Mean ± SD	217±30	255±42	0.426
Serum iron (pg\ml)	Mean ± SD	42.68±4.93	44.34±4.12	0.452
Serum ferritin (ng\ml)	Mean ± SD	11.71±3.36	11.57±3.94	0.721
Complications to treatment		0 (0%)	0 (0%)	------
Patients compliance	Compliant	10 (100%)	10 (100%)	------
	Non-compliant	0 (0%)	0(0%)	

Table 4. The CBC parameters before and after treatment with yogurt fortified with lactoferrin (*n*=10 each)

Parameters	Yogurt fortified by LF		
	Before treatment	After treatment	P-value
Hb(gm/dL)	9.34±0.63	10.81±0.78	<0.0001
HCT (%)	26.96±2.4	32.04±2.9	0.001
MCV (fl)	63.59±6.4	75.21±4.3	<0.0001
TLC ($\times10^3$/mcL)	6.70±1.6	6.50±1.39	0.524
Platelets ($\times10^3$/mcL)	217±30	255±42	0.394
Serum iron (pg\ml)	42.68±4.93	69.43±13.52	<0.0001
Serum ferritin (ng\ml)	11.71±3.36	36.34±17.26	<0.0001
TIBC (ug/dL)	461±53	312±51	<0.0001
Transferrin (%)	11.21±3.9	21.4±7.2	<0.0001

Values are expressed as mean ± standard deviation.

As lack of intracellular iron may evoke the increased expression of specific receptors on the surface of enterocytes and thereby elevated absorption of LF-bound iron. After LF is bound to the enterocyte, 90% of it is degraded and Fe^{3+} ions are released. The remaining intact 10% is transported through the cell membrane (Suzuki *et al.*, 2005)**.**

In addition, lactoferrin's capability of binding iron is two times higher than that of transferrin. This bond is very strong and can resist pH values as low as 4. LF has demonstrated remarkable resistance to proteolytic degradation by trypsin and trypsin-like enzymes (Lyer and Lonnerdal 1993)**.**

On comparing the rate of improvement in both groups, the Hb and red blood cell indices were significantly (P<0.05) higher in patients received LF with yogurt (group A) than those (group B) with LF only (Table 6).

This superior efficacy that noticed with the addition of yogurt may be related to the increased content of lactic acid in yogurt, which increases the solubility of iron and decreases the content of inhibitory phytate (Scheers et al., 2016). In addition, yogurt contains probiotics that can enhance bioavailability of iron by creating an acidic environment in the intestinal tract which makes iron more absorbable and degrading mineral complexing phytic acid from food (Sazawal *et al.*, 2010).

Table 5. The CBC parameters before and after treatment with lactoferrin only (*n*=10 each)

Commercial LF only			
Parameters	Before treatment	After treatment	P-value
Hb (gm/dL)	9.29±0.65	10.01±1.21	0.032
HCT (%)	26.38±2.7	29.90±1.8	0.049
MCV (fl)	58.36±5.1	69.31±1.48	<0.0001
TLC (×10^3/mcL)	6.50±1.39	6.20±1.7	0.396
Platelets (×10^3/mcL)	255±42	231±36	0.644
Serum iron (pg\ml)	44.34±4.12	56.94±10.14	<0.0001
Serum ferritin (ng\ml)	11.57±3.94	24.93±12.57	<0.0001
TIBC (ug/dL)	477±69	341±44	<0.0001
Transferrin (%)	11.07±3.6	18.9±4.2	<0.0001

Values are expressed as mean ± standard deviation.

Table 6. The CBC parameters after treatment in patients received Yogurt fortified with lactoferrin and lactoferrin only (*n*=10 each)

Parameter	Yogurt fortified by LF	Commercial LF only	P-value
Hb (gm/dL)	10.81±0.78	10.01±1.21	0.022
HCT (%)	32.04±2.9	29.99±1.89	0.040
MCV (fl)	75.20±4.3	69.31±1.48	<0.0001
TLC (×10^3/mcL)	6.50±1.3	6.20±1.7	0.266
Platelets (×10^3/mcL)	255±42	231±36	0.315
Serum iron (pg\ml)	69.43±13.52	56.94±10.14	0.002
Serum ferritin (ng\ml)	36.34±17.264	24.93±12.57	0.022
TIBC (ug/dL)	312±51	341±44	0.061
Transferrin (%)	21.4±7.2	18.9±4.2	0.187

Values are expressed as mean ± standard deviation.
Normal values of CBC parameters of children from 1-5 years old according to American Academy of pediatrics 2019 were: Hb 11-15 gm/dL, HCT 34-40%, MCV 75-87 fl, TLC 5-10×10^3/mcL, Platelets 150-400×10^3/mcL, serum iron 50-120 pg\ml, serum iron ferritin 20-200 ng/ml, TIBC 250-450 ug/dl and Transferrin 20-50%

In conclusion, fortification of milk with lactoferrin did not interfere with yogurt manufacture and the sensory properties of produced yogurt was acceptable. The yogurt produced from milk fortified with lactoferrin was effective well-tolerated treatment for the management of IDA in children. Further studies should be proceeded on a wider scale with all factors being controlled.

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