Chapter 9

Transglutaminase Applications in Dairy Technology

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ABSTRACT

Consumers’ expectations from a dairy product have changed dramatically during the last two decades. People are now more eager to purchase more nutritious dairy foods with improved sensory characteristics. Dairy industry has made many efforts to meet such expectations and numerous production strategies and alternatives have been developed over the years including non-thermal processing, membrane applications, enzymatic modifications of milk components, and so on. Among these novel approaches, transglutaminase (TG)-mediated modifications of milk proteins have become fairly popular and such modifications in dairy proteins offer many advantages to the dairy industry. Since late 1980s, a great number of researches have been done on TG applications in milk and dairy products. Especially, milk proteins-based edible films and gels from milk treated with TG have found many application fields at industrial level. This chapter reviews the characteristics of microbial-origin TG as well as its mode of action and recent developments in TG applications in dairy technology.

INTRODUCTION

Enzymatic modifications of food proteins to create a tailor-made end product have been of interest to food industry and food scientists for many decades (Gerard, 2002; Jaros, et al., 2006a; Buchert et al., 2010). During the last two decades, efforts to optimize the yield, shelf-life and quality characteristics of foods by enzymatic modifications of food ingredients have been intensified. These efforts have led to introduction of a large number of commercial enzymes into food industry. Among those enzymes,
transglutaminase (TGase; protein-glutamine γ-glutamyltransferase, EC 2.3.2.13) has been widely investigated by researchers and its commercial forms have reached a great market success (Kuraishi et al., 2001). TGase was identified about 50 years ago in guinea pig liver as cytoplasmic TGase 2 (Sarkar et al., 1957). TGase is an enzyme naturally present in most animal tissues and body fluids (Sharma et al., 2001). It has also been discovered in microorganisms including Streptoverticillium mobaraense, S. ladakanum, S. cinamnoneum, Physarum polycepharum and Bacillus subtilis (Ando et al., 1989; Klein et al., 1992; Tsai et al., 1996; Duran et al., 1998; Kobayashi et al., 1998). TGase extracted from tissues or body fluids of animals (i.e., cattle, swine and fish) have been investigated for their suitability for food productions at industrial scale. Factor XIII, a type of TGase extracted from blood of cattle and swine at slaughter, was found to be unsuitable for food applications since this enzyme needs thrombin for its activation. In the presence of thrombin red pigmentation often occurs and limits animal origin TGase for food applications (Motoki & Seguro, 1998). With the improvements in transgenesis procedures, mass production of microbial TGase with low cost and controlled enzyme activity is now possible (Lerner & Matthias, 2015). For this purpose, host microorganisms used were Eschericia coli (guinea pig liver TGase, Streptoverticillium TGase, fish TGase) (Ikura et al., 1980; Takehana et al., 1994; Yasueda et al., 1995), yeasts (human factor XIIIa) (Bishop et al., 1990) and Streptomyces spp. (Streptoverticillium TGase) (Washizu et al., 1994). In 1980s, guine pig liver enzyme was widely investigated for the feasibility of modifying food proteins for industrial applications (Motoki & Nio, 1983; Motoki et al., 1984; 1986, 1987a, 1987b, Nio et al., 1985, 1986). Although these studies yielded promising results, limited supply of guine pig liver and the social and technical barriers against its use in food applications have encouraged researches to find alternative sources. A great deal of research has been dedicated to screen enzyme-producing strains of microorganisms to find out natural and suitable source(s) of TGase. The TGase-like enzymes secreted by the microorganisms were screened for their ability to form glutamine-lysine bonding between food proteins, which is characteristic for TGase (Nonaka et al., 1989). Then the enzyme extracted from a variant of Streptoverticillium mobaraense was found to be suitable food applications (Washizu et al., 1994). Later, other microorganisms were also screened for production of microbial TGase (mTGase). Readers may refer to Kieliszek & Misiewicz (2014) for current list of microorganisms screened for mTGase production.

TGase catalyses an acyl transfer reaction between γ-carboxyamide groups of peptide-bound glutamine residues (acyl donor) and the primary amino groups glutamine and lysine residues (Liu & Damodaran, 1999). Ammonia is released as a by-product of this reaction and could be used as a marker to monitor the reaction (Kellerby et al., 2006). TGase is able to catalyse three different types of reactions depending on the reaction conditions. These are i) acyl transfer reaction, ii) cross-linking (polymerisation) reaction between glutamine and lysine and iii) deamidation (Zhu & Tramper, 2008; Han et al., 2009). These reactions lead to the formation of new intra- and intermolecular bonds between proteins, which further modify the structure and functionality of proteins, i.e. solubility, water holding capacity, emulsification capacity, rennetability, thermal stability, ethanol stability etc. (Dickinson, 1997; Lorenzen & Schlimme, 1998; Motoki & Seguro, 1998; Lorenzen, 2000; O’Sullivan et al., 2002; Huppertz & de Kruif, 2007a). mTGase is an extracellular enzyme of the class of transferases (Yokoyama et al., 2004) and show no requirement to cofactors (Macedo & Sato, 2005). Although mTGase may show some activity at pH 4 and 9, its pH optimum is between 5 and 8. The optimum activity temperature of mTGase is around 55 ºC and the enzyme losses its activity at 70 ºC for a few minutes (Yokoyama et al., 2004). TGase is accepted as GRAS by FDA since 1998 and food aid by EFSA and is widely used in dairy, meat and bakery industries (Romeih & Walker, 2017). This chapter will only focus on the dairy applications of mTGase.
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