RESEARCH ARTICLE
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605

Characterization of malabari goat lactoferrin and its pepsin hydro-lysate*

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Citation: Chandran, D., Uma, R. and Eldho, L. 2020. Characterisation of Malabari goat lactoferrin and its pepsin hydrolysate. *J. Vet. Anim. Sci.* **51**(1): 40-47.

Received : 02.08.2019 *Accepted : 11.11.2019* *Published : 01-01-2020*

Abstract

Lactoferrin, a minor whey protein present mainly in milk as well as in small quantities in most of the secretions of the body, has a wide range of biological activities to its credit including antimicrobial, antioxidant, anticancer and immunomodulatory properties. A low molecular weight peptideisolated from the pepsin hydrolysate of lactoferrin called as lactoferricin B, has been found to be more functionally active than its parent compound. The present work focussed on the isolation of lactoferrin from the colostrumof Malabari goats by cation exchange chromatography, followed by the assessment of its molecular weight by SDS-PAGE andits characterization by dot blot assay and western blotting.The concentration of lactoferrin as estimated by Lowry's method was found to be 15.103 mg/L of colostrum. Lactoferrin was hydrolysed by treatment with three per cent porcine pepsin under acidic conditions to form lactoferrin pepsin hydrolysate. The results of this study point to a single one step method to obtain pure lactoferrin from goats and further preparation of its pepsin hydrolysate.

Keywords: Whey proteins, lactoferrin, lactoferricin B, pepsin hydrolysate

Milkis a well-balanced source of all the essential nutrients needed for growth and development. Milk proteins are categorized into caseins and whey proteins according to theirphysio-chemicalcharacteristics. Caseins that contain phosphorus coagulate at a pH of 4.6 while the whey proteins that are devoid of phosphorus remain in solution in milk at the samepH. The whey protein family is made up of β-lactoglobulin, α-lactalbumin, immunoglobulins, lactoferrin, transferrin as well as many other minor proteins and enzymes (McGrath *et al.,* 2016).

Being secreted by epithelial cells, lactoferrin is present in all mucosal secretions of the body like nasal and bronchial secretions, saliva, tears, gastrointestinal fluids, bile and cervical mucus, as well as in the secondary granules of neutrophils (Legrand *et al*., 2008). Lactoferrin, also known as lactotransferrin, is the second most abundant milk protein, after caseins. It is an

** Part of the MVSc thesis submitted by the first author to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala*

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iron binding,non-hemic glycoprotein that belongs to the transferrin family and is alkaline in nature with an isoelectric pHof 8.7 (El-loly and Mahfouz, 2011). It occurs as a single polypeptide chainwith around 690 amino acids and has a molecular mass of 80kDa (Baker and Barker, 2005). It is salmon pink or pale red incolour and can exist in two forms, namely hololactoferrin (iron-rich) and apolactoferrin (iron-free) (Jameson *et al*., 1998).Recently, it has shot into utmost prominence due to its broad range ofbiologicalactivities, including roles in cell differentiation and proliferation, iron metabolism, antimicrobial, anti-inflammatory, antioxidant, anticancer and immunomodulatory properties (Tsuda *et al.*, 2010; Pan *et al.,* 2007; Rodrigues *et al.,* 2009).

Whey proteins have been proven to contain various functional peptides in their primary sequences, whose potentials have not been fully unravelled (Van Der Kraan*et al.*, 2006). Lactoferrin is one such minor whey protein from whichmore biologically active peptides like lactoferricin could be produced by pepsin mediated proteolytic digestion under acidic conditions(Tomita *et al*., 1991). Lactoferricinhas been proven to be more active in terms of its antimicrobial and immunomodulatory properties than the parent compound. It is encoded within the N-terminal lobe of lactoferrin and is abundant in hydrophobic amino acidslike phenylalanine and tryptophan, as well as basic amino acids like arginine and lysine (Bellamy *etal*., 1992).

