

# Phospholipase Applications in Cheese Production

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**Abstract:** Phospholipase is an enzyme that hydrolyzes phospholipids releasing a variety of products, like for example lyso-phospholipids, free fatty acids, di-acylglycerols, choline phosphate and phosphatidates, depending on the site of hydrolysis. In cheese production, lysophospholipids act as surface-active agents in the cheese curd, helping emulsification of water and fat during processing and reducing syneresis. Phospholipases are more specific and have little or no activity toward di- or triglycerides. As a result of phospholipid hydrolysis, flavor defects do not occur due to the main formation of palmitic, oleic, and stearic acids, which are non-volatile short chains fatty acids. According to the scientific studies the use of phospholipase is able to increase the yield of cheese and reduce the environmental impacts of cheese production. Protein and fat largely determine cheese yield. Depending on the milk composition, 75% to 78% of milk protein and 85% to 95% of milk fat are entrapped in the cheese curd. The remaining protein and fat are lost in the whey and, to a lesser extent, in the brine. Crucially in the production of pasta filata cheese fat losses occur in the hot stretching step, where the fresh curd is molded and stretched in hot water. The lysophospholipid-casein complexes should be studied to understand the mechanism leading to cheese yield improvements.

**Key words:** Phospholipase, cheese, yield.

## 1. Introduction

Phospholipases (A1, A2, B, C and D) are a group of enzymes that hydrolyze phospholipids (PLs) releasing a variety of products, like lyso-phospholipids, free fatty acids (FFAs), di-acylglycerols (DGs), choline phosphate and phosphatidates, depending on the site of hydrolysis [1].

Phospholipids having amphiphilic character account for 0.5%-1.0% of the total milk lipids [2]. Milk phospholipids are powerful emulsifiers [3]. About 65% of them are found in the milk fat globule membrane (MFGM), whereas the rest remain in the aqueous phase. Phosphatidyl choline, phosphatidyl ethanolamine and sphingomyelin are the major phospholipids of milk, which together comprise about 90% of the total [2]. During cheese manufacture, 85% to 95% milk fat and 75% milk protein are entrapped in the cheese curd and the rest lost in whey and brine. However, there occur fat losses during molding and stretching process of pasta filata cheeses [1].

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Increasing cheese yield is economically important commercially sense. Cheese yield is affected by milk composition and cheesemaking conditions [4]. Casein forms the structural matrix of cheese which retains fat and moisture [4]. Therefore, there are different methods for increasing cheese yield.

In this review, we will give an overview on phospholipase and use of phospholipase for increasing cheese yield.

## 2. Phospholipase Classification and Reactions

Phospholipases are hydrolytic enzymes that act on phospholipids are classified based on their substrate cleavage site as seen in Fig. 1.

The IUBMB enzyme nomenclature for phospholipase A<sub>1</sub>(PLA<sub>1</sub>) (EC: 3.1.1.32) catalyzes phosphatidylcholine + H<sub>2</sub>O = 2-acylglycerophosphocholine + a carboxylate reaction [5]. PLA<sub>1</sub> hydrolyzes of the ester bond at the *sn*-1 position of the phospholipid, thus producing a free fatty acid and 2-acyl lysophospholipid [6].

The IUBMB enzyme nomenclature for phospholipase A<sub>2</sub> (PLA<sub>2</sub>) (EC 3.1.1.4) catalyzes phosphatidylcholine + H<sub>2</sub>O = 1-acylglycerophosphocholine + a carboxylate reaction [5]. PLA<sub>2</sub> hydrolyzes of the ester bond at the *sn*-2 position of the phospholipid, thus producing a free fatty acid and 1-acyl lysophospholipid [6].

The IUBMB enzyme nomenclature for phospholipase B (PLB)(EC 3.1.1.5) catalyzes 2-lysophosphatidylcholine + H<sub>2</sub>O = glycerol phosphocholine + a carboxylate reaction [5]. PLB hydrolyzes both of the two fatty acids esterified at the *sn*-1 or *sn*-2 position of the phospholipid [6].

