CHAPTER 5

ENZYMATIC ACTIVITY OTHER THAN LIPASE

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ABSTRACT

In this chapter, enzymes (other than lipase) which are present in cream are discussed. The effects of heat treatments on the activities of these enzymes are described. The influence of residual enzyme activity, remaining after heating, on cream quality is also discussed.

5.1 INTRODUCTION

Many of the enzymes present in whole milk are also present in the cream phase. Here the enzymes exist predominantly as components of the milkfat globule membrane (MFGM) system in lipoprotein complexes with other membrane constituents. This is in contrast to their form in whole milk, where the enzyme may exist as soluble whey components or in association with casein micelles. The different environment of enzymes in cream compared to whole milk may influence the behaviour of these enzymes under certain heating conditions.

The major enzymes detected in cream or MFGM preparations and their distributions in the cream phase will be presented. Heat inactivation of the cream enzymes will be examined, in comparison with whole milk enzymes. The implications of residual enzyme activity after heat treatments on the quality of pasteurized cream will also be discussed.

5.2 CREAM ENZYMES

Table 5.1 lists the enzymes which have been detected in raw cream or MFGM preparations. The more extensively studied cream enzymes are discussed below.

Table 5.1: Enzymes in raw cream

Enzyme	Literature
β-N-Acetylglucosaminidase	Mellors (1968)
Acid phosphatase	Dowben et al. (1967); Kitchen et al. (1970); Kitchen (1974)
Aldolase	Polis & Schmukler (1950); Dowben et al. (1967); Kitchen et al. (1970)
Alkaline phosphatase	Hayashi et al. (1965); Dowben et al. (1967); Kitchen et al. (1970); Kitchen (1974)
Catalase	Mellors (1968); Kitchen et al. (1970); Ito (1972)
Cholinesterase	Dowben et al. (1967)
Diaphorase	Morton (1954); Kitchen (1974)
β-Galactosidase	Anderson & Cheeseman (1975)
Glucose-6-phosphatase	Dowben et al. (1967); Kitchen et al. (1970); Kitchen (1974)
β-Glucosidase	Anderson & Cheeseman (1975)
γ-Glutamyl transpeptidase	Majumder & Ganguli (1972); Kitchen (1974)
Inorganic pyrophosphatase	Kitchen (1974)
Mg^{2+} - ATPase	Dowben et al. (1967); Patton & Trams (1971); Kitchen (1974)
NADH: cytochrome c reductase	Dowben et al. (1967)
NADH dehydrogenase	Evans & Pillinger (1973); Kitchen (1974)
5'-Nucleotidase	Patton & Trams (1971); Huang & Keenan (1972); Kitchen (1974)
Nucleotide pyrophosphatase	Patton & Trams (1971); Kitchen (1974)
Plasmin	Hofmann et al. (1979)
Ribonuclease	Kitchen et al. (1970)
Sulfydryl oxidase	Kitchen (1974)
Xanthine oxidase	Dowben et al. (1967); Kitchen et al. (1970); Briley & Eisenthal (1974, 1975); Kitchen (1974)

Acid phosphatase has been shown to be strongly bound to MFGM components in cream (McPherson & Kitchen, 1982), in contrast to the enzyme in skim milk which exists in a free form (Bingham et al., 1961). Despite the differences in the environments of these enzymes, Bingham et al. (1961) reported no significant differences in the enzymatic properties of cream and skim milk acid phosphatase.

Alkaline phosphatase has been reported to be associated with MFGM lipids in cream (Groves, 1971). Release of this enzyme from cream to the skim milk phase has been used to assess agitation treatments of raw milk (Stannard, 1975). Copius Peereboom (1968) reported that the distribution of the isozymes of alkaline phosphatase in cream was different from that in whole milk, with the β-form of the enzyme apparently being closely associated with the lipoprotein complexes of the MFGM, while the α-form was more loosely complexed to the membrane. Further, the β-isozyme was the only enzyme fraction capable of reactivation and it was suggested that the reactivation of alkaline phosphatase in cream was influenced by the structure of the MFGM lipoproteins around the β-isozyme (Copius Peereboom, 1968). However, it is possible that these isozymes may simply be complexes of alkaline phosphatase bound to different sized membrane fractions (Kitchen, 1985).