Goat farming is a stable and reliable source of income for marginal level farmers of India. Kerala, the southern state of India, which is blessed with its rich biodiversity, is the abode of two promising goat breeds *viz.,*the Malabari and the Attappady Black. Currently the goat production scenario of Kerala is dominated by theMalabari goats, on account of their good production performance and adaptability to the warm tropical climatic conditions of the state. The genetic potential of this goat breed and the properties of its milk proteins have not been well studied. Perusal of literature revealed that the studies on the multifaceted lactoferrin protein and lactoferricin from Malabari goats are scarce. Hence the study was undertaken to isolate and characterize lactoferrin from the colostrum of Malabari goats as well as to prepare its pepsin hydrolysatein order to harness their varied potentials.

Materials and Methods

Isolation of Lactoferrin

Sample Collection and Processing

Around six litres of colostrum samples were obtained from newly kidded Malabari goats maintained at University Goat and Sheep Farm, College of Veterinary and Animal Sciences, Mannuthy, Kerala, India. The samples were processed as per the protocol of Vijayan *et al.* (2017) with minor modifications. The samples were initially centrifuged for 10,000 × *g* at 4°C for 30 min and the creamy layer formed on the top was removed. The skimmed colostrum obtained just above the white pellet of cells was filtered to remove traces of cream if any, and diluted twice with deionized water. Acid whey was prepared by acidifying diluted skimmed colostrum to pH 4.6 with 2*N* HCl. The precipitate formed was removed by centrifugation at 10,000 × *g* for 30 min at 4°C. The supernatant (acid whey) was filtered and neutralised to pH 6.8 with 2*N*NaOH. This was again centrifuged at 10,000 × *g* for 30 min at 4°C and the precipitate was discarded. The supernatant (neutralized whey) obtained was filtered and stored at -80°C till further use.

Ammonium Sulphate Protein Fractionation and Dialysis

The neutralized whey was subjected to ammonium sulphatebased protein fractionation by initial 0-45 per cent and further 45-80 per cent ammonium sulphate saturation. The precipitate formed was collected and diluted in 10ml of deionized water. The sample thus fractionated was then dialysed exclusively against several changes of distilled water and finally against 10m*M* sodium phosphate buffer with 250m*M* NaCl (equilibration buffer with pH 6.5).

Cation Exchange Chromatography

Cation exchanger CM Sephadex C-50 (GE Healthcare) resin was packed in a glass column of 300 ×18 mm size. The column was equilibrated and the sample was loaded.

After washing the column with equilibration buffer,the bound protein on the stationary phase was displaced by stepwise elution with 10m*M* sodium phosphate buffer containing 0.4*M* NaCl (elution buffer I), 0.6*M* NaCl (elution buffer II) and 0.8*M* NaCl (elution buffer III) respectively. A graph of fraction number versus absorbance at 280nm was plotted using the OD values of the eluted fractions. The samples with high OD values were further used for confirmation of the isolated protein.

Characterization of the Isolated Lactoferrin

Assessment of Molecular Weight by Sodium Dodecyl Sulphate- Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The eluted fractions were separated by SDS-PAGE on a mini vertical gel electrophoresis apparatus (Biorad, USA) in duplicates according to the procedure of Weber and Osborne (1969). A volume of 10µL each of the eluted samples having high OD values, commercially available bovine lactoferrin (standard lactoferrin) (Sigma Aldrich) and wide range protein marker (10-180kDa) (Puregene, Genetix) were loaded and the gel was initially run at a constant voltage of 50V/cm till the dye front crossed the stacking gel. The voltage was then increased to 100 V/cm till the dye front approached the end of the gel. 10-15 V/cm is normal voltage requirement for SDS-PAGE. Higher voltage was used here in order to compensate for the increased resistance that the gel developed during the course of running and also to increase the speed of protein electrophoresis without compromising the gel matrix. The protein bands formed on the gel was stained by Coomassie Brilliant Blue dye and photographed in ChemiDoc™MP imaging system (Biorad, USA).

