## THE USE OF ENZYMES IN EGG PROCESSING

Eggs provide valuable ingredients to the food industry with a variety of functional properties including emulsifying components in batters and mayonnaise; foaming in cakes and meringues; gelation in baked goods and quiches; adding nutritional value to foods such as dietary bars or powdered mixes (protein fortification); and improved texture of baked goods. The main components of eggs are proteins and lipids, and these are responsible for the functional attributes.

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Enzymes can be used to further improve the quality of these functional ingredients, from improving the emulsification of egg yolks, to increasing egg white foaming capacity and producing a non-bitter tasting protein hydrolysate. At Biocatalysts, our knowledge, expertise, and products mean all your enzyme requirements for processing eggs can be satisfied. This technical bulletin will take you through each enzyme available and detail the different ways which they can improve your egg processes, giving you an added competitive advantage.

The enzymes covered in this technical bulletin are from our current off-the-shelf range. If you find that these are not suitable, Biocatalysts has the enzyme development & manufacturing capabilities to create a completely unique enzyme for you, which can open endless possibilities.

## EGG PROCESSING OVERVIEW

Traditionally, egg ingredients were supplied in the form of whole (shell) eggs. However, today's food processors can choose from a wide range of egg ingredients where various processes are used to produce whole egg, egg yolks or egg whites which can be liquid or dried. In the past, it was thought that fresh whole shell eggs and liquid products had the best functionality. However, both liquid and dried egg products can be treated with enzymes to improve functionality and may also be supplemented with salt, sugar, or other ingredients to produce speciality egg products with improved functionality tailored for specific applications.



Dried egg products have the advantage that they can be easily pasteurised, have excellent shelf life and stability, are easier and cheaper to ship due to reduced volume and can be tailored with specific functionality.

Eggs are usually processed in a semi-continuous process. The entire process is best run in a chilled room to reduce the likelihood of microbial growth. Eggs are washed, cracked and the egg yolk and white separated if desired. The liquid egg is pumped into a tank where enzymes can be added to improve the functional properties of the egg. It can then be pasteurised and spray-dried.

The speed of the separator is critical to the quality of the egg white. A balance must be obtained between speed (and hence efficiency) and egg quality. The faster the separator is run the more yolk will contaminate the liquid egg white. A small amount of yolk lipid contaminating the egg white can significantly reduce its foaming capacity. Contaminating yolk lipids can be removed using Lipomod<sup>®</sup> 34P (see the section "Improving foaming capacity by removing lipids"). This allows production of "high-whip" egg white with improved foaming capacity whilst allowing faster throughput as the separator can be run at a higher setting.

The whole liquid egg, liquid egg white or liquid yolk is pumped from the separator to holding tanks. It can take several hours to fill a large tank, so it is important to keep the egg chilled to prevent microbial growth. Alternatively, if the liquid egg is to be stored for extended periods or at warmer temperatures, hydrogen peroxide may be added to prevent microbial growth. The hydrogen peroxide can then be removed with Catalase 929L immediately prior to pasteurisation (see section on "preventing microbial spoilage with peroxide and catalase").

The holding tank is an ideal location in which to add enzymes to improve the functional characteristics of the egg. If enzymes are to be added at this stage it is important that the holding tank can be stirred to ensure the enzyme is adequately distributed throughout the mixture. Biocatalysts' range of enzymes for egg processing will function below ambient conditions but a faster, more efficient reaction will be achieved if the process is carried out above ambient conditions. Continuous pH control is generally not necessary although most processors adjust the pH of the egg with citric acid prior to processing to adjust for the loss of carbon dioxide as the eggs age.



Egg yolks have extremely useful emulsifying and gelation properties due to the presence of various lipid and protein types and have been extensively used in recipes for products such as mayonnaise or baking ingredients. Enzymes can be used to modify the structure of the lipids present in egg yolks to improve their functionality. Egg yolk is a complex oil water emulsion composed of 50% water, 32% lipids and 16% protein. Approximately 28% of the lipids are phospholipids, of which roughly 80% is phosphatidylcholine, 12% is phosphatidylethanolamine

phosphatidylethanolamine (PE) 0 H

С-О-С-Н О Н Н Н | | | | |0 0 H-С-О-Р-О-С-С-N-N | | | | | | H О<sub>0</sub> Н Н Н

Figure 1: Structures of the main phospholipids in egg yolk

with other phospholipids such as sphingomyelin and lyso-phosphatidylcholine. The surface-active properties of these phospholipids can act a little like soap in stabilising oil water emulsions. Enzymatic conversion of the phospholipids into lyso-phospholipids increases the emulsion stability produced with these egg yolks.

Lipomod<sup>®</sup> 699L is a phospholipase A2 enzyme derived from porcine pancreas and is a highly cost-effective non-GM phospholipase A2. Enzymatically modifying the lipids in egg yolk greatly improves its emulsification properties, this means less modified-egg yolk is required to produce the same viscosity as non-treated egg yolk in mayonnaise and salad dressings.