Catalase detected in cream or MFGM preparations may be present in leucocytes which have either adhered to the fat globules (Peters & Trout, 1945) or which have ingested fat globules and separated with the cream phase (Anderson et al., 1975). However, at least some of the catalase appears to be bound to the membrane, as it can not be removed by washing (Mulder & Walstra, 1974). Ito (1971) reported that most of the catalase in buttermilk was complexed to MFGM lipoproteins.

Majumder & Ganguli (1972) presented data which suggested that γ-glutamyl transpeptidase occurred in milk as lipoprotein complexes with membrane components. Recent research on this enzyme has centred on examining its relationship to sulfydryl oxidase, another membrane constituent. Iwami et al. (1981) suggested that sulfydryl oxidase and γ-glutamyl transpeptidase activities were due to the same enzyme molecule, with sulfydryl oxidase activity being a non-enzymatic process initiated by γ-glutamyl transpeptidase. However, Sliwkowski et al. (1983) showed these two activities were distinct and could be separated.

5'-Nucleotidase is present in cream as a constituent of the MFGM (Kitchen, 1974). Two forms of this enzyme which have different substrate specificities and kinetic properties and are complexed to different amounts of membrane lipids have been detected in cream and MFGM preparations (Huang & Keenan, 1972).

Plasmin (natural milk proteinase) is mainly associated with the casein fraction of milk (Humbert & Alais, 1979). Low levels have been detected in MFGM preparations (Hofmann et al., 1979) and this enzyme has been implicated in the development of bitter flavours in UHT cream (Richter et al., 1979).

Xanthine oxidase is an important constituent of cream, representing approximately 10% of the total protein components of the MFGM (Briley & Eisenthal, 1975). Kitchen (1977) has demonstrated that xanthine oxidase is a constituent of a major membrane lipoprotein complex. Briley & Eisenthal (1975) have reported that two forms of the enzyme are present, one as a tightly bound membrane constituent and the other in a lipoprotein complex which can be released in a soluble form. These may represent the inactive and active forms of xanthine oxidase proposed by Greenbank & Pallansch (1962). Further work (Sullivan et al., 1982) has shown that xanthine oxidase exists in the membrane in a number of forms. These could be genetic variants, different forms of the enzyme (that is, de-sulfo, de-molybdo), immunologically distinct proteins or could be due to different degrees of association with MFGM components.

The distributions in raw cream of some of the enzymes, expressed as a percentage of their activities in whole milk, are shown in Table 5.2.

The variations in the distribution values of individual enzymes may reflect the extent of milk separation (that is, yielding a low or high fat cream). Some

Table 5.2: Distribution of enzymes in raw cream

Enzyme	% Of whole milk activity	Literature
Acid phosphatase	21	Bingham et al. (1961)
	23	Kitchen et al. (1970)
Aldolase	19	Kitchen et al. (1970)
Alkaline phosphatase	30	Morton (1953)
	40	Zittle et al. (1956)
	31	Kitchen et al. (1970)
	49-91	Stannard (1975)
Catalase	24	Kitchen et al. (1970)
γ-Glutamyl transpeptidase	47	Majumder & Ganguli (1972)
	23	Baumrucker (1979)
Ribonuclease	3	Kitchen et al. (1970)
Xanthine oxidase	20-81	Gudnason & Shipe (1962)
	21	Kitchen et al. (1970)
	41-85	Stannard (1975)

researchers have also reported different levels depending on sample treatment. Gudnason & Shipe (1962) reported that the distribution of xanthine oxidase in freshly separated cream ranged from 40 to 62% which increased to 72-81% on storing the separated cream for 24 h at 4°C. When whole milk was subjected to the same storage conditions prior to separation, the percentage of xanthine oxidase in the cream fraction decreased to 20-24%. The authors attributed this change in distribution on storage to the release of the enzyme from the cream membrane to the skim milk milk phase in a more active form. Stannard (1975) also observed that the distributions of xanthine oxidase and alkaline phosphatase in cream were substantially reduced on cooling milk before separation. This treatment may have caused a loss of the loosely bound α-form of alkaline phosphatase from the membrane environment (Copius Peereboom, 1968).