Confirmation of the Isolated Protein by Dot Blot Assay

Dot blot assay was carried out for confirming the reactivity of commercially available primary anti-lactoferrin antibody and Horse Radish Peroxidase (HRP) conjugated secondary anti-rabbit IgG antibody (Sigma Aldrich, USA) prior to conduction of western blotting. One microliter of standardlactoferrin and Malabari goat lactoferrin was coated in the centre of activated to polyvinylidene difluoride (PVDF, 0.45µm) membrane strip (Biorad, USA). The air dried strips were incubated in blocking buffer (five grams of dried skim milk powder dissolved in 100mL phosphate buffered saline) at 37ºC for one hour. The strips were then incubated withprimary antibody (1:1000 dilution of bovine anti-lactoferrin antibody in blocking buffer) at room temperature for one hour and then washed six times with Tris Buffered Saline with Tween-20 (TTBS) for 10 min each. Further PVDF membrane strips were incubated with the HRP conjugated secondary antibody (anti-rabbit IgG) diluted in blocking buffer (1:2000) at room temperature for one hour. The strips were washed four times with TTBS as described earlier and were immersed in chromogenic visualisation solution (containing 100m*M*Tris hydrochloride, 40µg/mL diaminobenzidinestock and three per cent hydrogen peroxide(H_2O_2) with mild rocking at room temperature in dark for two to three minutes. The reaction was terminated by washing the strips with distilled waterand the strips were further air dried and photographed. Development of a brown colour indicated a positive reaction while no colour formation indicated a negative reaction.

Confirmation of Isolated Protein by Western Blotting

Theunstained gel after electrophoresis was equilibrated in transfer buffer for 15 min. The protein was subsequently transferred to PVDF membrane using a Trans Blot Turbo transfer system (Biorad, USA) as per the manufacturer's instructions and the transfer was done at 55mA for 120 min. The membrane was then removed and subjected to immunoblotting. Initially the blot was blocked with 20mL of five per cent dried skim milk powder in phosphate buffered saline at room temperature for two hours and washed twice with TTBS in 10 min intervals. The PVDF membrane was incubated with diluted primary antibody (1:1000) for one hour at 37ºC, followed by washing for four times; 15 min each with TTBS continuously with a mild shaking. Later the membrane was incubated for one hour at 37ºC in HRP conjugated secondary antibody (anti-rabbit IgG) diluted in blocking buffer (1:2000) with constant agitation and washed four times with TTBS as explained

earlier. The transfer membrane was immersed in chromogenic visualisation solution in a dark room with mild rocking in room temperature until the blot developed. The reaction was terminated by adding distilled water and blot was photographed. The standardlactoferrin served as the positive control.

Estimation of Iron Content of the Isolated Lactoferrin

Total iron content of Malabari goat lactoferrin(both undigested and digested) was estimated according to Lee and Clydesdale (1979) method using atomic absorption spectrophotometry. Total iron concentration in the sample was calculated as given below:

$$\text{Total iron concentration (ppm)} = (AAs - AAb) \times \frac{\text{Final sample volume}}{\text{Sample weight (g)}}$$

