Available online at www.sciencedirect.com
SCIENCE DIRECT
International Dairy Journal 16 (2006) 707–715
INTERNATIONAL DAIRY JOURNAL
www.elsevier.com/locate/idairyj

Review

Indigenous enzymes in milk: A synopsis of future research requirements

A.L. Kelly*, P.F. Fox

Department of Food and Nutritional Sciences, University College, Cork, Ireland

Received 31 May 2005; accepted 1 October 2005

Abstract

Milk contains a large number of indigenous enzymes, with differing functions, stability to processing, impact on dairy products, and significance for consumer safety (e.g., antimicrobial enzymes). Some enzymes are of interest for their beneficial activity (e.g., lactoperoxidase), some for use as indices of processing (e.g., alkaline phosphatase) and some for effects on the quality of dairy products (e.g., plasmin, lipoprotein lipase), which may be either positive or negative for different products. The study of enzymes in milk is a key specialisation within both the fields of biochemistry and dairy science, and remains an active research subject. Many questions remain to be answered about the nature and significance of milk enzymes, and progress is in some cases hampered by inconsistencies in assay methodologies being used; some of these issues are discussed in this article, the content of which was generated in part by discussions at the First IDF Symposium on Indigenous Enzymes in Milk, Cork, Ireland, 20–22 April 2005.

Keywords: Milk; Enzymes; Dairy products; Quality

Contents

*Corresponding author. Tel.: +353 21 4903405; fax: +353 21 4270001.
E-mail address: a.kelly@ucc.ie (A.L. Kelly).

0958-6946/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.idairyj.2005.10.018

1. Introduction

Study of the indigenous enzymes in milk has a long history, dating from the earliest days of enzymology and pre-dating by 50 years recognition that enzymes are proteins. About 70 indigenous enzymes have been identified in milk and several of these are significant in milk and milk products from a number of viewpoints; the best-characterised enzymes in milk are probably:

- *N*-Acetylglucosaminidase
- Acid phosphatase
- Alkaline phosphatase
- Amylase
- Bile-salts-stimulated lipase
- Catalase
- α-L-Fucosidase
- γ-Glutamyl transferase
- Glutathione peroxidase
- Lactoperoxidase
- Lipoprotein lipase
- Lysozyme
- Plasmin
- Ribonuclease
- Sulphydryl oxidase
- Superoxide dismutase
- Xanthine oxidoreductase

Andrews (1992) suggested that the days are over when enzymes in milk would be studied just because they were there. While this may be true in certain respects, the indigenous enzymes in milk are still an active area of research. Probably most, perhaps all, of the principal enzymes in bovine milk have been discovered but it is likely that some additional enzymes remain to be demonstrated as new, more sensitive, assays are developed.

About 20 enzymes have been isolated from milk and characterised; these are the enzymes present at high levels and/or that are significant from technological or other viewpoints. Many aspects of the principal enzymes in milk have been described in detail in various papers in these Proceedings. In this review, some potentially interesting areas for future research on milk enzymes, based on recent findings and suggested, in part, during discussions at the First IDF Symposium on Indigenous Enzymes in Milk, will be discussed. This is not intended to be an exhaustive review, but rather to raise some potentially interesting questions about the enzymes in milk. Many questions pertaining specifically to the proteolytic enzymes in milk are considered separately in a short review (Kelly, O'Flaherty, & Fox, 2006) in these Proceedings.

2. Overview of key aspects of milk enzymes

When considering enzymes in milk, a number of key issues should be considered:

- What enzymes are present in milk?
- What is the level of activity of each of these enzymes and how and why does activity vary?
- What processes applied to milk affect the activity of the indigenous enzymes?
- What is the practical significance of milk enzymes, i.e., do they affect the manufacture or quality of dairy products, or impact on food safety or consumer health?

3. What enzymes are present? Diversity of enzymes in milk

While there may be enzymes in milk which have not yet been identified, it is probable that the most significant activities are known, at least for good quality (i.e, low somatic cell count, SCC) milk. There have been sporadic (often only a single study) reports of certain indigenous enzymes in bovine milk; for example, Reimerdes (1983) reported the presence of an indigenous lysine aminopeptidase, which has not been confirmed. The presence of one enzyme reported previously in bovine milk, glutathione peroxidase, has been questioned (see Stagsted, 2006); the measured activity previously ascribed to glutathione peroxidase is probably due to the activity of sulphydryl oxidase.

To date, enzymes in only bovine and human milk have been studied in detail; there has been only limited and sporadic research on the enzymes of goat, sheep and buffalo milk and very little on milk of the horse, pig or other mammals. Considering the large inter-species differences in the level/activity of indigenous enzymes that have been demonstrated in the milk of the few species that have been studied, it is very likely that extending the range of species investigated should lead to interesting results. For example, human and mares' milk contain around 3000 times more lysozyme than cows' milk, for unknown reasons, although this may be related to the antibacterial function on this enzyme. There are also large inter-species differences in the activity of lactoperoxidase (LPO), and bile-salts-stimulated lipase occurs in the milk of only a few species.

4. What is the level of activity of indigenous enzymes in milk?

When considering measurement of the activity of any enzyme in milk, it is important first to define the objective of the measurement in terms of the aims of the study in question. For example, the level or activity of any enzyme could be considered from at least three perspectives:

- Total concentration of the enzyme in milk, e.g., measured by an enzyme-linked immunosorbent assay (ELISA) or after isolation of the enzyme from the milk. These techniques avoid the effects of inhibitory substances in the milk which could interfere with enzyme activity, but ELISA methods may not distinguish between active and inactive enzyme molecules.