HOW DO ENZYMES ENHANCE THE FLAVOUR OF DAIRY INGREDIENTS?

This technical bulletin will describe how Biocatalysts Ltd.'s current off-the-shelf range of enzymes is specifically formulated for the manufacture of Enzyme Modified Dairy Ingredients (EMDIs). It will discuss how Enzyme Modified Cheese (EMC), Lipolysed Butter Oil (LBO) and accelerated cheese ripening can be used in your processes to develop and enhance the flavour profiles of your dairy ingredients.

Biocatalysts Ltd have extensive knowledge in developing enzymes for dairy flavour enhancement and if you are looking for something uniquely specific to your requirements, we can use our enzyme identification, development & manufacturing capabilities to create a completely exclusive enzyme for you.

Other technical bulletins that may be of interest include 'The Use of Enzymes in the Production of Dairy Protein Hydrolysates.'

INTRODUCTION

Enzymes have been used for centuries to produce flavour in dairy products. Traditionally flavour is generated by enzymes produced from microflora present in cheese, butter, or cream and time is required for these complex flavours to evolve. In more recent times the addition of exogenous (external source) enzymes to immature cheese, butter oil or cream has been used to speed this process up intensifying the flavour in hours and days instead of weeks and months. These EMDI's are stable, cost-effective flavour compounds that can be used in a variety of food applications.

TRADITIONAL CHEESE MAKING

The important starting point for dairy flavours is milk. Traditional cheese making processes rely on enzymes (namely lipases and proteases) to produce the characteristic cheese flavour, aroma, and texture through the modification of milk fat and milk proteins. Firstly, a protease (rennet) turns the milk into curds and whey, creating the texture of cheese. Secondly, a whole range of enzymes turn the bland immature cheese curd into the complexity of cheese flavours that we have in the world today. This maturation phase is dependent on the type of cheese being produced and it can take months or years to develop the desired characteristic flavour.

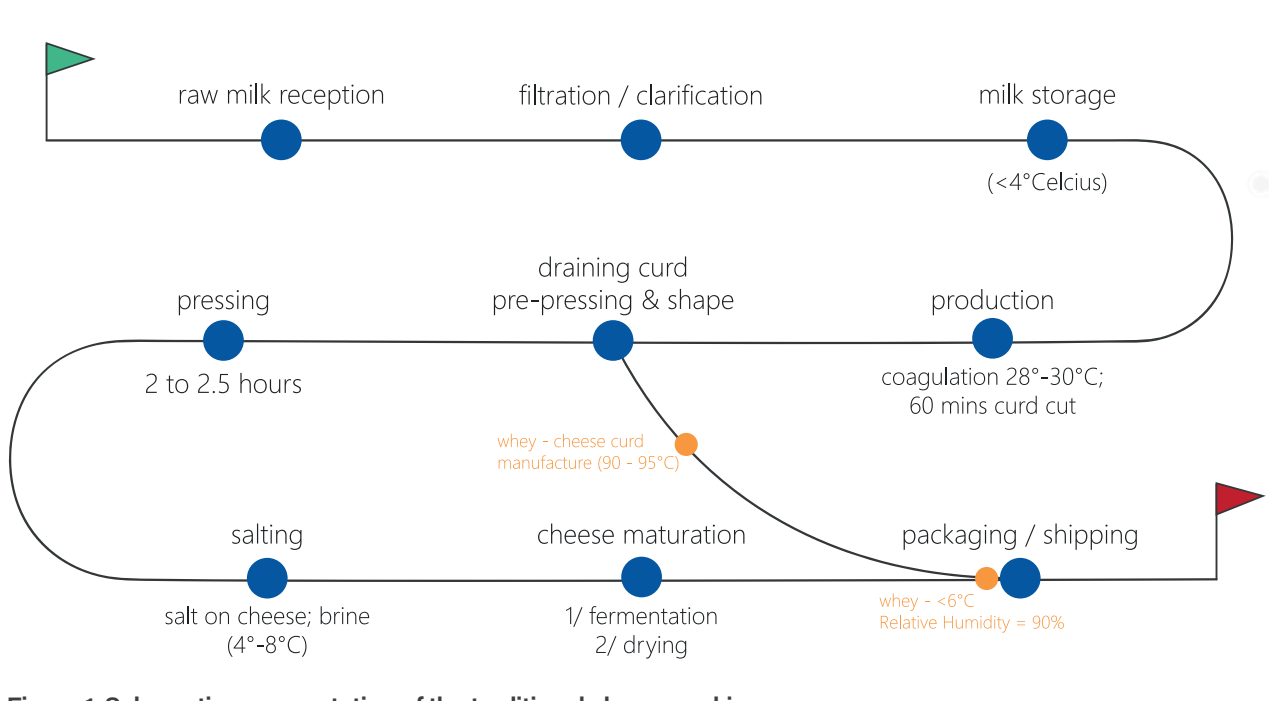


Figure 1. Schematic representation of the traditional cheese making process

WHAT IS THE PROCESS FOR PRODUCING ENZYME MODIFIED CHEESE?