Where, AAs - Atomic absorption for sample and

AAb - Atomic absorption for blank

Estimation of Carbohydrate Content of the Isolated Lactoferrin

Total carbohydrate content of Malabari goat lactoferrin was estimated according to Phenol-sulphuric acid method as determined by DuBois *et al.* (1956). Standard glucose solution (stock) was prepared by dissolving 100mg glucosein 100mL of distilled water. A volume of 10mL of the stock solution was then diluted to 100mL with distilled water to prepare the working standard.Weighed 10mg gLf into a test tube, which was hydrolysed by keeping the tube in boiling water bath for three hours with five millilitres of 2.5*N* HCl and cooled to room temperature. Neutralized with solid Na_2CO_3until the effervescence ceased. Made up the volume to 100mL and centrifuged. Pipetted out 0.2, 0.4, 0.6, 0.8 and one millilitre of working standard into a series of test tubes. Pipetted out 0.1 and 0.2mL of the sample solution into two separate test tubes. Made up the volume in each test tube to one millilitre with distilled water. A blank solution was further set up with one millilitre of distilled water. Added one millilitre of five per cent phenol in water (w/w) and five millilitres of concentrated sulphuric acid to each test tube. After allowing the test tubes to stand for 10 min, they were vortexed for 30 sec and placed for20 min in a water bath at 25-30°C for colour development. Then, light absorption at 490nm was recorded on a spectrophotometer. Total carbohydrate content was calculated as follows:

Absorbance corresponds to 0.1 mL of the test = x mg of glucose

100mL of the sample solution contains =

$0.1x \times 100$ mg of glucose

= % of total carbohydrate present.

Estimation of Concentration of the Isolated Lactoferrin

The eluted protein fractions containing lactoferrinas identified by SDS-PAGE, and confirmed by dot blot assay and western blotting were dialysed against several changes of distilled water and were frozen using -120°C (Operon cold trap) and then subjected to lyophilisation in a freeze drier (Operon FDU 7003, Korea). The lyophilised samples were diluted with minimum quantity phosphate buffered saline and then subjected to estimation of the protein content by Lowry's method (Lowry *et al.,* 1951) using commercially available bovine serum albumin (BSA) as standard. The absorbance was measured spectrophotometrically at 620nm. The standard graph was plotted and the protein concentration was calculated from the graph.

Preparation of Lactoferrin Pepsin Hydrolysate(LPH)

Pepsin hydrolysate was prepared from the isolated lactoferrinby digesting it with porcine pepsin according to the procedure as described by Bellamy *et al*. (1992). Lactoferrin was dissolved in sterile water (Millipore) to a concentration of five per cent and pH adjusted to 3.0 using filter sterilized 1*N* HCl. Porcine pepsin was added to achieve a final concentration of three per cent (w/w of substrate) and the reaction mixture wasincubated at 37°C for four hours. The reaction was terminated by incubation in a pre-heated water bath (80°C) for 15 min followed by cooling at 5°C for 15min and then subsequent reduction of the

pH of the reaction mixture to 7.0 using 1*N* NaOH. Insoluble peptides were removed by centrifugation at 15000 ×*g* for 30 min. The resultant supernatant liquid was lyophilised and stored at -80°C till further use. The digestion of lactoferrin by pepsin was confirmed by SDS-PAGE.

Results and Discussion

Isolation of Lactoferrin by cation exchange chromatography

The sample obtained after dialysis was loaded onto the chromatography column packed with CM Sephadex C-50 at a flow rate of 0.33 ml/min and was eluted with a step gradient of 0.4, 0.6 and 0.8*M* NaCl. The optical density (OD) of the eluted fractions was monitored at 280 nm and their elution profile was plotted. Among the fractions eluted with 0.8*M* NaCl, six fractions i.e., fraction numbers 63 – 68, showed high OD_{280} values and they formed a single peak in the elution profile. Results of this study is in agreement withYounghoon*et al.* (2009) who reported elution of caprine lactoferrin using 0.8M NaCl, while Vijayan *et al.* (2017) and Abbas *et al.* (2015) reported that maximum absorbance, indicative of the presence of the adsorbed protein was detected in the fractions eluted with 0.6*M* NaCl and 0.5*M*NaCl respectively.

