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# Industrial Application of Lipases in Cheese Making: A review

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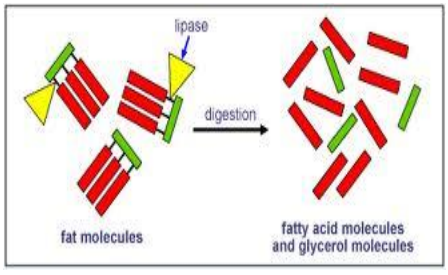
**Abstract**

*Lipases are extensively used in the dairy industry for the hydrolysis of milk fat. The dairy industry uses lipases to modify the fatty acid chain lengths, to enhance the flavours of various cheeses. Current applications also include the acceleration of cheese ripening and the lipolysis of butter, fat and cream. The free fatty acids generated by the action of lipases on milk fat endow many dairy products, particularly soft cheeses with their specific flavor characteristics. Lipolysis is an important biochemical event occurring during cheese ripening and has been studied quite extensively in varieties such as Blue and hard Italian cheeses where lipolysis reaches high levels and is a major pathway for flavour generation. However, in the case of cheeses such as Cheddar and Gouda, in which levels of lipolysis are moderate during ripening, the contribution of lipolytic end products to cheese quality and flavour has received relatively little attention. The progress of lipolysis and its effect on flavour development during cheese ripening is reviewed. FFA are important precursors of catabolic reactions, which produce compounds that are volatile and contribute to flavour. The aim of this work is to review the existing knowledge of the way in which lipolysis and FFA catabolism proceed during cheese ripening, how lipolysis may be measured and monitored and also how this biochemical event contributes to cheese flavour.*

**Key words:** ***Lipolysis; Cheese ripening; Catabolic products; Flavour***

## Introduction

Lipases (EC 3.1.1.3) are enzymes that catalyse the hydrolysis of triglycerides (triacylglycerols), the major lipid components of milk. This hydrolysis is commonly referred to as lipolysis. The products of the reaction are free, or nonesterified fatty acids, and partial glycerides (mono- and diglycerides) and, in some cases, glycerol. By definition, lipases act at the lipid–water interface of emulsions of longchain, insoluble triglycerides, while the related esterases act on esters of short-chain fatty acids (and other acids) and soluble esters (Jaeger et al., 1994).




The significance of lipolysis in milk is two-fold: flavor production and altered functionality. Free fatty acids (FFAs), particularly those of short and medium chain length, have strong flavours that, in most cases, are considered undesirable. The flavour of whole milk with an elevated FFA level, 4-1.5 mmol L-1, is unacceptable to most people (IDF, 1987) and is variously described as rancid, butyric, astringent or even bitter, although this last flavour is usually attributable to products of proteolysis not lipolysis.

The term ‘‘rancid’’ is also used to describe the off-flavour due to lipid oxidation. In general, oxidative and hydrolytic rancidities are not related, although there is some evidence that FFAs are more susceptible to oxidation than the parent triglycerides. Flavours due to lipolysed fat can also be desirable; some cheeses such as certain hard Italian and Blue varieties owe their characteristic flavour to the presence of FFAs (Deeth & Fitz-Gerald, 1995).

A major functionality effect of lipolysis in milk is depression of its foaming ability when injected with steam

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demonstrates the relationship between foaming capacity (steam frothing value) and degree of lipolysis (as FFA). This effect is manifested in difficulty in producing acceptable foam when making cappuccino coffee. It is due to the partial glycerides produced during lipolysis, which are surface active and displace the foam-stabilizing proteins at the air–water interface of the foam bubbles. Some other functionality defects, such as impaired creaming ability during separation and increased churning time in the manufacture of butter, have been reported (Deeth & Fitz-Gerald, 1995).

**Sources of lipases**

**1. Animal lipases**

The most animal lipases from Porcine Pancreas (EC 3.1.1.3) are derived from kid-goats, lambs, and/or calves sources. The component that animal lipase is made from is generally proprietary, but sources in literature say drying and grinding of pre-gastric glands at the base of the calves tongues.

**2. Microbial lipases**

The lipase/esterase system of Lactic Acid Bacteria (LAB) has received much less attention then their proteolytic. Lactococcus sp. Are only weakly lipolytic, but lactococci may be responsible for the liberation of quite high levels of FFA when present in high cell numbers or over extended ripening periods. Lipase/esterase of Lactococcus strains, which appear to be intercellular, have been studied (Chich et al., 1997). Obligately homofermentative lactobacilli used as starter (Lb. helveticus, Lb. delbrueckii subsp. bulgaricus and Lb. delbrueckii subsp. lactis) also produce esterase, some of which have been studied (El-Soda et al., 1986; Gobbetti et al., 1996 and Khalid et al., 1990). Facultatively heterofermentative lactobacilli (e.g., Lb. casei, Lb. paracasei and Lb. plantarum) (Gobbetti et al., 1997). Table 5 describes the use of lipase in cheese making and accelerated cheese ripening. The free fatty acids take part in simple chemical reactions that initiate the synthesis of other flavour ingredients, such as acetoacetate, b-keto acids, methyl ketones, flavour esters and lactones.

**3. Plant Lipases**

Lipases from plants are broadly classified into: 1- Triacylglycerol lipases (EC 3.1.1.3) or 'true' lipases that hydrolyze the ester bonds of storage triacylgycerols of seeds, such as oilseeds and cereal grains. 2- Non-specific lipid acylhydrolases exhibiting combined action of phospholipases A1 (EC 3.1.1.32), A2 (EC 3.1.1.4), B (EC 3.1.1.5), glycolipase, sulfolipase and monoacylglycerol lipase, which occur in diverse plant tissues; and 3- Phospholipases C (EC 3.1.4.3) and D (EC 3.1.4.4), the latter being more widely distributed in plants. Several reviews have extensively covered the occurrence, properties and physiological role of various lipases in plants (Mukherjee, 1994). El-Hofi and Ismail (1998-2000) Utilizated purified plant lipase from Papaya (Carica papaya) in acceleration of Ras cheese slurry. Degheidi et al., (1998) used lipase extracted from jack fruit seeds for improving the quality of Domiati cheese (Fig.1)..