The starting substrate (in this case, cheese off cuts) is shredded and mixed with water and emulsifying salts (phosphates and/or citrates) to form a cheese slurry of approximately 60-85% of cheese (40-45% of dry cheese solids). The EMC slurry is then pasteurised (72ºC, 10 min) and cooled to 30-45ºC. At this stage, enzymes should be added. The EMC slurry is then incubated at approximately 30-50ºC for 8-48 hours (dependent on the enzymes used). Following this incubation, the EMC is heated at 80°C for 10-20 minutes (to deactivate the enzymes) and then cooled to 30ºC before final packaging as a paste or spray dried into a powder.

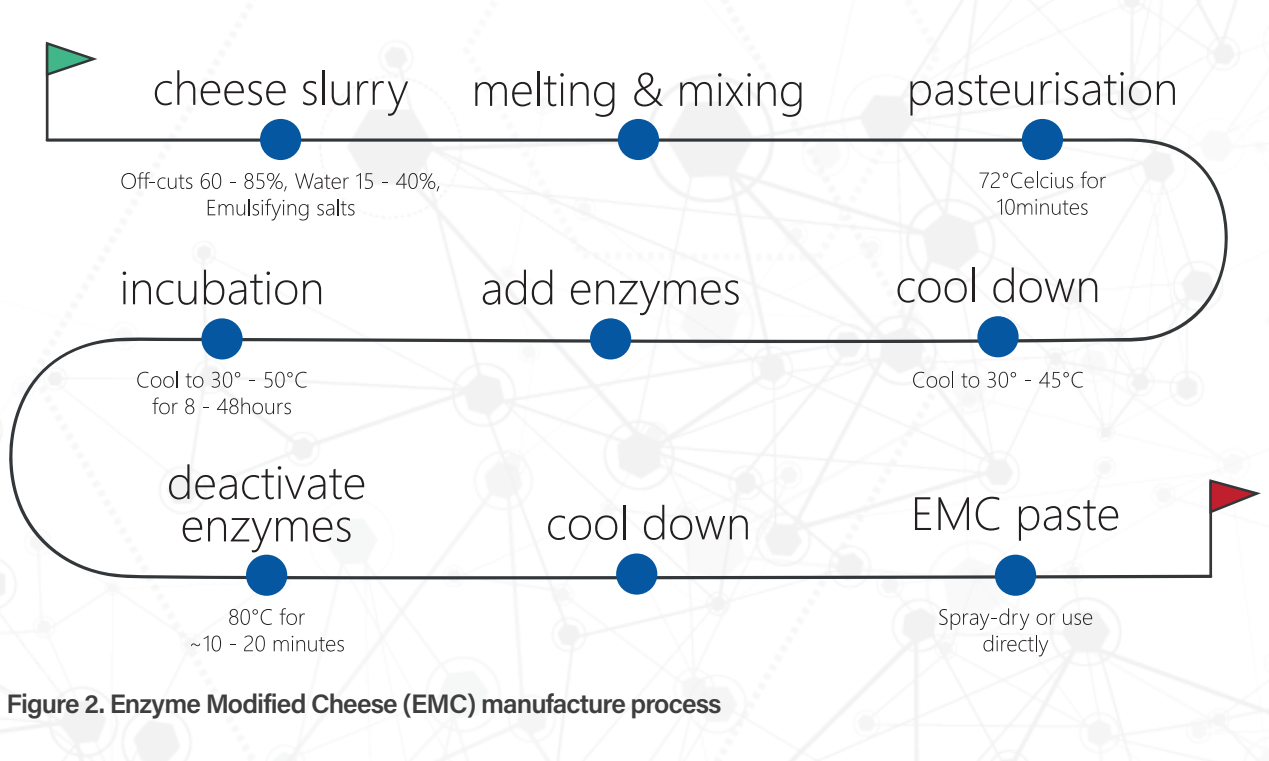


Figure 2. Enzyme Modified Cheese (EMC) manufacture process

BENEFITS OF USING ENZYME MODIFIED CHEESE

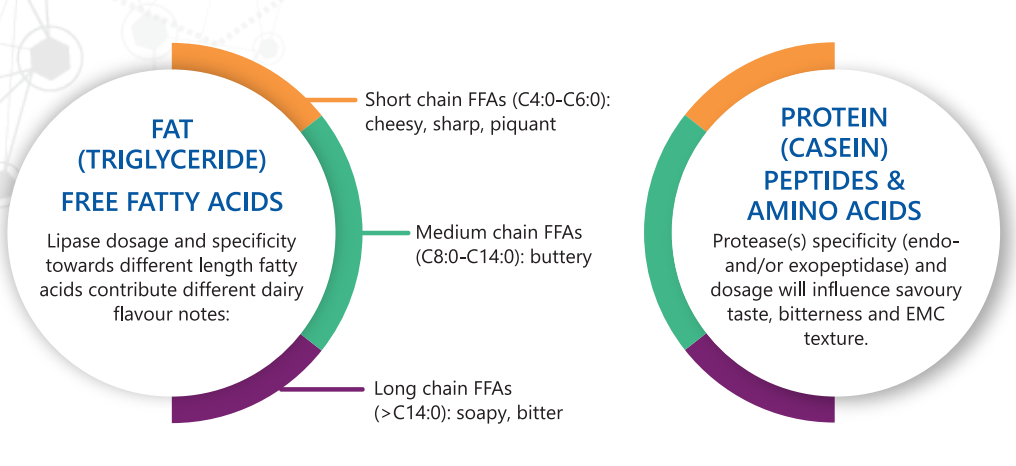
- Up to 90% of the flavour can be generated by the enzyme reaction when an immature cheese is used
- Greater diversity of flavours available to create unique dairy product flavourings including Cheddar, Swiss and Blue Cheese
- Enhanced cheese flavour developed in days rather than months/years
- Stronger cheese flavour (x30 stronger than regular cheese)
- More cost effective than using mature cheese to impart flavour
- Highly reliable & reproducible products
- Where there are limits on how much cheese can be used compared to how much flavour is needed
- EMC powder formats give extended shelf life

TABLE 1: COMMON PROBLEMS ENCOUNTERED IN EMC PROCESSING

Problem	Enzyme Solution