Characterization of the Isolated Lactoferrin

Assessment of molecular weight of lactoferrin by SDS-PAGE

The eluted fractions from Malabari goat colostrum which showed peak absorbance at 280nm were analysed using 12 per cent SDS-PAGE to identify their electrophoretic mobility in correspondence with the referencelactoferrin. The fractions eluted with the 0.8*M* NaCl buffer could be visualized as a single 80kDa Coomassie Brilliant blue-stained band at the same position as that of the reference. Absence of any other proteins in these fractions facilitated the identification of the protein by SDS-PAGE. The presence as well as purity of lactoferrin could hence be confirmed as a single band in SDS-PAGE and the similar electrophoretic mobility to that of standard lactoferrin (Fig1). The results obtained in this study are similar and analogous to the reports of Younghoon*et al*. (2009), Le Parc *et al.* (2014), Abbas *et al*. (2015) and Vijayan *et al.* (2017)who employed SDS-PAGE to confirm the molecular weight and purity of caprine lactoferrin, whose results confirmed the molecular weight of goat lactoferrin to be 80 kDa.

Confirmation of isolated protein as lactoferrin by Dot Blot Assay

Development of a brown colour spot at the same position as that of standard lactoferrin (Fig 2) due to its reactivity with commercially available primary anti-lactoferrin antibody and Horse Radish Peroxidase (HRP) conjugated secondary antibody (anti-rabbit IgG) indicated a positive reaction and thus confirmed theidentity of the protein under study.

Confirmation of the isolated protein as lactoferrin by Western blotting

The presence of lactoferrin in the eluted fractions was further assured by Western blotting. The presence of prominent thick band corresponding to the same position as that of positive control (standard lactoferrin) confirmed the identity of isolated protein as lactoferrin (Fig 3). A few higher molecular weight non-specific bands were obtained in thelanes of both isolated protein and positive control in the western blot. These non-specific bands were caused by technical artefacts and hence were not scientifically relevant. Moreover, SDS-PAGE (Fig 1) does not show the presence of any proteins corresponding to those non-specific bands in western blot.Results obtained were similar and compatible with the many previous reports. Nakamura *et al.* (2001) reported the production of recombinant N-lobe of bovine lactoferrin in cultured insect cells wherein they utilised Western blot analysis to study the molecular weight of proteins isolated from culture medium. Western blotting technique was utilised by Garcia-Montoya *et al.* (2013) to confirm the identity of lactoferrin while purifying the recombinant bovine lactoferrin expressed in *Escherichia coli* cells. Ragehe*t al.* (2016) investigated the expression of lactoferrin in the ocular tissues of humans and mice and reported that Western blot analysis confirmed

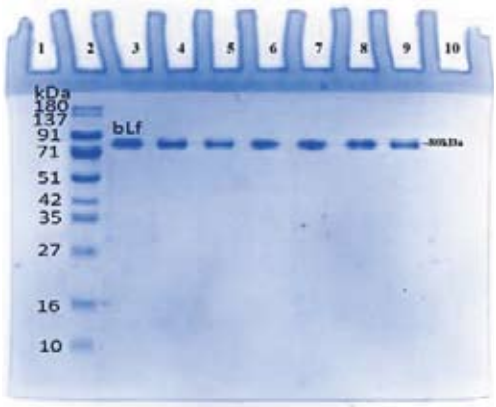

Fig 1. Electrophoretic profile of the eluted fractions with high OD_{280} values from Malabari goat colostrum Lane 2: Wide range molecular weight marker,Lane 3: Commercially available bovine lactoferrin, Lane 4-9: Eluted fractions

Fig 2. Dot Blot assay of the eluted protein fractions containing lactoferrin from Malabari goat colostrum

the presence of lactoferrin in multiple ocular tissues of both the species.

Estimation of Iron Content of the Isolated Lactoferrin

The total iron content in the undigested and digested lactoferrin isolated from Malabari goat breed, as estimated by atomic absorption spectrophotometry was230 ppm and 600 ppm respectively. The value obtained was more than that reported by Abbas *et al.* (2015), according to whom the total iron content of goat colostrum lactoferrinwas123 ppm. Sinchu (2017) stated that the total iron content of the lactoferrin isolated from the colostrum of Malabari goat was 820 ppm.