5.3 EFFECTS OF HEATING ON CREAM ENZYMES

While the effects of heat treatments on the enzymes in whole milk and skim milk have been studied quite extensively, little research has been conducted on the enzymes in cream. In addition to fat globules, cream also contains a skim milk phase, with the level of these components being dependent on the degree of separation from whole milk. Therefore, the enzyme complement of cream would arise from constituents in both free and membrane-bound forms. It is largely unknown if heat treatments would have similar effects on these enzymes.

Acid phosphatase is a relatively heat-stable enzyme which survives normal pasteurization treatments of whole milk. More severe heating conditions (88°C, 30 min, 100°C, 1 min) are required for complete inactivation (Mullen, 1950). This enzyme has been reported to survive UHT sterilization under certain conditions (Andrews & Pallavicini, 1973). The different forms of acid phosphatase present in cream appear to be affected by heating to a similar extent, with approximately 70% of the activity remaining in cream and skim milk after a heat treatment of 65°C for 15 min (Bingham et al., 1961). The temperature of storage after heating may also affect residual acid phosphatase activity. Mullen (1950) found that storage of whole milk for 48 h at 17°C resulted in a 40% reduction in activity. Similar losses may occur in UHT creams which have extended storage periods at ambient temperatures.

Aldolase has been reported to be heat-labile, being unstable at 37°C (Polis & Schmukler, 1950) and is therefore unlikely to be present in pasteurized creams.

Alkaline phosphatase has been studied in detail because of its use in the milk pasteurization test. The enzymes in cream and skim milk appear equally affected by pasteurization with complete inactivation observed after heating at 65°C for 15 min (Bingham et al., 1961). Reactivation of alkaline phosphatase was initially observed during storage of pasteurized creams (Brown & Ellikey, 1942), and subsequently in milks and creams which had been heated at higher temperatures (Copius Peereboom, 1966; Murthy et al., 1976). The optimum storage temperature for reactivation has been reported to be 30-34°C (Wright & Tramer, 1953; Murthy et al., 1976), with the extent of reactivation increasing as the fat level in the product increased (Murthy et al., 1976). This may be due to a higher initial enzyme concentration in high-fat products or to different isozymes being present, as proposed by Copius Peereboom (1968). From these studies it would appear that of the commercial dairy products UHT creams may exhibit the greatest extent of reactivation.

While some information is available on the heat inactivation of catalase in a partially purified form (Ito, 1973), the presence of other milk components and the environment of the enzyme (that is, membrane-bound or free) may alter the inactivation characteristics of this enzyme in cream. γ-Glutamyl transpeptidase and sulfydryl oxidase are not completely inactivated in whole milk under pasteurization conditions (Swaisgood, 1980; Kitchen, 1985) and therefore would probably be present in pasteurized creams. Huang & Keenan (1972) reported that heating purified 5'-nucleotidase at 60°C for 30 min resulted in a loss of 20% of enzyme activity. Snow et al. (1980) have shown that soluble 5'-nucleotidase has different kinetic properties to the membrane-bound form which may affect the heat stability of this enzyme on pasteurization of cream. Plasmin has been shown to be extremely heat-stable, surviving pasteurization and certain UHT treatments (Humbert & Alais, 1979; Snoeren et al., 1979). Its presence in pasteurized creams may lead to flavour and stability problems on storage of these products. Ribonuclease has been shown to survive heating at 90°C for 5 min (Zittle, 1964) and some residual activity may be present in pasteurized creams, although the levels associated with the fat phase are very low (Kitchen et al., 1970).