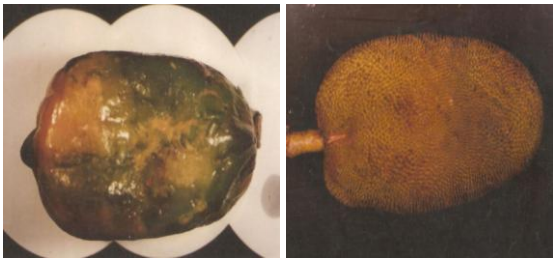

Fig. 1. Papaya (Carica papaya) and Jack fruit (Artocarpus integrifolis)

Table 5—Examples of lipase in cheese production

| Cheese type | Lipase source |
|---|---|
| Romano | Kid/lamb pre-gastric |
| Domiati | *Mucor miehei* |
| Feta | |
| Camembert | *Penicillium camemberti* |
| Mazarella | Calf/kid pre-gastric |
| Parmesan | |
| Provolone | |
| Fontina | *Mucor miehei* |
| Ras | |
| Romi | |
| Roque fort | *Penicillium roqueforti* |
| Cheddar | *Aspergillus oryzae/A. niger* |
| Manchego | |
| Blue | |

**Characteristics of Lipase**

According to Lee and Lee (1990), esterolytic and lipolytic enzymes were produced by cell lysis of Lb. casei subsp. casei LLG. Maximum lipolytic activity was observed at pH 7.2 and 37℃; enzyme activity was inhibited by Ag+ and Hg+2 ions and stimulated by Mg+2 and Ca+2. Activators: Bile salts, Ca+2 is required for activity [Sr+2 and Mg+2 are less effective activators. Inhibitors: Versene, Zn+2, Cu +2, Hg+2, iodine. Stabilizers: DFP may be used to stabilize impure preparations containing proteinases in solutions. Stability: Highly purified, homogenous preparations of hog pancreas lipase are extremely labile

**Lipase addition**

Lipase is always added to the milk before it starts to coagulate. So, if you are making a fresh cheese (where the starter and rennet are mixed together), you add it before the starter. (That goes for any cheese, like a lactic acid cheese, where the curds coagulate quickly from the action of the starter.)

If you are making a cheese with rennet that is separate from the starter, you need to add the lipase right before you add the rennet. (This assures that the lipase does not interfere with the starter.)

Always dissolve your lipase in chlorine-free (or distilled) water before adding it to your milk (up to 1/2 cup of water).

**Assay of lipase activity**

The lipase determination was carried out according to procedure of Tigerstrom and Stelmashuk, 1989 by using the following reagents:

**Substrate:**

(a) 2% Tween 20 (v/v) solution in 20 mM Tris HCl buffer at pH 7.0

(b) 120 mM $CaCl_2$

(c) Standard curve of pure lipase with different concentration.

**Procedure:**

The reaction mixture had the following composition:

3.6 ml of substrate, 0.1 ml of 120 m M CaCl2 and 0.3 ml of enzyme extract. The mixture was incubated at 30℃ for 30 min. The reaction was followed periodically by measuring the increase in the optical density at 500 nm due to the fatty acids from Tween 20 and then precipictation as the activity was expressed as mg pure lipase / ml.

Unit Definiton: One Unit releases one micromole of fatty acid per minute from emulsified olive oil at 25℃, pH 8.0

## Lipolysis

**1. Lipolysis and related events**

Lipids have a major effect on the flavor and texture of foods, including cheese. Lipids contribute to cheese flavor in three ways: 1- They are a source of fatty acids, especially short chain fatty acids, which have strong and characteristic flavors. Fatty acids are produced through the action of lipases in a process referred to as Lipolysis. In some varieties, the fatty acids may be converted to other sapid and aromatic compounds, especially methyl ketones and lactones. 2- Fatty acids, especially polyunsaturated fatty acids undergo oxidation, leading to the formation of various unsaturated aldehydes that are strongly flavored and cause a flavor defected as oxidative rancidity. Lipids oxidations appears to be very limited in cheese, probably owing to its low redox potential (-250 mV). 3- Lipids function as solvents for sapid and aromatic compound produced not only from lipids but also from proteins and lactose. Lipids may also adsorb from the environment compounds that cause off-flavors. Of the various possible contribution of lipids to cheese flavor, lipolysis and modification of the resultant fatty acids are the most significant. The degree of lipolysis in cheese varies widely between varieties, from about 6 mEq free fatty acids in Gouda to 45 mEq/100g fat in Danish Blue (Gripon, 1993).

**2. Lipolytic Agents in milk and cheese**

It is well established that milk fat is essential for the development of correct flavour in cheese during ripening. This was demonstrated in studies with cheeses made from skim milk, or milk in which milk fat had been replaced by other lipids; such cheeses did not develop correct flavour (El-Safty & Isamil, 1982; Wijesundera et al., 1998). Lipids present in foods may undergo oxidative or hydrolytic degradation (McSweeney & Sousa, 2000). Polyunsaturated fatty acids are especially prone to oxidation, which leads to the formation of various unsaturated aldehydes that are strongly flavoured and result in the flavour defect referred to as oxidative rancidity (Fox et al., 2000). Lipolytic enzymes may be classified as esterases or lipases, which are distinguished according to three main characteristics: (1) length of the hydrolysed acyl ester chain, (2) physico-chemical nature of the substrate and (3) enzymatic kinetics. FFA are released upon lipolysis and contributes directly to cheese flavour, especially short- and intermediate-chain FFA. The proportions of free C6:0 to C18:3 in Cheddar cheese appear to be similar to those in milk fat. However, free butanoic acid (C4:0) occurs at a greater relative concentration in cheese than in milk fat, suggesting its selective release by lipases present in cheese or its synthesis by the cheese microflora (Fox et al., 2000; McSweeney & Sousa, 2000). Lipases in cheese originate from six possible sources: (1) the milk, (2) rennet preparation (rennet paste), (3) starter, (4) adjunct starter, (5) non-starter bacteria and, possibly, (6) their addition as exogenous lipases (McSweeney & Sousa, 2000).