Estimation of Carbohydrate Content of the Isolated Lactoferrin

The total carbohydrate content in the isolated lactoferrin as estimated by phenol-sulphuric acid method was 9.3 per cent. This result was consistent with that ofShimazaki (2000) who found the carbohydratecontent in lactoferrinfrom variable sourcesto range

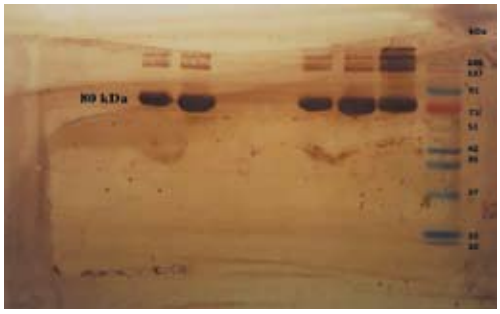

Fig 3. Western blot analysis of the protein isolated from Malabari goat colostrum

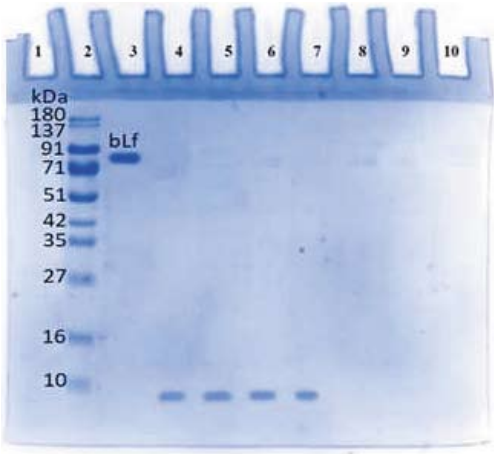

Fig 4. Electrophoretic profile of the lactoferrin pepsin hydrolysate solution
Lane 2: Wide range molecular weight marker,Lane 3: Commercially available bovine lactoferrin,Lane 4-7: Lactoferrin pepsin hydrolysate (gLPH) solution

between 7and 11.5 per cent.Brisson *et al*. (2007) found that bovine lactoferrin contained 11.2 per cent carbohydrate, human lactoferrin contained 6.4 per cent, horse 3.5 per cent and camel 11 per cent. Al-Hatim (2012) found the carbohydrate content in cow and sheep milk to be 11.1 per cent and 8.2 per cent respectively. Abbas *et al.* (2015) reported that the carbohydrate content of lactoferrin isolated from goat colostrum, as estimated by phenol-sulphuric acid method was 10.4 per cent.

Estimation of Concentration of the Isolated Lactoferrin

The protein content in the lyophilised sample was estimated by Lowry's method and the concentration as calculated from the standard curve was found to be 2.485 mg/mLof reconstituted sample. It corresponded to a yield of 15.103 mg/L of colostrum, which

is close to the yield of 10.94 mg/L as obtained by Vijayan *et al.* (2017). Drackova*et al.* (2009) estimated lactoferrin content of goat milk by reverse phase high-performance liquid chromatography and reported the average concentration to be 120±18 µg/mL.Moradian*et al.* (2014) isolated lactoferrin from bovine colostrum by fast protein liquid chromatography and determined its concentration by Bradford assay to be 2.4 mg/mL. Abbas *et al.* (2015) stated that the concentration of lactoferrin isolated by combined cation exchange gel filtration protocol from colostrum, was found to be 250 mg/L of colostrum using Bradford assay. According to Zainab *etal*. (2015), the lactoferrin concentration in goat milk was 300mg/Las found out by Bradford method.

Preparation of Lactoferrin Pepsin Hydrolysate

Digestion of Lactoferrin by three per cent porcine pepsin to form lactoferrin pepsin hydrolysate (gLPH) was confirmed by 12 per cent SDS-PAGE. A band representing small peptide with molecular weight of less than 10kDa called as lactoferricin B was observed (Fig 4). The methodology followed and the results obtained were compatible with the reports of many authors. Saito *et al*. (1991) had reported that the pepsin hydrolysate of lactoferrin can be produced by treatment of lactoferrin with porcine pepsin under acidic conditions. Tomita *etal*. (1991) performed pepsin hydrolysis of lactoferrin and by SDS-PAGE, found out that molecular weight of most peptides in the hydrolysate were less than six kilodaltons. Chantaysakorn and Richter (1999) reported that the digestion of lactoferrin by pepsin was confirmed by SDS-PAGE, where in which they observed numerous bands representing small peptides with molecular weights of less than six kilodaltons.