EMC texture is inconsistent	Ensure cheese is fully melted and homogenous prior to enzyme addition. Add additional phosphate emulsifying agents.
EMC is too 'runny' (low viscosity)	Protease level is too high. Try reducing the dosage of protease (Biocatalysts Ltd.'s technical team can advise).
EMC flavour lacks blue notes	Switch to a high butyric lipase such as Lipomod® 338MDP.
EMC has too much bitterness	Use a de-bittering enzyme such as Flavorpro® 937MDP in combination with Promod® 215MDP.

WHAT ARE BIOCATALYSTS LTD RANGE OF DAIRY FLAVOUR ENHANCING ENZYMES?

Lipase and protease enzymes from Biocatalysts Ltd are known as Lipomod® and Promod® respectively. They help to release the valuable flavour compounds in dairy fats and dairy proteins.



Lipases release free fatty acids which are associated with cheese flavours, while proteases release peptides and amino acids which give savoury and bitter notes. Biocatalysts Ltd has considerable experience in providing enzymes to produce a wide range of flavour profiles for EMC from sharp blue to Cheddar-type flavours. Our extensive range of Lipomod® and Promod® enzymes will develop a variety of different flavours that can be selected and adapted to our customers' requirements. Figures 3 and 4 illustrate the difference lipases and proteases have on the impact of the flavours generated using Lipomod® 801MDP and Promod® 215MDP respectively in producing EMCs.

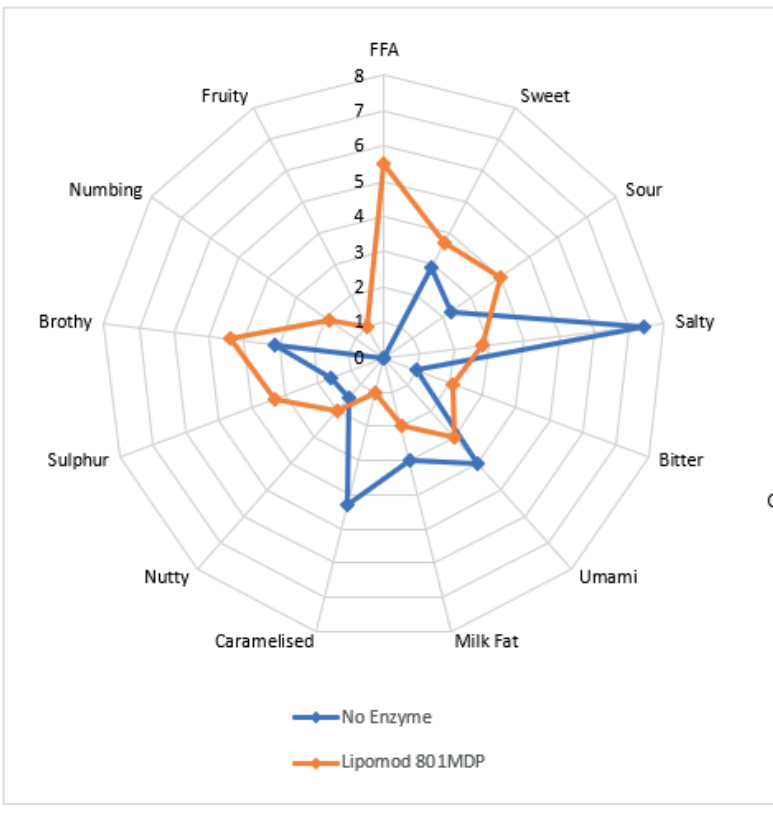


Figure 3: Sensory Analysis of EMC made with Lipomod® 801MDP (by an external flavour analyst company)