**2.1. Indigenous milk lipase**

Milk contains a very potent indigenous lipoprotein lipase (LPL), which normally never reaches its full activity in milk (Fox & Stepaniak, 1993). The enzyme is present in milk due to leakage through the mammary cell membrane from the blood where it is involved in the metabolism of plasma triacylglycerides. Bovine milk contains 10–20 nm L-1 lipase which, under optimum conditions (37℃, pH 7) with addition of an apolipoprotein activator, apo-CII, could theoretically release sufficient FFA acids within 10 s to cause perceptible hydrolytic rancidity. Hydrolysis of as little as 1–2% (w/v) of the milk triacylglycerides to fatty acids gives a rancid or ‘‘lipolysed’’ flavour to the milk. This does not occur under normal circumstances as LPL and fat are compartmentalized; ca. 90% (w/v) LPL in milk is associated with the casein micelles and the fat, occurring in globules, is surrounded by a lipoprotein membrane (MFGM). If the MFGM is damaged, e.g., due to agitation, foaming, homogenization, inappropriate milking or milk-handling techniques, significant lipolysis may occur resulting in off-flavours in cheese and other dairy products (Fox et al., 2000). LPL has been shown to be relatively non-specific for fatty acid type, but is specific for the sn-1 and sn-3 positions of mono-, di- and triacylglycerides (Olivecrona et al., 1992). Therefore, short- and medium-chain fatty acids are preferentially released by LPL. LPL is of more significance in raw milk cheeses than in cheeses

made from pasteurized milk, since its activity is not reduced by pasteurization. According to Deeth and Fitz-Gerald (1995), it is generally accepted that high-temperature short-time (HTST) treatment (72℃ for 15 s) inactivates the enzyme very extensively. However, it is still thought to contribute to lipolysis in pasteurized-milk cheese, as 78℃ for 10 s is required for its complete inactivation . More recently, Chavarri et al., (1998) studied the enzymology of industrial raw and pasteurized ewes' milk and Idiazabal cheese made from these milks.

**2.2. Rennet paste**

Commercial rennets are normally free from lipolytic activity. However rennet paste, used in the manufacture of some hard Italian varieties (e.g., Provolone, Romano), contains the lipase, pregastric esterase (PGE) (Nelson et al., 1977). PGE is highly specific for short chain acids esterified at the sn-3 position. Suckling stimulates the secretion of PGE at the base of the tongue, and it is washed into the abomasa with the milk. Rennet paste is prepared from the abomasa of calves, kids or lambs slaughtered after suckling. The abomasums is partially dried and ground into a paste, which is slurried in milk before being added to cheese milk (Fox & Stepaniak, 1993). Some interspecies differences in specificity have been reported for calf, kid and lamb PGEs which result in slight differences in flavor characteristics of the cheese, depending on the source of PGE ( Fox & Stepaniak, 1993).

**2.3. Microbial lipase**

Lipases and esterases of lactic acid bacteria (LAB) appear to be the principal lipolytic agents in Cheddar and Dutch-type cheeses made from pasteurized milk (Fox et al., 2000). Evidence for this comes from the very low levels of FFA in aseptic starter-free cheeses made using glucono acid-d-lactone, rather than in cheese made using starter culture. Despite the presence of these enzymes, LAB, especially Lactococcus and Lactobacillus spp. are generally considered to be weakly lipolytic in comparison to species such as Pseudomonas, Acinetobacter and Flavobacterium (Chich et al., 1997). However, because of their presence in cheese at high numbers over an extended ripening period, LAB are considered likely to be responsible for the liberation of significant levels of FFA. To date, lipases/esterases of LAB appear to be exclusively intracellular and a number have been identified and characterized (Chich et al., 1997; Castillo et al., 1999; Liu et al., 2001). El-Soda et al., (1986) found intracellular esterolytic activities in four strains of lactobacilli: Lb. helveticus, Lb. delbrueckii subsp. bulgaricus, Lb. delbrueckii subsp. lactis and Lb. acidophilus. All lactobacilli showed activity against substrates up to C5:0; Lb. delbrueckii subsp. lactis and Lb. acidophilus displayed the highest esterolytic activities. None of the strains tested hydrolysed o- and p-nitrophenyl (p-NP) substrates containing fatty acids of the even numbered carbon atoms from 6 to 14. Esterase activity was higher than lipase activity in all strains. Kamaly et al., (1990) reported the presence of lipases in the cell-free extracts of a number of strains of Lc. lactis subsp. lactis and Lc. lactis subsp. cremoris; these lipases were, in general, optimally active at 37℃ and pH 7 to 8.5. Lc. lactis subsp. cremoris showed the highest lipolyticac tivity of the strains studied on tributanoic acid and milk fat emulsions. Activity of all lipases was stimulated by reduced glutathione and low (Ca+2, 2g 100mL-1) concentrations of NaCl but inhibited by high concentrations of NaCl (Ca+2, 20g 100 mL-1). Khalid and Marth (1990) reported the quantitative estimation of the lipolyticac tivity of Lb. casei L-7, Lb. casei L-14, Lb. plantarum L-34 and Lb. helveticus L-53. Milk fat, olive oil and tributanoicac id emulsions were used as substrates; the three emulsions were hydrolysed by the four strains of lactobacilli with the exception of Lb. casei L-7, which failed to hydrolyse olive oil. Chich et al., (1997) reported the presence of esterolytic activities in an intracellular extract of Lactococcus lactis subsp. Lactis NCDO 763. Activity was detected using b-naphthyl butanoicac id (C4:0) as substrate and the purified enzyme was active on p-NP from C2 to C12 with pH and temperature optima of 7.0–8.0 and 55℃, respectively. Lb. fermentum, a species found in the starter used in the manufacture of Parmesan cheese (Battistotti & Bosi, 1987), contains a cell surface-associated esterase specific for C4:0 which can hydrolyse b-naphtyl esters of fatty acids from C2:0 to C10:0 (Gobbetti et al., 1997). Liu et al., (2001) identified three intracellular esterases in Streptococcus thermophilus, two of which were purified to homogeneity and designated esterase I and II with molecular masses of B34 and 60 kDa, respectively. Differences in substrate specificities between esterase I and II were noted, with esterase Ihydrolyzing p-nitrophenyl esters of short chain FFA C2 to C8 while esterase II hydrolysed C2–C6 p-nitrophenyl esters. Both enzymes had maximum activity on p-NP butanoicac id. Esterase I, which was tested against a range of glyceride substrates, hydrolysed di- and monoacylglycerides up to C14:0. Recently, Collins et al., (2003) examined the influence of starter autolysis on lipolysis during a 238 d ripening period, as measured by the concentrations of FFA from C4:0 to C18:3 in Cheddar cheese made using either Lc. lactis subsp. cremoris AM2 or Lc. lactis subsp. cremoris HP as starters. These workers found that Cheddar cheese made using the highly autolytic Lc. lactis subsp. cremoris AM2 developed significantly higher levels of a number of FFA including octanoic acid (C8:0), tetradecanoic acid (C14:0), hexadecanoic acid (C16:0), and octadecanoic acid (C18:0) during ripening compared with cheese made with the less autolyticstra in Lc. lactis subsp. cremoris HP. Cell free extracts prepared from both strains had generally similar levels of activity on lipase (tri cis-9-octadecenoic acid emulsion) or esterase (p-NP butanoicac id) substrates and these workers concluded that there was preliminary evidence for a relationship between autolysis of starter bacteria and lipolysis in cheese. Kakariari et al., (2000) purified and characterized an intracellular esterase from