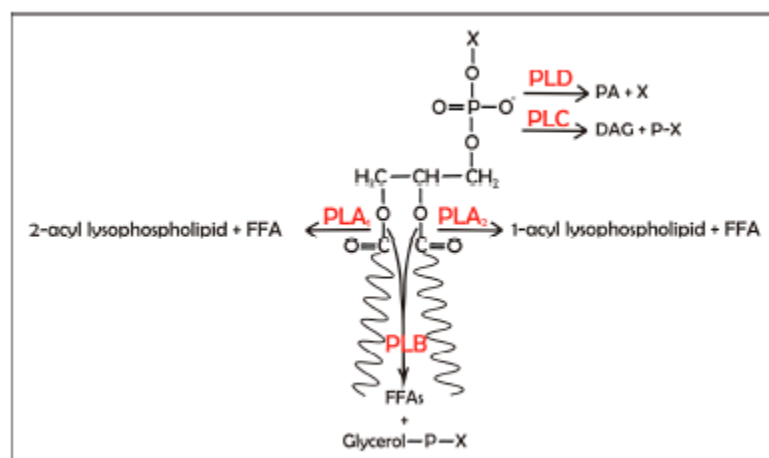
The IUBMB enzyme nomenclature for phospholipase C (PLC) (EC: 3.1.4.3) catalyzes phosphatidylcholine + H<sub>2</sub>O = 1,2-*sn*-diacylglycerol + phosphocholine reaction [5]. PLC cleaves the glycerophosphate bond, thus releasing diacylglycerol and the phosphorylated head-group [6].

The IUBMB enzyme nomenclature for phospholipase D (PLD) (EC: 3.1.4.4) catalyzes phosphatidylcholine + H<sub>2</sub>O = choline + a phosphatidate reaction [5]. PLD cleaves the terminal phosphodiesteric bond of the phospholipid, thus releasing phosphatidic acid (PA) along with the head-group [6].

### 3. Phospholipase Applications in Dairy

Milk is the complex fluid secreted in the udder of female mammals, whose constituents are water, proteins, fat, hydrocarbons, minerals, enzymes, and vitamins. Milk fatglobules (MFGs) consist mainly of triacylglycerol (TAG) (95%-98% w/w) and lipids are present as dispersed droplets [7]. However, there are also minor amounts of diacylglycerols, monoacylglycerols, free fatty acids, phospholipids (PLs) and sterols and trace amounts of fat-soluble vitamins, b-carotene and fat-soluble flavouring compounds in milk lipids [2].

MFGs have different technologic roles in dairy products. As examples, in milk processed to instant milk powder by co-spray drying, PLs coat the powder particles and improve the heat stability of reconstituted milk [7]. PLs provide higher volumes to aerated products such as ice cream, icings and whipped toppings [7]. PLs are used as co-emulsifiers for stabilization of low-fat dairy spreads contain 20%-60% fat [10]. During production of butter PLs have a significant influence on fat crystallization behavior. PL is acting as nucleating agent and retards crystals' growth during butter churning thus occurs smaller crystals [11].



**Fig. 1 Reactions catalyzed by the phospholipases [6].**

DAG, diacylglycerol; FFA, free fatty acid; PA, phosphatidic acid; P-X, phosphorylated head-group; X, head-group. X can be choline, ethanolamine, glycerol, inositol or serine.

The milk lipid fraction is known to contribute to the textural characteristics of many dairy products [8, 9].

#### 4. Use of Phospholipase in Cheese Production

In the cheese industry there has been an intense interest for increasing cheese yield. The application of phospholipase is one of the methods which can be applied to increase the cheese yield without decreasing the quality. The lysophospholipids that occur due to enzymatic hydrolysis of phospholipids increase cheese yield by improving fat and moisture retention in the curd, suggesting interactions of lysophospholipids with the protein matrix [12]. Lysophospholipids are more water-soluble [12]. Normally, 75% of milk protein and 85%-90% of milk fat are entrapped in the cheese curd. The rest is lost in whey and brine and fat is lost during molding and stretching process [7, 13]. Researches show that lysophospholipids improve the yield in a range of 0.7%-3.8% in total cheese [7, 14-16].

According to Sorensen et al. [17], a suitable dosage of phospholipase is in the range 0.003-0.7 mg enzyme protein per g milk fat.

*Fusarium venenatum* PLA1 has the highest activity in milk [12] and is commercially available.

The study of Lilbæk et al. [12] showed that the surface properties of milk change by PLA1 through release of surface-active lysophospholipids from the MFGM.

In a study [14] where PLA1 added into the cheese milk at a rate of 5 LEU per gram of milk fat with EPS-producing culture cheese yield is reported to increase.

Lilbæk et al. [16] added PLA1 in a dose corresponding to 5 lecithase units/g of milk fat to cheese milk and reported that the total cheese yield was increased by 3.2% in the phospholipase vats compared with the control vats.

#### 5. Results and Suggestions

It is obvious that enzymatic hydrolysis of milk phospholipids by phospholipases has increased the yield of Mozzarella cheese, Pizza cheese, Chihuahua-type cheese.

The effect of the oil and water retained in the cheese matrix as a result of enzymatic hydrolysis of milk phospholipids on ripening cheeses such as Kashar, Cheddar, Colby, Edam, Muenster, Gruyere, Emmenthal, Camembert, Parmesan and Romano cheeses can be studied.

The interaction of lysophospholipids with the protein matrix and the surface activity of the lysophospholipid-protein complexes should be studied in more detail to understand the mechanism leading to yield improvements.

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