Xanthine oxidase has been implicated in lipid peroxidation in dairy products (Aurand & Woods, 1959; Aurand et al., 1977). This enzyme is not completely destroyed by pasteurization of whole milk, with 18-31% of the xanthine oxidase activity remaining after processing (Cerbulis & Farrell, 1977; Zikakis & Wooters, 1980). The purified enzyme can be inactivated by milder heat treatments (Bergel & Bray, 1959), which indicates that the presence of milk components may exert a stabilizing effect on the enzyme. Greenbank & Pallansch (1962) found that xanthine oxidase was considerably more resistant to the effects of heat in cream, with activity still detected after 15 min at 77°C, whereas the skim milk enzyme was completely inactivated after 10 min. It would appear that the free enzyme in skim milk is more heat sensitive than the membrane-bound form.

Storage of cream at 4°C resulted in an increase in xanthine oxidase activity possibly due to release of the enzyme from the membrane environment in a more active form (Gudnason & Shipe, 1962). However, Bhavadasan & Ganguli (1980) have proposed that this observed increase in xanthine oxidase activity could be due to an increase in activity of the membrane-bound form as a result of structural changes in the membrane induced by cold storage.

5.4 IMPLICATIONS FOR QUALITY

It is apparent that some residual enzyme activity could remain after pasteurization of cream and could affect the quality of the product, particularly on storage.

Acid phosphatase removes phosphate groups from casein, causing changes in micelle stability (Bingham et al., 1972). Since casein is present in the skim milk phase of cream, residual acid phosphatase activity may lead to problems such as gelling after UHT treatments (Andrews & Pallavicini, 1973). Reactivation of alkaline phosphatase during storage would not be expected to cause degradative changes in cream, since the action of this enzyme is inhibited by the presence of certain milk components, for example, lactose and β-lactoglobulin (Lorrient & Linden, 1976). The major technological significance of alkaline phosphatase reactivation is that it diminishes the reliability of the phosphatase test, making it difficult to assess whether correct pasteurization conditions have been applied. Catalase has been reported to oxidize unsaturated fatty acids (George, 1952). MFGM lipids contain a significant proportion of unsaturated fatty acids (Kitchen, 1977) and degradation of these components could destabilise the membrane structure which may result in the appearance of free fat in the product.

The reduction in quality due to proteolysis is particularly important in UHT products which are stored for extended periods at ambient temperatures. Driessen & van der Waals (1978) have shown that plasmin preferentially attacks β-casein which could cause gelling of the product on storage (Humbert & Alais, 1979). Plasmin has also been implicated in the development of bitter flavours in milks and dairy products (Richter et al., 1979). This enzyme has been shown to rapidly hydrolyze one of the components of the MFGM (Hofmann et al., 1979), which could cause breakdown of the membrane around the fat globules in cream.

Sulfydryl compounds which yield cooked flavours can be produced by heating milk (Kiermeier & Hamed, 1961). A large proportion of these compounds may originate from cysteine groups in the MFGM (Mulder & Walstra, 1974). These residues appear much more reactive in the membrane than in skim milk (Mulder & Walstra, 1974), therefore these flavours may be more pronounced in pasteurized creams. Sulfydryl oxidase oxidises sulfydryl groups to disulfides (Shipe et al., 1975). Since this enzyme survives pasteurization, the residual enzyme may act to reduce the level of these flavour compounds during storage.

Xanthine oxidase has been implicated in the development of oxidized flavours in dairy products as a result of lipid peroxidation (Aurand & Woods, 1959; Aurand et al., 1977; Bruder et al., 1980). The lability of unsaturated fatty acids towards enzymatic oxidation increases with the degree of unsaturation (Aurand et al., 1967). Therefore it appears likely that MFGM lipids would be susceptible to oxidative degradation and may be involved in the initial steps of lipid peroxidation. This was confirmed by Allen & Humphries (1977) who also found that the lipid oxidizing ability of xanthine oxidase was increased by heat denaturation of the protein components of the membrane. A similar effect may occur in pasteurized creams.

Owing to the nature and reactivity of the membrane around the fat globules, pasteurized creams would appear to be more susceptible than pasteurized milks to certain changes induced by residual enzyme activity.

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