Propionibacterium freudenreichii subsp. freudenreichii. Esterase activity was assayed for in the cell-free growth medium, cell wall fractions and a sonicated intracellular extract. In contrast to Dupuis et al., (1993) esterase activity was not found in the cell-free medium, or associated with the cell wall fractions. The enzyme purified by Kakariari et al., (2000) was either cytoplasmic or cell membrane associated.

**Comparison of lipoprotein lipase and bacterial lipase**

The lipases produced by these bacteria have different characteristics from LPL and it is important to recognize these differences in order to be able to determine the cause of lipolysis in particular situations. A major difference in their mode of action is that the MFGM does not appear to be a barrier to access of the bacterial lipases to the fat in intact fat globules. While this is true for the lipases produced by psychrotrophic bacteria, some other microbial lipases do not have this characteristic. For example, the lipase produced by Candida cylindracea cannot access the fat in intact fat globules, a property exploited in the test for free or lipase-accessible fat (Fitz-Gerald & Deeth, 1983). The reason for this difference between lipases is unclear. Another significant difference is their heat stabilities. Normal HTST pasteurization inactivates LPL, while most of the lipases from psychrotrophic bacteria are heat resistant, with many of them surviving even intense heat treatments such as UHT treatments (140°C for 4 s) (Fitz-Gerald & Deeth, 1983). Because only trace amounts of these heat-resistant enzymes are likely to be produced in milk during normal commercial operations, their effects are only observed in products such as UHT milk, butter,

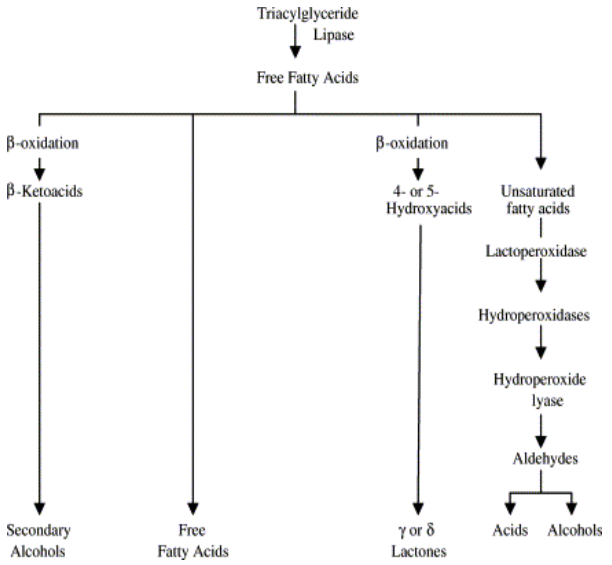


Fig. 2. Catabolism of free fatty acids

cheese and milk powders after a period of storage. Affected products, or products manufactured from them, develop rancid off flavours and may become unacceptable for human consumption.

Catabolism of free fatty acids

While short-chain fatty acids contribute directly to cheese flavour, free fatty acids (FFAs) also contribute indirectly to cheese flavour by acting as precursors for the production of volatile flavor compounds through a series of reactions known collectively as metabolism of fatty acids. Pathways for the metabolism of FFAs in cheese during ripening are summarized in Fig. 2. Esters are found commonly in many cheese varieties and are produced by the reaction of an FFA with an alcohol. While methyl, propyl and butyl esters have been found in cheese (Meinhart & Schreier 1986), the most common alcohol available for this reaction is ethanol and hence ethyl esters are the dominant esters in cheese. Ethanol is the limiting reactant in the production of esters; this alcohol is derived from the fermentation of lactose or from amino acid catabolism. Holland et al., (2002) suggested that esters are formed during cheese ripening by transesterification of an FFA from partial glycerides to ethanol. Thioesters are compounds formed by the reaction of FFAs with sulphydryl compounds, usually methanethiol (CH3SH; thus forming methylthioesters) (McSweeney & Sousa, 2000; Collins et al., 2003). Lactones are cyclic compounds formed from hydroxyacids following intramolecular esterification. Both γ- and δ-lactones (with five- and six-sided rings, respectively) have been found in cheese. The production of lactones during ripening is limited by the levels of their precursor compounds, hydroxyacids. The mammary gland is reported to possess a δ-oxidation system for fatty acids, or hydroxyacids may be produced by reduction of ketones (Collins et al., 2003). The presence of large amounts of high molecular weight lactones in rancid. Dodecalactone may be produced from long-chain unsaturated fatty acids by P. roqueforti while hydroxyacids may be produced by the action of lipoxygenases and other enzymes present in members of the rumen microflora (Collins et al., 2003). FFA metabolism is of most significance in blue-mould cheese in which FFAs are converted to 2-methyl ketones (alkan-2-ones) via a pathway corresponding to the early stages of β-oxidation caused by the action of spores and vegetative mycelia of P. roqueforti (Collins et al., 2003) and perhaps other fungi (e.g. P. camemberti or G. candidum) (Molimard & Spinnler, 1996). The rate of production of methyl ketones is affected by a number of factors, including temperature, physiological state of the mould and concentration of precursor FFA. The rate of production is maximal between pH 5 and 7; this range of pH values encompasses most blue cheeses (Gripon, 1993). Although up to 11 methyl ketones have been identified in cheese (Collins et al., 2003), the most common are pentan-2-one, heptan-2-one and nonan-2-one (Collins et al., 2003). Methyl ketones may be reduced to the corresponding secondary alcohol (e.g. pentan-2-ol,

heptan- 2-ol and nonan-2-ol) by the action of enzymes of P. roqueforti (Fig.3).