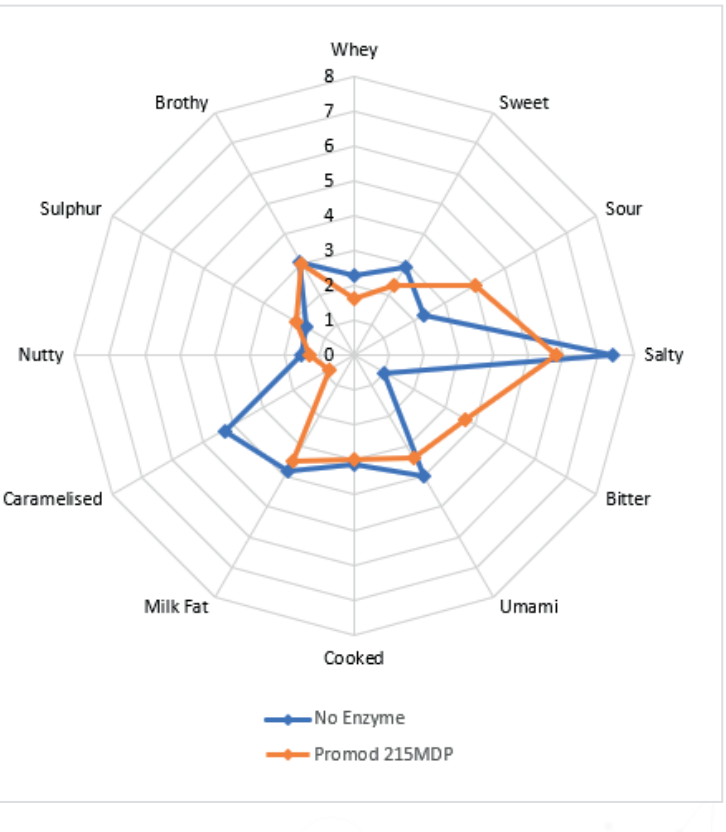


Figure 4: Sensory Analysis of EMC made with Promod® 215MDP (by an external flavour analyst company)

HOW DOES THE COMPOSITION OF THE SUBSTRATE AFFECT THE FLAVOUR PROFILE CREATED?

The composition of the raw material will greatly influence the flavour and the texture of the EMC obtained. The EMC flavour profile will therefore be dependent on the enzymes used, the process conditions and the varieties of substrates and ages of cheese used. Cheese for example, has a much higher content of milk proteins than butter and cream which contains mostly milk fat with some proteins. Therefore, when using enzymes to enhance the flavour of dairy ingredients such as butter or cream the overall flavour generation is a much softer, buttery flavour that contributes a fatty mouthfeel texture without the addition of savoury notes and bitterness that can form from the hydrolysis of dairy proteins present in cheese. Lipomod® 4MDP can be used to achieve a mild hydrolysis in butter and cream substrates to produce a well-rounded buttery flavour with an enhanced creamy mouthfeel, without the production of bitter or soapy flavour notes.

CAN BIOCATALYSTS LTD ENZYMES BE USED TO CREATE VEGETARIAN, KOSHER & HALAL DAIRY PRODUCTS?

EMCs were historically made solely using pig pancreatic enzymes (a complex mixture of lipase and protease enzyme activities). Due to customer demands, the range of flavours needed for EMCs has considerably increased and the production methods used now are much more sophisticated. The regulatory requirements (vegetarian, Kosher, Halal) have also strongly influenced the development of non-animal enzymes. Biocatalysts Ltd specialise in the supply of microbial enzymes for EMC manufacture, our substantial range enable the creation of a wide range of flavour profiles whilst adhering to regulatory requirements.

ADVANTAGES OF BIOCATALYSTS' MICROBIAL LIPASES & PROTEASES:

1. Lipases can be supplied protease free which does not produce bitterness typically associated with pancreatic lipase use.
2. Proteases that have been specifically developed to reduce bitter notes.
3. Fatty acid flavour profiles can be altered using tailor-made Biocatalysts Ltd.'s products.
4. Amino acid composition can be changed using Biocatalysts Ltd.'s specially developed proteases
5. Concern regarding possible contamination of animal products (pancreatic enzymes) with viruses and prions is not a problem with microbial enzymes.
6. No supply chain issues using microbial enzymes that could come from animal-derived enzymes.
7. EMCs can be used in vegetarian products.
8. EMCs are suitable for use in Kosher and Halal certified products.

HOW DO LIPASES / LIPOMOD® WORK?