Lipomod<sup>®</sup> 833L2 is a microbial phospholipase A2 that is also capable of modifying the lipid components of egg yolk to improve its emulsifying characteristics. Biocatalysts developed Lipomod<sup>®</sup> 833L2 to be able to offer an enzyme that is Kosher, Halal and vegetarian compliant, to meet the growing market needs.

In addition to improving the emulsification properties of egg yolk, enzymatically modifying the yolk's structure also makes the mayonnaise produced more heat stable, which means it can be pasteurised without risk of separating.

The enzyme phospholipase A2 cuts at the 2 position on the glycerol backbone (see Fig. 2) to produce new molecules with different and superior emulsifying properties. Other phospholipase enzymes such as phospholipase A1 and phospholipase D are generally not as effective at improving the emulsification properties of egg yolk so phospholipase A2 should be used for this application.

Lipomod<sup>®</sup> 699L or Lipomod<sup>®</sup> 833L2 are both ideal enzymes to improve the emulsifying properties of egg yolk, whole egg, or purified lecithin. As a guide, whole egg or a 65 - 80% w/v aqueous solution of egg yolk can be prepared. The solution should be dosed with either Lipomod<sup>®</sup> 699L or Lipomod<sup>®</sup> 833L2 at 200 to 1,000 ml per ton of egg product. To ensure the enzyme is evenly distributed throughout the mixture the solution should be gently mixed. The reaction takes 2 - 4 hours at 40 - 60°C to reach completion.

To prevent damage to the egg, some processors prefer to incubate the reaction at lower temperatures for longer periods (overnight).



Figure 2: Phospholipase A2 specificity

Following the addition of Lipomod<sup>®</sup> 699L or Lipomod<sup>®</sup> 833L2, phospholipids are rapidly hydrolysed to produce lyso-phospholipids and free fatty acids. The lyso-phospholipids are more hydrophilic than the phospholipids and therefore have superior emulsifying properties. Titration methods can be used to estimate the end point of the reaction by measuring the concentration of free fatty acids released, alternatively lyso-lecithin can be measured by Nuclear Magnetic Resonance.

Under optimum conditions, over 80% of the phospholipids can be hydrolysed within the first hour. Once the reaction is complete the modified egg yolk can be pasteurised.

## EXTERNAL EVALUATION MEASURING THE VISCOSITY AND FIRMNESS OF MAYONNAISE MADE WITH LIPOMOD<sup>®</sup> L833L2

An independent study was conducted to compare the functional properties of mayonnaise manufactured using egg yolk modified by Lipomod<sup>®</sup> 833L2 and egg yolk modified using a commercially available microbial phospholipase A2 enzyme. Both enzymes were added to the egg yolk at 2 different dose levels: 250ml and 450ml per ton of egg yolk. All batches of egg yolk were incubated at 50°C for 4 hours.

The viscosity and texture of all four mayonnaise samples were measured: the viscoelastic properties were measured using a rheometer and the firmness was determined using a texture analyser. In addition to the enzyme-modified egg yolk mayonnaise samples, a mayonnaise was also made with untreated egg yolk and tested as a base line control in the evaluation trials.

# MAYONNAISE

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The mayonnaise was produced by a 2-stage process, the egg yolk, water, salt sugar, mustard and vinegar were pre-mixed in a pilot scale Silverson high shear mixer. Then the crude emulsion was passed through a colloid mill to create a finer emulsion.

The study shows that egg yolk treated with Lipomod<sup>®</sup> 833L2 is capable of making a mayonnaise that overall has the same viscosity (see Fig. 3) and firmness (see Fig. 4) as a mayonnaise made with a similar commercially available microbial phospholipase A2.



Note: These mayonnaise trials and results originate from independent studies carried out by Campden BRI, United Kingdom (www.campdenbri.co.uk)

COMPARISON OF MAYONNAISE VISCOSITY (at a shear rate of 1 s<sup>-1</sup>)



## IMPROVE FOAMING PROPERTIES OF EGG WHITE BY REMOVING CONTAMINATING YOLK LIPIDS WITH LIPOMOD<sup>®</sup> 34P

The main functional property of egg white is its high foaming capacity. Any cross contamination of egg white with egg yolk lipids greatly reduces its foaming capacity. In a high throughput egg processing plant it is impossible to avoid cross contamination. The solution is to remove any egg yolk lipids from the egg white using Biocatalysts' Lipomod<sup>®</sup> 34P (L034P). This enzyme breaks down the lipid complexes reducing any contamination and ensuring the egg white maintains full foaming capacity.

The enzyme may be added to the liquid egg white. As an initial guide Lipomod<sup>®</sup> 34P can be dosed at 10 - 30 mg per kg of egg white. The reaction can be allowed to proceed with mixing at 40°C for 2 to 5 hours. After incubation, the egg white can be pasteurised and, if desired, spray dried.