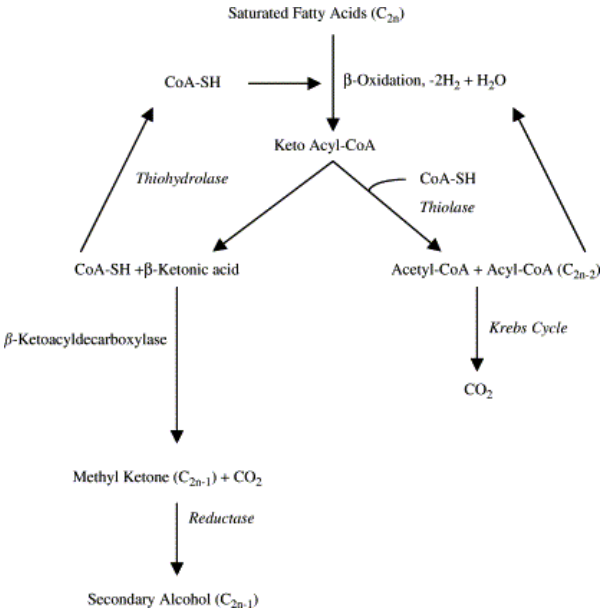


Fig. 3. Catabolism of fatty acids by Penicillium spp.

**Contribution of lipolysis and metabolism of FFA to cheese flavor**

The flavour of mature cheese is the result of a series of biochemical changes that occur in the curd during ripening, caused by the interaction of starter bacteria, enzymes from the milk, enzymes from the rennet and accompanying lipases and secondary flora (Urbach, 1997). The numerous compounds involved in cheese aroma and flavour are derived from three major metabolic pathways: catabolism of lactate, protein and lipid (Molimard & Spinnler, 1996). Lipid hydrolysis results in the formation of FFA, which may, directly, contribute to cheese flavour and also serve as substrates for further reactions producing highly flavoured catabolicend products. Cheese flavour is very complex and differs from one cheese variety to another. In the case of hard Italian cheeses, FFA are significant contributors to the flavour (Brennand et al., 1989). For mould ripened cheeses, methyl ketones are important flavour contributors (Molimard & Spinnler, 1996). However, in the case of Cheddar cheese and similar varieties, little is known about the exact contribution of individual compounds to flavour (Wijesundera & Drury, 1999). In this respect, the physical presence of fat in cheese is important for flavour development. Long-chain FFA (>12 carbon atoms) are considered to play a minor role in cheese flavour due to their high perception thresholds (Molimard & Spinnler, 1996). Short and intermediate-chain, even-numbered fatty acids (C4:0–C12:0) have considerably lower perception thresholds and each gives a characteristic flavour note. Butanoic acid contributes ‘‘rancid’’ and ‘‘cheesy’’ flavours. Hexanoic acid has a ‘‘pungent’’, ‘‘blue cheese’’ flavour note, octanoic acid has a ‘‘wax’’, ‘‘soap’’, ‘‘goat’’, ‘‘musty’’, ‘‘rancid’’ and ‘‘fruity’’ note. Depending on their concentration and perception threshold, volatile fatty acids can either contribute positively to the aroma of the cheese or to a rancidity defect. The flavor effect of FFA in cheese is regulated by pH. In cheeses with a high pH, e.g., surface bacterially ripened cheese, the flavour effect of fatty acids may be negated due to neutralization (Molimard & Spinnler, 1996). Fatty acids and secondary alcohols are also major flavour components. While methyl ketones are more important in relation to the flavour of Blue cheeses, they are also present in Camembert cheese at 25–60 mmol 100 g_1 of fat. The two major methyl ketones in Blue and Camembert cheeses are nonan-2- one and heptan-2-one (Gripon, 1993). The homologous series of odd-chain methyl ketones, from C3:0 to C15:0, constitute some of the most important components in the aroma of surface-mould ripened cheese, e.g., St. Paulin, Tilsiter and Limburger. The significance of methyl ketones to Cheddar flavour has not been established. Significant levels of pentan-2-one and heptan-2-one in the headspace of Cheddar has been attributed to mould contamination (Urbach, 1997). Wijesundera and Watkins (2000) provided evidence of the significance of methyl ketones to Cheddar cheese flavour, as cheese made with milk containing vegetable fat was low in characteristic Cheddar flavour and in methyl ketones.

**Application of lipases in cheese making**

Cheese manufacture is one of the classical examples of food preservation, dating from 6000-7000 BC. World production of cheese is roughly 15x106 tones per annum (about 35% of total milk production) and has increased annual at a rate of about 4% per annum. The basic steps are common to most cheese manufacture varieties, these are: acidification, coagulation, dehydration (cutting the coagulum, cooking, stirring, pressing, salting and other operations that promote gel syneresis, shaping (molding and pressing) and salting. Hassan et al., (1996) screened ten microbiological sources for abilities to produce proteolytic and lipolytic activities. Three organisms were selected from them for proteolytic activity and lipolytic activity. Both of them were used to accelerate cheese slurries ripening with concentrations of 0.5, 1.0 and 1.5 ml/100g curd. Slurries were incubated at 37◦C for 7 days. The best results were obtained when Lactobacillus delbrueckii subsp. bulgaricus was used for both protease and lipase. Ismail et al., (1996) used of mixture enzyme (protease and lipase) shortened the ripening period of Ras cheese and saved about 50% of ripening period. Also hasted the flavor development and improved the characteristics of body and texture. Degheidi et al., (1998) used lipase extracted from jack fruit seeds for improving the quality of Domiati cheese. Addition of jack

fruit lipase at the rate of 600 or 900 units/100ml of cheese milk improved the organoleptic properties of Domiati cheese during pickling. Akın et al., (2003) studied the effects of commercial pregastric lipase enzyme on the accelerated ripening of white pickled cheese. Sensory evaluation of cheese samples was also performed. Generally, when the pregastiric lipase levels increased, volatile free fatty acids (VFFAs) and free fatty acids (FFAs) also significantly increased ($P<0.05$). Generally, sensory scores (appearance, flouvour, body and texture and odour) were significantly higher in both 4 g/100 l treated milk added and after 20 days ripening ($P<0.05$). Hernández et al., (2005) studied three commercial lipases (lipases A1 and A2 were pregastric lipases and lipase A3 was a fungal lipase) from three different Idiazabal (sheep's raw milk) cheese. It be concluded that lipase A1 is an adequate commercial lipase to develop the characteristic flavour of Idiazabal cheese, both in artisan and industrial fabrications. Atasoy and Türkoğlu, (2009) evaluated the lipolysis in Urfa cheese made from raw and pasteurized goats' and cows' milk with mesophilic or thermophilic cultures. Total free fatty acid (FFA) contents of goats' milk cheese were significantly ($P < 0.001$) lower than that of cows' milk cheese throughout ripening, whereas goats' milk cheese flavour was higher ($P < 0.05$) than cows' milk cheese.

