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Review

Effect of technological treatments on bovine lactoferrin: An overview

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ABSTRACT

Lactoferrin (LF) is a multifunctional protein that exerts important activities in the neonate through its presence in milk, and also in other external mucosas, acting as a defense protein of innate immunity. The addition of bovine LF to infant formula and also to other functional products and cosmetics has increased during the last decades. Consequently, it is essential to know the effect that the technological processes, necessary to elaborate those products, have on LF activity. In this study, we have revised the effect of classical treatments on lactoferrin structure and activity, such as heat treatment or drying, and also of emerging technologies, like high pressure or pulsed electric field. The results of the studies included in this review indicate that LF stability is dependent on its level of iron-saturation and on the characteristics of the treatment media. Furthermore, the studies revised here reveal that the non-thermal treatments are interesting alternatives to the traditional ones, as they protect better the structure and activity of lactoferrin. It is also clear the need for research on LF encapsulation by different ways, to protect its properties before it reaches the intestine. All this knowledge would allow designing processes less harmful for LF, thus maintaining all its functionality.

1. Introduction

Lactoferrin (LF) is an iron-binding glycoprotein, belonging to the transferrin family, along with serum transferrin, ovotransferrin, melanotransferrin and carbonic anhydrase inhibitor (Farnaud & Evans, 2005). LF is produced by mucosal epithelial cells in various mammalian species, including bovine, ovine, caprine, equine and canine species, and also rodents and humans. LF is mainly found in milk and colostrum, as well as in other secretory fluids, such as tears and saliva, and also in white blood cells (Connelly, 2001). LF is a single polypeptide chain glycoprotein with a molecular weight of around 78 kDa. Structural studies about this protein have reported 691 and 696 amino acids in human and bovine LF, respectively (Baker, Baker, Smith, & Baker, 2000; Moore, Anderson, Groom, Haridas, & Baker, 1997). The molecule of LF is folded into two homologous N- and C-terminal lobes, each of them being able to bind one atom of ferric iron (Baker & Baker, 2005). There are several forms of LF depending on the number of iron atoms bound to the molecule: the holo-LF form, saturated with iron in both sites; the apo-LF form, devoid of iron, and the intermediate forms, which contain iron only in either of the two lobes. The structure of LF and its two lobes, with the different possibilities of conformation of the apo-form lobes are shown in Fig. 1. The molecule or LF is more compact when its lobes have iron bound and consequently, more resistant to heat and proteolysis (Sánchez, Peiró, et al., 1992; Steijns, Brummer, Troost, & Saris, 2001). LF presents several sites for glycans depending on the species; in particular, bovine LF has five sites although only four are normally glycosylated. The glycan moieties in LF may contribute to some of its biological roles (Karav, German, Rouquié, Le Parc, & Barile, 2017). In 1992, some researchers from the Japanese dairy company Morinaga discovered that pepsin released an antimicrobial peptide from native bovine LF that was called lactoferricin (Bellamy et al., 1992) (see Fig. 1). This peptide showed to be extremely basic, characteristic that is related with its antibacterial activity (Vogel, 2012). Lactoferricin has been found as naturally being produced in the human gut (Kuwata, Yip, Tomita, & Hutchens, 1998; Kuwata, Yip, Yip, Tomita, & Hutchens, 1998) and proved to be more potent against some bacteria than intact LF (Bellamy et al., 1992).

Denaturation in proteins occurs when strong environmental conditions are present, such as strong acid/base, extreme temperatures and concentrated organic/inorganic salt. These conditions transform the conformation of the protein and cause the breakdown of forces (*e.g.* hydrogen and disulfide bonds) that give rise and stabilise higher order (secondary, tertiary and quaternary) structural features. In LF, denaturation alters 3D structure and consequently its functional properties, as the iron binding capacity and antibacterial activity. Therefore, processing parameters have to be considered and studied to preserve LF

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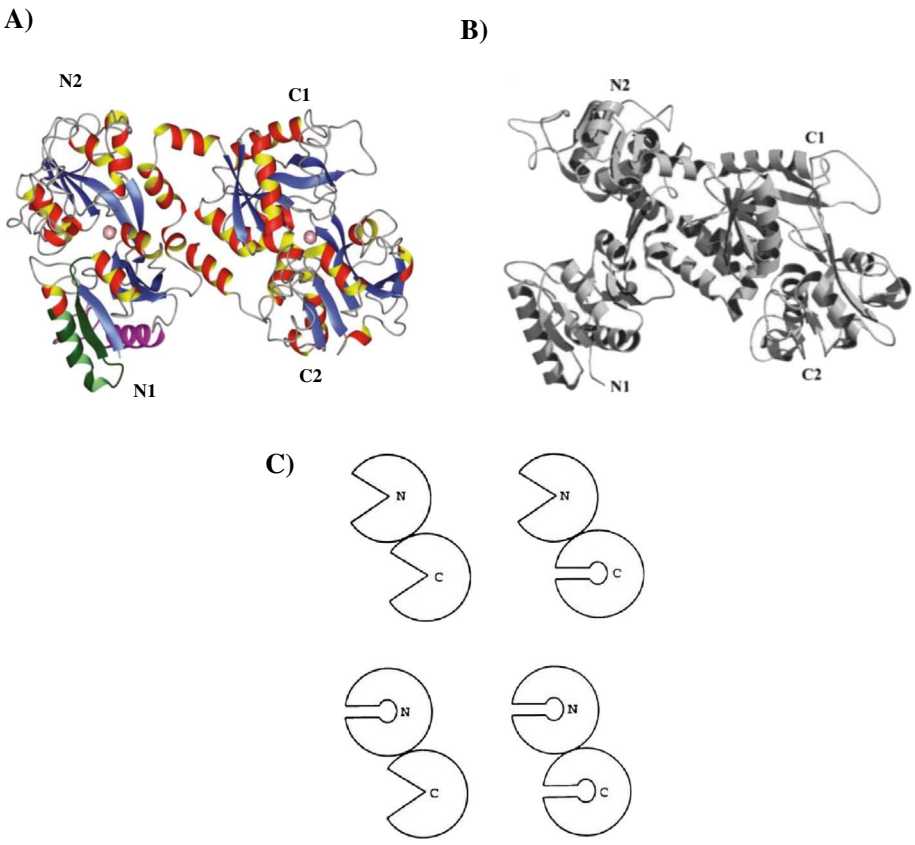