Lipases break down the lipids (fats and oils) in the cheese releasing free fatty acids which give the cheese a pungent flavour. Different lipases will preferentially release different fatty acids allowing each lipase to produce a distinct flavour profile. Figure 5 illustrates the action of lipases in hydrolysing the triglyceride structures in fats, to release the fatty acids from the glycerol backbone.

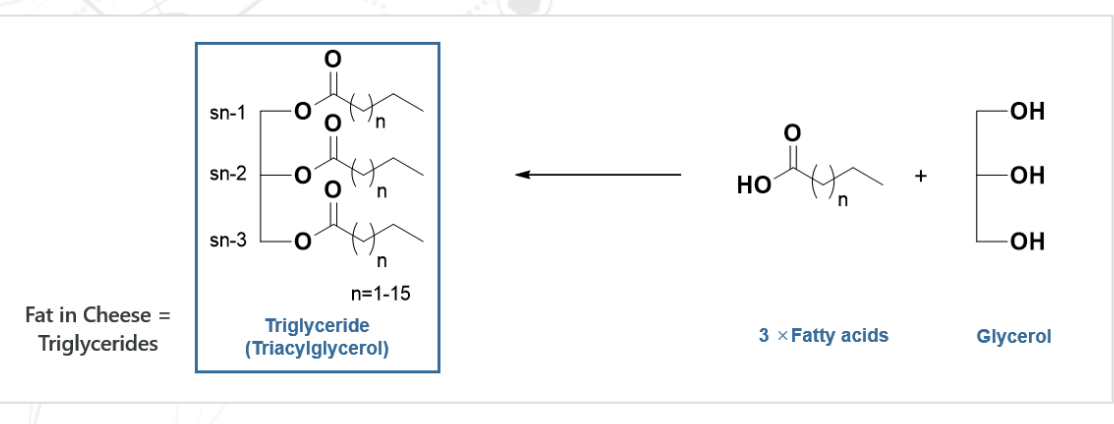


Figure 5. Hydrolysis of triglycerides in cheese fat using lipases to release fatty acids

WHY DOES FATTY ACID CHAIN LENGTH MATTER?

There are approximately 15 to 20 different fatty acids in dairy fat. The length of a fatty acid refers to the number of carbon atoms on the chain, determining the carbon chain length. As such these are categorised into Short Chain Fatty Acids (SCFAs) C4:0 - C6:0, Medium Chain Fatty Acids (MCFAs) C8:0 - C14:0, and Long Chain Fatty Acids (LCFAs) >C14:0. The different carbon chain lengths of the fatty acids contribute a range of different flavour profiles. For example, hydrolysis liberating the short chain fatty acids (C4:0 and C6:0), release butyric and caproic acid, the two fatty acid flavours identified as producing cheesy, fatty and sharp acetic flavours typically associated with dairy-type applications.

Lipases have varying specificities towards different substrates as well as different length fatty acids (Figure 6). This means different lipases can be used, on their own or in combination with other lipases, to achieve diverse flavour portfolios to produce uniquely flavoured dairy products. Lipomod® 4MDP is a unique microbial lipase developed by Biocatalysts Ltd, to have a high specificity towards the release of short chain fatty acids with very minimal release of any medium to long-chain fatty acids. EMCs produced using Lipomod® 4MDP have a well-rounded and balanced mature cheese flavour without any bitter or soapy flavour notes, often associated with the release of longer chain fatty acids. Whereas Lipomod® 338P is a fungal enzyme with a broader specificity towards release of the different length fatty acids. The combination of releasing short chain fatty acids and a higher proportion of long chain fatty acids results in a strong and pungent EMC flavour profile of blue cheese flavour notes.