Cation-exchange chromatography-based separation and purification is an effective way to isolate lactoferrin from milk even though the yield of the protein has been found to be less. The characterizedlactoferrin from goat colostrum and its pepsin hydrolysate could be utilized as immunomodulatory, therapeutic agents as well asfunctional food ingredients beneficial to humans and animals.

References

Abbas, Z. H., Doosh, K. S. and Yaseen, N. Y. 2015. Isolation, purification and characterization of lactoferrin from goat colostrum whey. *Pak J. Nutr.* **14**: 517-523.

Adlerova, L.,Bartoskova, A. and Faldyna, M. 2008. Lactoferrin: a review. *Vet. Med*. **53**: 457-468.

Al-Hatim, R.R. 2012. Separation and purification lactoferrin from bovine and ovine colostrums and studying of some its properties and applications. *Master's Thesis, College of Agric. university of Basra-Iraq*.

Baker, E. N. and Baker, H. M. 2005. Molecular structure, binding properties and dynamics of lactoferrin. *Cell. Mol. Life Sci.* **62**:2531-2539.

Bellamy, W., Takase, M., Wakabayashi, H., Kavase, K. and Tomita, M. 1992. Antibacterial spectrum of lactoferricin B, a potent bactericide peptide derived from the N-terminal region of bovine lactoferrin. *J. Appl. Microbiol.* **73**: 472–479.

Brisson, G., Britten, M. and Pouliot, Y. 2007. Heat-induced aggregation of bovine lactoferrin at neutral pH: effect of iron saturation. *Int. Dairy J.* **17**: 617-624.

Chantaysakorn, P. and Richter, R.L. 2000. Antimicrobial properties of pepsin-digested lactoferrin added to carrot juice and filtrates of carrot juice. *J. Food Prot.* **63**: 376-380.

Drackova, M., Borkovcova, I., Janstova, B., Naiserova, M., Pridalova, H., Navratilova, P. and Vorlova, L. 2009. Determination of lactoferrin in goat milk by HPLC method. *Czech. J. Food Sci.* **27**: 102-104.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.T. and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28**: 350-356.

El-Loly, M.M. and Mahfouz, M.B. 2011. Lactoferrin in relation to biological functions and applications: a review. *Int. J. Dairy Sci*. **6**: 79-111.

Garcia-Montoya, I., Salazar-Martínez, J., Arevalo-Gallegos, S., Sinagawa-Garcia, S. and Rascon-Cruz, Q. 2013. Expression and characterization of

recombinant bovine lactoferrin in *E. coli*. *Biometals.* **26**: 113-122.

Jameson, G. B., Anderson, B. F., Norris, G. E., Thomas, D. H. and Baker, E. N. 1998. Structure of human apolactoferrin at 2.0 Å resolution- Refinement and analysis of ligand-induced conformational change. *ActaCryst. D.* **54**:1319-1335.

Lee, K. and F. M. Clydesdale. 1979. Quantitative determination of the elemental, ferrous, ferric, soluble and complexed iron in food. *J. Food Sci*. **44**: 549-554

Legrand, D., Pierce, A., Elass, E., Carpentier, M., Mariller, C. and Mazurier, J., 2008. Lactoferrin structure and functions. In: *Bioactive components of milk* Springer. New York, pp. 163-194.

LeParc, A., Dallas, D. C., Duaut, S., Leonil, J., Martin, P. and Barile, D. 2014. Characterization of goat milk lactoferrin N-glycans and comparison with the N-glycomes of human and bovine milk. *Electrophoresis***35**: 1560–1570.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem*. **193**: 265-275.