Karami et al., (2009) used a commercial pregastric lipase to accelerate lipolysis of Iranian ultrafiltered-Feta (UF-Feta) cheese. An increase in the lipase level or ripening period resulted in an increase in the rate of disappearance of fat globules. After 20 days of ripening, no apparent fat globules were observed and fewer fingerprints and voids of free fat were detected, compared to when no enzyme was used. Hernández et al., (2009) describe the effect of the addition of pregastric lipase on the composition and sensory properties of Idiazabal cheese. Free fatty acids (FFA), partial glycerides, free amino acids (FAA), gross composition and sensory characteristics were determined at different ripening times in cheeses manufactured with three different amounts of commercial animal lipase or with lipase-containing artisanal lamb rennet paste. It indicated that aroma and flavour parameters of Idiazabal cheese and the content of short-chain FFA and diglycerides were highly correlated to first principal component (PC1), while texture parameters, compositional variables and FAA were correlated to the second principal component (PC2). Bruinenberg (2010) reviewed the innovations in Dairy cultures and Enzymes. Flavour development in dairy fermentations, most notably cheeses, results from a series of (bio)chemical processes in which lactic acid bacteria (LAB) play a pivotal role. Flavour compounds in cheese arise from the action of added enzymes such as rennet, indigenous milk enzymes, enzymes originating from added primary and secondary (adjunct) starter organisms and the non-starter bacteria (so-called NSLAB). Flavour compound formation by LAB have shown that three main processes can be distinguished: conversion of lactose and citrate (carbohydrate metabolism), degradation of fat (lipolysis) and degradation of caseins (proteolysis). This review opportunities offered by the adjunct starter concept for modulating the cheese characteristics. In addition, modification of cheese and whey processing can be achieved by using selected fungal enzymes: i.e. a carboxypeptidase is found to accelerate the flavour development andreduce bitterness in cheese and a novel peroxidase gives reduced levels of annato-derived colour in whey without adverse side-effects on flavour and functionality of the whey. Drake, et. al., (2010) Studied influence of fat on flavour and flavour development in Cheddar cheese. Flavour and volatile components of low fat Cheddar cheeses were distinct from full fat cheeses ($p<0.05$). Decreases in fat content resulted in decreases in milkfat flavour, higher whey flavour and a decrease in the rate of aged cheese flavour development compared to full fat cheeses. Sensory thresholds of isolated key compounds revealed >100 fold increases for some compounds in a fat based matrix (full fat cheese) compared to a water based matrix (83% fat reduced cheeses) ($p<0.05$). As a result, odor activity values for certain compounds including homofuraneol and phenylacetaldehyde increased in low fat cheese compared to full fat cheeses. These results demonstrate that fat reduction in Cheddar cheeses alters volatile compound production as well as volatile compound release to collectively impact flavor perception. Karami et al., (2010) investigated the effects of a commercial pregastric lipase enzyme (calf lipase) on the free fatty acid profile of Iranian UF (ultrafiltered)-Feta cheese during ripening period. Sensory evaluation and physico-chemical analysis of cheese samples were also performed. Commercial pregastric lipase (at the levels of 0, 2, 4 and 6 g 100 kg-1 of retentate) together with rennet was added to retentate. With an increase in lipase level and ripening period, the main components of cheese did not changed significantly but water soluble nitrogen increased during ripening period ($P<0.05$). With an increase in the pregastric lipase level, the percentages of C4:0-C8:0 fatty acids decreased significantly while that of C12:0-C18:0 and C18:1 increased. The percentages of C10:0, C18:2 and C18:3 did not change. With an increase in ripening period, the percentages of C4:0-C14:0 decreased while the percentages of C16:0, C18:0, C18:1 and C18:2 increased ($p<0.05$) and C18:3 percentage did not show any changes. Overall, sensory scores (appearance, flavour, body and texture and piquant taste) indicated that combination of 20 days with 6 g 100kg-1, 40 days with 4 g 100kg-1 and 60 days with 2 g 100kg-1 retentate were the best ripening conditions. The addition of lipase to cheese milk could be recommended for the acceleration of flavour development in UF. McMahon (2010) This paper discusses research on improving low fat cheeses and the role of salt in cheese flavor. Successfully producing lower fat cheese requires mimicking fat's role in texture, performance, flavor and color of cheese as well as compensating for the lower salt

concentration when moisture content is increased. Fat influences cheese texture by acting as a reinforcing filler when the cheese is cold, forming characteristic stringiness of pasta filata cheeses, and providing weak points within the protein network that allow strain weakening and formation of a smooth mass during chewing of full fat cheeses. In heated applications, fat softens when warmed, while release of fat from within the cheese lubricates the cheese surface as well as inhibiting cheese moisture loss during baking. Only a small amount of salt is needed to influence physical characteristics of cheese but with low salt levels there is a change in microbiology activity during cheese aging. So apart from a loss of milky and buttery flavors contributed by milk fat there are significant challenges in controlling microbial development in low fat cheeses which tend to have pronounced rosey, burnt and bitter flavors. Lowering salt content allows longer survival of lactococci and more rapid growth of non-starter lactobacilli. As well as changing flavor development by lowering salt content, consumers have a preference for salty taste in cheese and react negatively to large reductions in salt. McSweeney, et. al., (2010) have been studied biochemical changes during ripening Cheese include lipolysis and proteolysis together with the metabolism of residual lactose, and of lactate and citrate. Lipolysis and proteolysis result ultimately in the liberation of fatty acids and amino acids, respectively, which may be metabolized to a range of volatile flavour compounds. Walter Bisig (2010) studied the comparison between PDO Emmental cheese and industrial Swiss-type cheese production in Europe. Swiss Emmentaler with a protected designation of origin (PDO) is mostly produced with aspartate negative propionic acid bacteria to avoid late fermentation. PDO had higher contents of acetate and propionate and less lactate, butyrate and succinate compared to Emmental made of thermised milk. It also showed a more pronounced proteolysis. As key odorants 4 ketones and aldehydes, 3 esters, 1 lactone and 2 furans were identified. They were characterised as butterlike (diacetyl), cheese-factory-like, fruity, caramel, spicy, smoky or mushroom-like. For diacetyl, a higher content could be found in Emmental from thermised milk (not significant). Volatile compounds which were significantly higher in PDO were 3-hydroxybutanone, hexanal, butanoic-acid-ethyl-ester and 3-methylheptane. Significantly lower were propan-2-ol
and butan-1-ol. A technique to proof the origin of PDO based on the use of specific FHL strains and their identification in matured cheese with the selection of exclusive PCR-primers was tested successfully. Wolf et al., (2010) studied the Characterization commercial Reggianito cheeses from different dairy plants (global composition, lipolysis, proteolysis and volatile compound profiles). A total of 53 compounds were identified, the majority belonging to the groups of ketones, alcohols, acids, esters and aldehydes. All these compounds have been reported in Italian grana-type cheeses. Visualization of the analytical results was performed by principal component analysis. This analysis clustered cheese samples according to dairy plants. This fact could be, among other factors, consequences of differences in technologies and ripening time of different manufacturers.