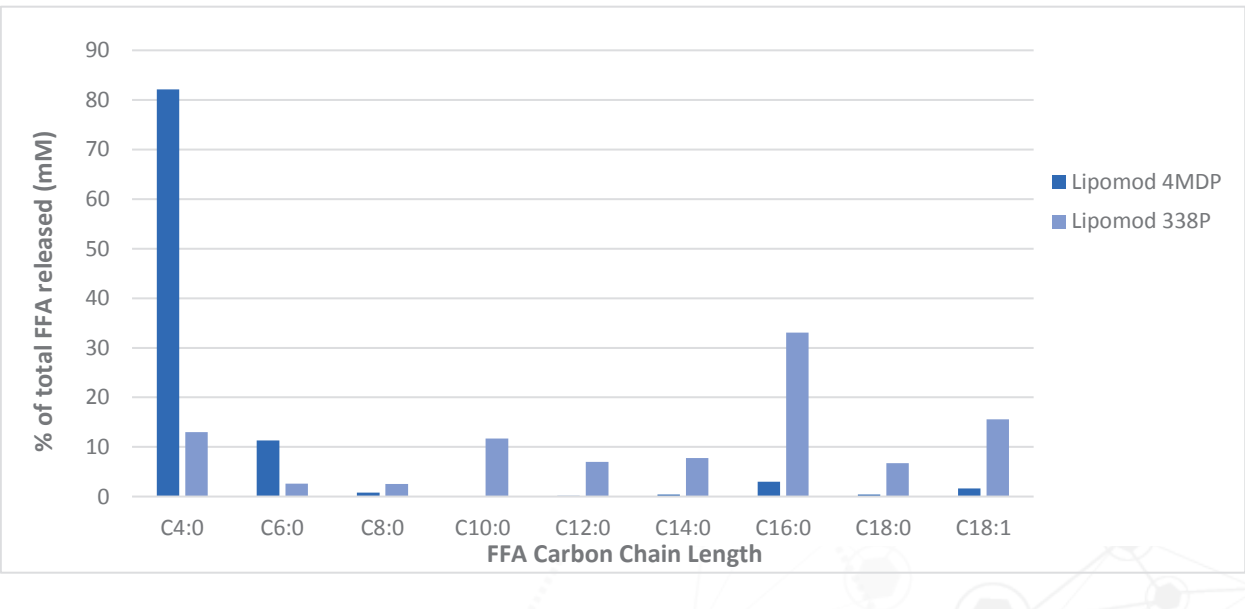


Figure 6. Free Fatty Acid Profile of Butter Oil Hydrolysis with Lipomod® 4MDP and Lipomod® 338P

Biocatalysts Ltd produces a wide range of Lipomod® enzymes containing different lipases and esterases which can be used to produce EMCs with differing flavour notes.

The table below shows our extensive range of Lipomod® enzymes and their benefits.

TABLE 2: BIOCATALYSTS LTD.'S RANGE OF LIPOMOD® ENZYMES FOR EMDI & EMC MANUFACTURING

Product	Dose rate (% w/w on cheese in the slurry)	Benefit
Lipomod® 4MDP	0.1 - 0.5	High specificity microbial lipase for producing a wellrounded and balanced mature cheese flavour.
Lipomod® 34MDP	0.01 - 0.1	Produces mild buttery flavours.
Lipomod® 187MDP	0.05 - 0.15	Microbial lipase for cheddar-type EMC.
Lipomod® 338MDP	0.1 - 0.5	Microbial productsuitable for production of blue flavour notes.
Lipomod® 621MDP	0.05 - 0.15	Mixed fungal esterase and protease for Swiss style notes.
Lipomod® 691MDP	0.05 - 0.15	Microbial product suitable for production of rounded cream notes.
Lipomod® 768MDP	0.1 - 0.2	Mixed fungal lipases and esterase for more aged flavour profile, dominated by free fatty acids, with subtle sweet undertones.
Lipomod® 801MDP	0.1 - 0.5	Microbial alternative to pancreatic lipase (contains endopeptidase activity). A well-balanced flavour profile dominated by free fatty acid flavours with some protein notes, including brothy and sulphur undertones.
Lipomod® 957MDP	0.1 - 0.5	Microbial alternative to porcine pancreatin (contains endopeptidase activity) producing a flavour profile dominated by strong free fatty acid notes with sulphur and sweet undertones.

HOW DO PROTEASES / PROMOD® WORK?

Endopeptidases (also referred as endo-proteases) and exopeptidases break down the proteins in cheese into peptides and amino acids. The composition of the peptides and amino acids present are responsible for the different flavour notes produced. Protein breakdown is responsible for two important flavour notes in EMC and EMDI production:

1. Developing a savoury background flavour by generating hydrophilic peptides and amino acids. The production of hydrophilic peptides enhances flavour generation due to the increased solubility, this can impart a savoury, sweet or sour flavour.
2. The accumulation of short hydrophobic peptides can create some bitterness, which is a normal component of cheese flavours, however at high levels bitterness is undesirable. Excessive bitterness can be controlled using exopeptidases.