McGrath, B.A., Fox, P.F., McSweeney, P.L. and Kelly, A.L. 2016. Composition and properties of bovine colostrum: a review. *Dairy Sci. Technol.*, **96**: 133-158.

Moradian, F., Sharbafi, R. and Rafiei, A. 2014. Lactoferrin: isolation, purification and antimicrobial effects. *JOMB.***3**:203-206.

Nakamura, I., Watanabe, A., Tsunemitsu, H., Lee, N.Y., Kumura, H., Shimazaki, K.I. and Yagi, Y. 2001. Production of recombinant bovine lactoferrin N-lobe in insect cells and its antimicrobial activity. *Protein Expr. Purif.* **21**: 424-431.

Pan, Y., Rowney, M., Guo, P. and Hobman, P. 2007. Biological properties of lactoferrin: an overview. *Aust. J. Dairy Technol.* **62**: 31p.

Rageh, A.A., Ferrington, D.A., Roehrich, H., Yuan, C., Terluk, M.R., Nelson, E.F. and Montezuma, S.R. 2016. Lactoferrin expression in human and murine ocular tissue. *Curr. Eye Res.* **41**: 883-889.

Rodrigues, L., Teixeira, J., Schmitt, F., Paulsson, M. and Månsson, H.L., 2008. Lactoferrin and cancer disease prevention. *Crit. Rev. Food Sci. Nutr.* **49**: 203-217.

Saito, H., Miyakawa, H., Tamura, Y., Shimamura, S. and Tomita, M. 1991. Potent bactericidal activity of bovine lactoferrin hydrolysate produced by heat treatment at acidic pH. *J. Dairy Sci.* **74**: 3724-3730.

Shimazaki, K. 2000. Lactoferrin: A Marvelous Protein in Milk?.*Anim. Sci. J.***71**: 329-347.

SinchuVijayan. 2017. Isolation and characterization of lactoferrin from colostrum of goats. *MVSc Thesis* submitted to Kerala Veterinary and Animal Sciences University, Pookode, 45p.

Tomita, M., Bellamy, W., Takase, M., Yamauchi, K., Wakabayashi, H. and Kawase, K. 1991. Potent antibacterial peptides generated by pepsin digestion of bovine lactoferrin. *J. Dairy Sci.* **74**: 4137-4142.

Tsuda, H., Kozu, T., Iinuma, G., Ohashi, Y., Saito, Y., Saito, D., Akasu, T., Alexander, D.B., Futakuchi, M., Fukamachi, K. and Xu, J. 2010. Cancer prevention by bovine lactoferrin: from animal studies to human trial. *Biometals*. **23**: 399-409.

Van der Kraan, M.I., Nazmi, K., van't Hof, W., Amerongen, A.V.N., Veerman, E.C. and Bolscher, J.G. 2006. Distinct bactericidal activities of bovine lactoferrin peptides LFampin 268–284 and LFampin 265–284: Asp-Leu-Ile makes a difference. *Biochem. Cell Biol*. **84**: 358-362.

Vijayan, S., Radhakrishnan, U., Eldho, L. and Jayavardhanan K. K. 2017. Isolation and purification of lactoferrin from colostrum of malabari goats. *J. Exp. Biol.* **5**: 4-10.

Weber, K. and Osborne, M. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.***244**:4406-4412.

Younghoon, K., Mae J., Kyoung S. H., Jee, Y. I, Sejong, O. and Sae, H. K. 2009. Anticancer activity of lactoferrin isolated from caprine colostrum on human cancer cell lines. *Int. J. Dairy Tech*. **62**:277-281.

Zainab, H.A., Kifah, S.D. and Nahi, Y.Y. 2015. Isolation, purification and characterization of lactoferrin from goat colostrum whey. *Pakistan J. Nutr.***14**: 517-523. ■