## References


Akın, N., Aydemir, S., Koçak, C. and Yıldız, M. A. (2003). Changes of free fatty acid contents and sensory properties of white pickled cheese during ripening. Food Chemistry, 80:77–83.

Atasoy, A. F. and Türkoğlu, H. (2009). Lipolysis in Urfa cheese produced from raw and pasteurized goats' and cows' milk with mesophilic or thermophilic cultures during ripening. Food Chemistry, 115: 71–78.

Brennand, C. P., Ha, J. K., & Lindsay, R. C. (1989). Aroma properties and thresholds of some branched-chain and other minor volatile fatty acids occurring in milkfat and meat lipids. Journal of Sensory Studies, 4, 105–120.

Bruinenberg, P.G. (2010). Innovations in Dairy Cultures Enzymes. IDF, New Zealand

Castillo, I., Requena, T., Fernandez de Palencia, P., Fontecha, J., & Gobbetti, M. (1999). Isolation and characterization of an intracellular esterase from Lactobacillus casei subsp. casei. IFPL731. Journal of Applied Microbiology, 86, 653–659.

Chich, J.-F., Marchesseau,K., & Gripon, J.-C. (1997). Intercellular esterase from Lactococcus lactis subsp. lactis NCDO 763: Purification and characterization. International Dairy Journal, 7, 169-174.

Collins, Y. F., McSweeney, P. L. H, & Wilkinson, M. G. (2003). Evidence for a relationship between autolysis of starter bacteria and lipolysis in Cheddar cheese. Journal of Dairy Research, 70, 105–113.

Deeth, H. C., & Fitz-Gerald, C. H. (1995). Lipolytic enzymes and hydrolytic rancidity in milk and dairy products. In P. F. Fox (Ed.),Advanced dairy chemistry, Vol. 2: Lipids (2nd ed., pp. 247–308). London, UK: Chapman & Hall Publishers.

Deeth, H. C., & Smith, R. A. D. (1983). Lipolysis and other factors affecting the steam frothing capacity of milk. Australian Journal of Dairy Technology, 38, 14–19.

Deeth, H. C., & Touch, V. (2000). Methods for detecting lipase activity in milk and milk products. Australian Journal of Dairy Technology, 55,153–168.

Degheidi, M. A., Abd Rabou, N. S. and Ismail, A. A. (1998). Improvement of Domiati cheese quality during pickling using jack fruit lipase. Egyptian Journal of Dairy Science, 26: 103-115.

Drake1, M.A., R.E. Miracle1, D. J. McMahon (2010). The influence of fat on flavour and flavour development in Cheddar cheese. IDF, New Zealand

Dupuis, C., Corre, C., & Boyaval, P. (1993). Lipase and esterase activities of Propionibacterium freudenreichii subsp. freudenreichii. Applied and Environmental Microbiology, 59, 4004–4009.

El-Hofi, M. A. and Ismail, A. A. (1998-2000). Utilization of purified and characterized lipase from Papaya (Carica papaya) in acceleration of Ras cheese slurry. Egyptian Journal of Food Science, 26-28: 61-72.

El-Safty, M. S., & Isamil, A. A. (1982). Addition of free fatty acids to improve the flavour and accelerate the ripening of Domiati cheese made from reconstituted milk. Journal of Dairy Research, 49, 671–675.

El-Safty, M. S., Khorshid, M. A. and Ismail, A. A. (1979). The effect of soymilk on lipase activity and organoleptic properties of Ras cheese. Milchwissenschaft, 34, (1):22-23.

El-Soda, M., Ezzat, N., Hassanein, S., El-Abassy, F. and Wahba, A. (1990a). Acceleration of Ras cheese ripening 1- Addition of commercial enzymes preparation. Egyptian Journal of Dairy Science, 18: 171-182.

El-Soda, M., Ezzat, N., El-Abassy, F., Wahba, A. and Hassanein, S. (1990b). Acceleration of Ras cheese ripening II-Combination of gross proteolytic agent with the cell free extract of some Lactobacilli. Egyptian Journal of Dairy Science, 18: 183-193.

El-soda, M., Abd El-Wahab, H., Ezzat, N., Desmazeaud, M.J., & Ismail, A. (1986). The esterolytic and lipolytic activities of the lactobacilli. II, Detection of esterase system of Lactobacillus helveticus, Lactobacillus bulgaricus, Lactobacillus lactis and Lactobacillus acidophilus. Lait, 66, 431-443.

Fernandez, L., Beerthuyzen, M. M., Brown, J., Siezen, R. J., Coolbear, T., Holland, R., & Kuipers, O. P. (2000). Cloning, characterization, controlled overexpression, and inactivation of the major tributyrin esterase gene of Lactococcus lactis. Applied and Environmental Microbiology, 66, 360–1368.

Fitz-Gerald, C. H., & Deeth, H. C. (1983). Factors influencing lipolysis by skim milk cultures of some psychrotrophic microorganisms. Australian Journal of Dairy Technology, 38, 97–103.

Fox, P. F., Guinee, T. P., Cogan, T. M., & McSweeney, P. L. H. (2000). Fundamentals of cheese science. Gaithersburg, MD: Aspen Publishers Inc. Fox, P. F., Law, J.,

Fox, P.F., & Stepaniak, L. (1993). Enzymes in cheese technology. International Dairy Journal, 3, 609.