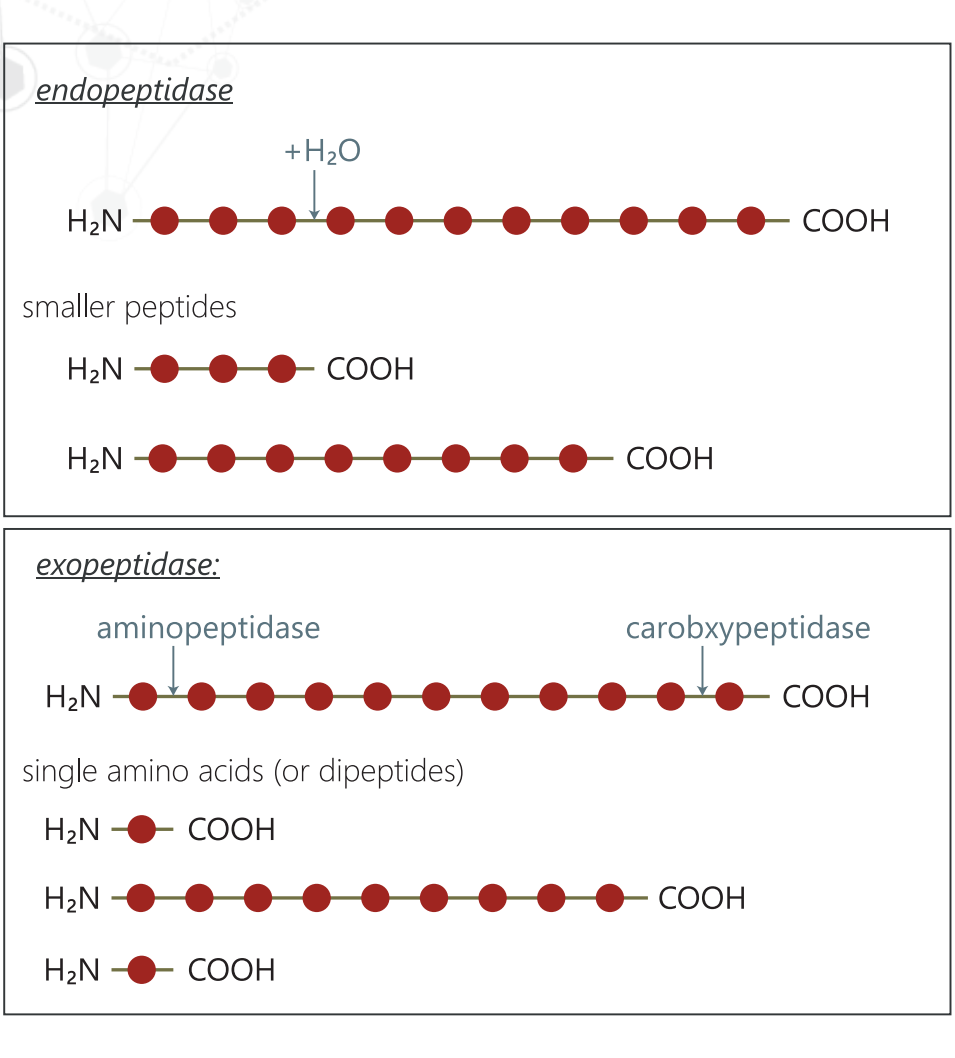


Figure 7. Peptide production following hydrolysis using an endopeptidase or exopeptidase

Biocatalysts Ltd.'s proteases, Promod® 215MDP and Flavorpro® 937MDP can be used to develop proteolytic savoury notes typical of cheese, in the production of EMDI and EMCs. Flavorpro® 937MDP is a high-performance exopeptidase for reducing bitterness, which can be used in combination with other enzymes. Promod® 845MDP is a protease particularly suited for the manufacture of EMCs where creamy protein notes are required. Promod® 845MDP generates cooked, caramel, and nutty flavours with very low bitterness, producing a creamy savoury taste in EMC application. Promod® 903MDP has been specifically designed to generate a sharp (aged cheese), savoury taste in EMC applications. Flavorpro® UMAMI 852MDP can be used for the manufacture of EMCs where strong mature and mouth filling savoury protein notes are required. Flavorpro® UMAMI 852MDP releases high levels of glutamic acid compared to other exo- and endopeptidases (Figure 8). Glutamic acid is an amino acid giving strong umami flavour commonly found in fermented or aged foods. Umami heightens the flavour profile which helps increase the apparent saltiness in EMC (Figure 9), therefore EMCs made with Flavorpro® UMAMI 852MDP can be used for salt reduction in processed cheese applications. Flavorpro® UMAMI 852MDP characteristically does not impart a significantly bitter flavour and is therefore an ideal protease for use in flavour generation for EMCs.