Fig. 1. A) Crystal structure of bovine holo-lactoferrin, each lobe of the protein presents a site for binding one Fe^{3+} iron (pink spheres, light grey in the print version). Lactoferricin region (residues 17–41) that is cleaved by pepsin in the gut is highlighted in green (dark grey in print). Lactoferrampin region (residues 265–284) is highlighted in purple (medium grey in print) (adapted from Vogel, 2012). B) In the apo-lactoferrin structure determined by Anderson, Baker, Norris, Rumball, and Baker (1990) shown here, the N-lobe appears iron-free and open, while the C-lobe was closed despite being without iron bound. C) The C-lobe can adopt open forms, through the same kind of conformational change as was seen for the N-lobe, the possible structures that could exist for the apo-form in solution are shown schematically, including structures with both lobes open, one open one closed, and both closed (adapted from Baker & Baker, 2004).

biological activity. The most important parameters which affect the structure of LF are pH, temperature, high pressure, ionic strength and the presence of other proteins and polysaccharides (Bengoechea, Peinado, & McClements, 2011; Sreedhara et al., 2010).

In 2000, LF was approved by the US Food and Drug Administration (FDA) as a Generally Recognized as Safe (GRAS) substance to be used in different food categories. However, in Europe it was nine years later, when the company Friesland Campina, formerly DMV International, made a request to place bovine LF on the market as novel food ingredient. The European Commission (EC) approved the use of bovine LF in different food categories in 2012 under Regulation (EC) No 258/97 of the European Parliament and of the Council. This regulation includes the specification of bovine LF and the maximum levels established for foods. Table 1 shows the maximum levels of bovine LF use in food, approved by FDA and EU. It is necessary to indicate that before the approval of LF as a novel food ingredient by the EC, the European Food Safety Authority issued the report “Scientific Opinion on bovine lactoferrin”. This report revealed that heat treated bovine LF, added as ingredient mainly in a non-denatured state, though with a certain percentage of denatured protein, had been demonstrated to have no toxicity in a wide variety of studies (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2012).

Table 1
Intended uses and maximum levels authorized for bovine lactoferrin (FDA, 2012; EUR-Lex 2012).

Food group	Maximum use level (USA)	Maximum use level (EU)
Yoghurt	100 mg/100 g	80 mg/100 g
Powdered milk	400 mg/100 g	330 mg/100 g
Infant formulae	100 mg/100 g	200 mg/100 g
Milk dessert	200 mg/100 g	130 mg/100 g
Chewing gum	3000 mg/100 g	3000 mg/100 g
Non-alcoholic drinks	n.r.	120 mg/100 g
Products based on cheese	n.r.	2000 mg/100 g
Cakes and pastries	n.r.	1000 mg/100 g
Candies	n.r.	750 mg/100 g

n.r: not reported values.

The number of publications with LF as the main subject increased markedly since 1992, as it is shown in Fig. 2. The approval of LF as food ingredient in 2000 by the FDA and in 2012 by the European Commission has probably increased the interest in the applications of LF, and consequently, those years mark an increase of the number of the studies on this protein. The number of publications registered in ScienceDirect in 2016 approaches one thousand publications. The accumulated number of publications on LF from 1990 until 2017 reaches 7456, 9237 and 15,262 in PubMed, Web of Science and ScienceDirect, respectively, which indicates the relevance of this protein in different scientific areas.

Several reviews have been published on the biological functions of LF, mainly on its antimicrobial activity against a great variety of microorganisms, and on its protective activity as anti-tumoral, anti-inflammatory and as immunomodulatory agent (Brock, 2012; González-Chávez, Arévalo-Gallegos, & Rascón-Cruz, 2009; Levay & Viljoen, 1995; Sánchez, Calvo, & Brock, 1992; Vogel, 2012; Weinberg, 2007). It has been proposed that milk LF is responsible for innate protection against intestinal illnesses in the neonate and therefore, it has a great potential to be used as a nutraceutical compound for infants as well as for adults. Actually, LF is administered as nutraceutical in tablets, and also as functional component in several types of products, such as infant formula, yoghurt, and dietary supplements for humans and pets (Franco, Castillo, Pérez, Calvo, & Sánchez, 2010; Satue-Gracia, Frankel, Rangavajhyala, & German, 2000; Steijns & van Hooijdonk, 2000; Tomita et al., 2009). Food products supplemented with LF are usually subjected to technological treatments, such as pasteurization or spray-drying, in order to make them safe for human consumption and to extend their shelf life. However, those treatments may have a deleterious effect on LF activity, effect that needs to be considered. Although, there are also some studies about the effect of technological treatments