Gobbetti, M., Fox. P.F., Smacchi, E., Stepaniak, L., & Damiani, P.(1997). Purification and characterization of a lipase from Lactobacillus plantarum 2739. journal of Food Biochemistry, 20, 227-246.

Gripon, J. C. (1993): Mould-ripened cheeses. In P. F. Fox (Ed.), Cheese: Chemistry, physis and microbiology (2d ed., Vol. 2). London : Chaman & Hall.

Hernández, I., Renobales, M. D., Virto, M., P´erez-Elortondo, F. J., Barrón, L. J. R., Flanagan, C. and Albisu, M. (2005). Assessment of industrial lipases for flavour development in commercial Idiazabal (ewe's raw milk) cheese. Enzyme and Microbial Technology, 36: 870–879.

Hernández, I., Barrón, L. J. R., Virto, M., Pérez-Elortondo, F. J., Flanagan, C., Rozas, U., Nájera, A. I., Albisu, M., Vicente, M. S. and Renobales, M. D. (2009). Lipolysis, proteolysis and sensory properties of ewe's raw milk cheese (Idiazabal) made with lipase addition. Food Chemistry, 116:158–166.

Holland R., S.-Q. Liu, T. Wang, M. Bennett, G. Norris, M.-L. Delabre, M.W. Lubbers, J.W. Dekker and V.L. Crow,(2002). Esterases of lactic acid bacteria. Australian Journal of Dairy Technology. 57: 116.

IDF (1987). Significance of lipolysis in the manufacture and storage of dairy products. IDF Bulletin No. 144. Brussels, Belgium: International Dairy Federation.

Ismail, A. A., Hassan, A. A. and El-Hofi, M. A. (1996). Studies on Ras cheese ripening with enzymes addition. II. Application of bacterial proteases and lipases in acceleration ripening of Ras cheese. Egyptian Journal of Food Science, 24, (3): 401-416.

Jaeger, K.-E., Ransac, S., Dijkstra, B. W., Colson, C., van Heuvel, M., & Misset, O. (1994). Bacterial lipases. FEMS Microbiology Reviews, 15, 29–63.

Kakariari, E., Georgalaki, M. D., Kalantzopoulos, G., & Tsakalidou, E. (2000). Purification and characterization of an intracellular esterase from Propionibacterium

freudenreichii ssp. Freudenreichii ITG 14. Le Lait, 80, 491–501.

Kamaly, K. M., Takayama, K., & Marth, E. H. (1990). Acylglycerol acylhydrolase (lipase) activities of Streptococcus lactis, Streptococcus cremoris, and their mutants. Journal of Dairy Science, 73, 280–290.

Karami, M. ,P. Sokhanparvar, A.R. Seydmoradi (2010). The effect of added calf lipase on the lipolysis during the ripening of ultrafiltered Feta cheese. IDF, New Zealand.

Karami, M., Ehsani, M. R., Mousavi, S. M., Rezaei, K. and Safari, M. (2009). Microstructural properties of fat during the accelerated ripening

Khalid, N. M., & Marth, E. H. (1990). Lactobacilli-their enzymes and role in ripening and spoilage of cheese: A review. Journal of Dairy Science, 73, 2669–2684.

Lee, S. Y., & Lee, B. H. (1990). Esterolyticand lipolyticac tivities of Lactobacillus casei subsp-casei LLG. Journal of Food Science, 55, 119–126.

Liu, S-Q., Holland, R., & Crow, V. L. (2001). Purification and properties of intracellular esterases from Streptococcus thermophilus. International Dairy Journal, 11, 27–35.

McMahon, D. J. (2010). Issues with Lower Fat and Lower Salt Cheeses. IDF, New Zealand

McSweency, P. L. H., & Sousa, M. J. (2000): Biochemical pathways for the production of flavour compounds in cheeses during ripening: A reviewg. Lait, 80, 293-324.

McSweeney, P. L. H., V. Caldeo, A. Topcu, D.R. Cooke (2010). Ripening characteristics of cheese. IDF, New Zealand.

Molimard, P., & Spinnler, H. E. (1996). Review: Compounds involved in the flavour of surface mould-ripened cheeses: Origins and properties. Journal of Dairy Science, 79, 169–184.

Mukherjee, K. D. (1994) Plant lipases and their application in lipid biotransfomations. Prog. Lipid Res. Vol.33 No.1 : 165-174.

Nelson, J. H., Jensen, R. G., & Pitas, R. E. (1977). Pregastricesterase and other oral lipases—a review. Journal of Dairy Science, 60, 327–362.

Olivecrona, T., Villaro, S., & Bengtsson-Olivecrona, G. (1992): Indigenous enzymes in milk. II. Lipases in milk. In P. F. Fox (Ed.), Advanced Dairy Chemistry (Vol. 1). London: Elsevier Science Publishers.

Tigerstrom,R.G. and Stelmaschuk,S. (1989). The use of Tween 20 in a sensitive turbidimetricassay of lipolytic enzyme. Can. J. Microbial. 35, 511

Urbach, G. (1997). The flavour of milk and dairy products: II. Cheese:Contribution of volatile compounds. International Journal of Dairy Technology, 50, 79–89.

Walter Bisig (2010). Comparison between PDO Emmental cheese and industrial Swiss-type cheese production in Europe. IDF, New Zealand.

Wijesundera, C., & Drury, L. (1999). Role of milk fat in production of Cheddar cheese flavour using a fat-substituted cheese model. Australian Journal of Dairy Technology, 54, 28–35.

Wijesundera, C., Drury, L., Muthuku-marappan, K., Gunasekaran, S., & Everett, D. W. (1998). Flavour development and the distribution of fat globule size and shape in Cheddar-type cheese made from skim milk homogenized with AMF or its fractions. Australian Journal of Dairy Technology, 53, 107.

Wijesundera, C., & Watkins, P. (2000). Milkfat is essential for the development of Cheddar cheese flavour. Australian Journal of Dairy Technology, 55, 86.

Wolf, I.V., M.C. Perotti, S.M. Bernal, C.A. Zalazar (2010). Study of the chemical composition, proteolysis, lipolysis and volatile compounds profile of commercial Reggianito Argentino cheese: Characterization of Reggianito Argentino cheese. Food Research International, 43, 1204–1211