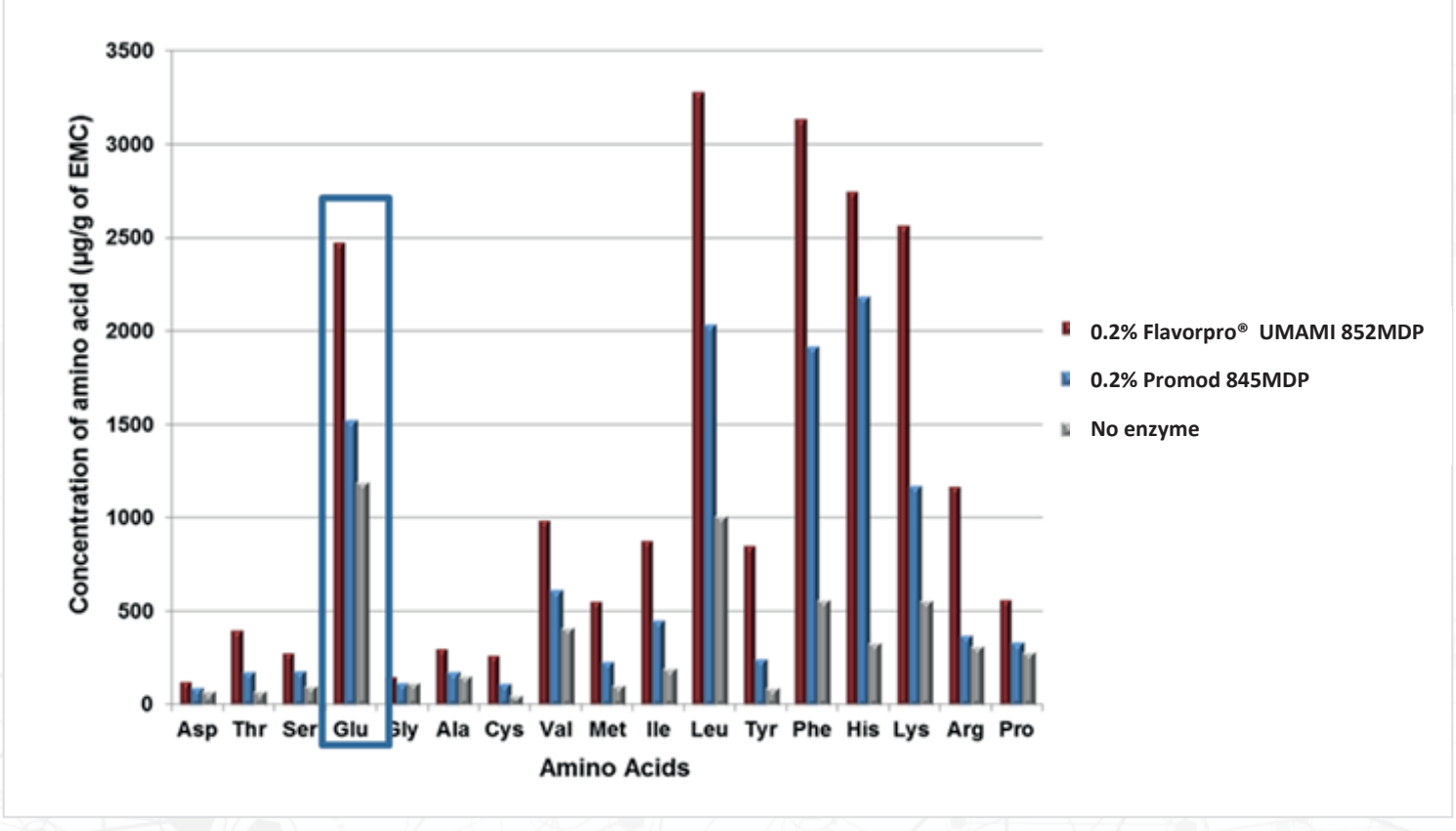


Figure 8. Amino Acid Profile of EMCs made with Promod® 845MDP and Flavorpro® UMAMI 852MDP (0.2% w/w on cheese in the EMC, 40°C, 22h)

Figure 9. Sensory analysis of EMC made with Flavorpro® UMAMI 852MDP and Promod® 215MDP (by an external flavour analyst company)

Biocatalysts Ltd.'s range of protease enzymes includes Promod® and Flavorpro® enzymes all can be used to develop proteolytic savoury notes typical of cheese, in both EMC and accelerated ripening applications.

The table below shows our range of Promod® and Flavorpro® enzymes and their benefits.

TABLE 3: BIOCATALYSTS LTD.'S RANGE OF PROTEASE ENZYMES FOR EMDI & EMC MANUFACTURING

Product	Dose rate (% w/w on cheese in the slurry)	Benefit
Promod® 215MDP	0.05 - 0.1	Microbial endopeptidase for mature savoury notes with low level bitterness.
Promod® 845MDP	0.05 - 0.2	Microbial protease for producing a creamy savoury taste in EMC application.
Promod® 903MDP	0.05 - 0.2	Microbial protease suited for the manufacture of EMCs where sharp protein notes are required.
Flavorpro® 937MDP	0.05 - 0.1	High performance microbial exopeptidase primarily used to reduce bitterness or produce mature savoury notes.
Flavorpro® 373MDP	0.05 - 0.1	Mixture of micobia endopeptidase activities with a high level of glutaminase side activity for high release of glutamic acid.
Flavorpro® UMAMI 852MDP	0.1 - 0.3	Micobial exopeptidase with endopeptidase and glutaminase side activities used for the manufacture of EMCs where strong, mature and mouth filling savoury protein notes are required. EMC made with Flavorpro® UMAMI 852MDP can be used for salt reduction in processed cheese applications.

CONCLUSION

If you would like to discuss further how enzymes will help improve your product, arrange a sample to test or find out more about our unique enzyme development & manufacturing service please get in contact.