Figure 5. Improved foaming properties of egg white by removing contaminating yolk lipids with Lipomod® 34P

## FLAVORPRO<sup>®</sup> 786MDP FOR THE PRODUCTION OF EGG WHITE HYDROLYSATES

Egg white protein is a valuable product with important nutritional and functional properties. The most abundant protein in egg white is ovalbumin. The proteins present in egg white are high quality proteins, relatively easy to digest and efficiently absorbed into the body. Biocatalysts' Flavorpro<sup>®</sup> 786MDP is designed to efficiently hydrolyse egg white proteins to produce a bland, non-bitter tasting egg white hydrolysate. The single step enzymatic treatment of egg white proteins using Flavorpro<sup>®</sup> 786MDP results in a reduction in the size of the egg white proteins, thus allowing them to be more efficiently digested and absorbed into the body. This is very useful for protein fortification of foods such as nutritional bars or powdered mixes, used by athletes, where clarity of the egg white hydrolysate in the final product is not important.

The benefits of using Flavorpro® 786MDP to hydrolyse egg white protein include:

- Improved absorption and digestion of egg white proteins.
- Improved heat stability (heat stable up to 90°C).
- Improved foaming properties of egg white proteins.
- Efficient hydrolysis of ovalbumin.
- Clean tasting cloudy egg white hydrolysate produced.

Flavorpro<sup>®</sup> 786MDP can also be used in combination with other enzymes, such as Flavorpro<sup>®</sup> 937MDP, if an egg white protein hydrolysate with a high degree of hydrolysis is desirable.

# PREVENTING MICROBIAL SPOILAGE USING

## **HYDROGEN PEROXIDE AND CATALASE 929L**

Processed eggs should be pasteurised to eliminate the presence of possibly harmful bacteria and prevent spoilage. Micro-organisms in the egg can be killed by exposure to heat or sterilizing chemicals. It is virtually impossible to completely eliminate all the micro-organisms but the longer the egg is exposed to heat or a sterilizing agent, and the higher the temperature or concentration of the sterilant, the more micro-organisms are killed and the longer it will take them to grow back. Under UK law, the method of pasteurisation should achieve the same reduction in micro-organisms as heating to 64.4°C for at least 2 minutes and 30 seconds.

Eggs are usually pasteurised by heating. Unfortunately, the temperatures typically used to pasteurise eggs can damage the egg proteins impacting their functional characteristics. To lessen this damage, hydrogen peroxide can be used to chemically sterilise the egg solution before thermal pasteurisation allowing shorter time and/or lower temperature combinations to achieve the desired reduction in micro-organisms. If hydrogen peroxide has been utilised to assist pasteurisation of egg ingredients Catalase 929L should then be used to remove residual peroxide, breaking it into harmless water and oxygen.

To sterilise egg products using hydrogen peroxide, approximately 1.3 litres of 35% hydrogen peroxide is added slowly with mixing to each tonne of liquid egg product. The hydrogen peroxide should be added slowly to avoid damage to the egg proteins by high peroxide concentrations. The mixture is held for at least 20 minutes to allow the hydrogen peroxide to kill vegetative micro-organisms. Low levels of hydrogen peroxide can also be used to prevent growth during long incubations in holding tanks. Once sterilization is complete, residual hydrogen peroxide must be eliminated with Catalase 929L. As a guide, 100 - 150 ml of Catalase 929L is added per tonne of liquid egg and mixed. The mixture may bubble for a while as the hydrogen peroxide is broken down to water and oxygen gas, so it is advisable to allow space in the tank for foaming.

It is important to consider that once catalase has been added and the hydrogen peroxide removed, conditions are suitable once again for the growth of micro-organisms. So, the egg must be kept cold and in clean containers. Ideally, we recommend the liquid egg is then pasteurised by traditional heating methods. The combination of hydrogen peroxide and heat pasteurisation achieves a much greater reduction in microbial numbers than either technique can independently. Interestingly, the hydrogen peroxide treatment sensitises spore formers making them much easier to kill by heating. The net effect is improved shelf life resulting from greater reduction in microbial numbers whilst maintaining the functional characteristics of the egg by reducing exposure to heat damage.

NOTE: Since the nature of the substance to be processed can be variable and processes may operate in different manners, this will influence the performance of the enzyme. The above are therefore guidelines only and in all cases, trials should be carried out in order to determine exact conditions necessary to achieve the product with the desired characteristics. The dose of enzyme, temperature, pH, and time of incubation are important factors to consider in any trial. Biocatalysts cannot accept any liability if the above information is used to produce product without having first performed adequate trials.

### BIOCATALYSTS' RANGE OF ENZYMES FOR EGG PROCESSING

Product	Benefit
Lipomod <sup>®</sup> 34P	Improves emulsification properties by modifying phospholipids.
Lipomod <sup>®</sup> 699L	Improves emulsification properties by modifying phospholipids.
Lipomod <sup>®</sup> 833L2	Microbial phospholipase that improves emulsification properties, can be used to produce Kosher, Halal, and vegetarian products.
Catalase 929L	Removes hydrogen peroxide that is used prior to pasteurisation to prevent microbial spoilage of the egg ingredients.
Flavorpro <sup>®</sup> 786MDP	Used to produce clean tasting egg white hydrolysates for a variety of end applications. Flavorpro® 786MDP can also be used in combination with Flavorpro® 937MDP if an egg white protein hydrolysate with a high degree of hydrolysis is desirable.

#### COMMON PROBLEMS ENCOUNTERED IN EGG PROCESSING

Problem	Enzyme Solution
Egg white does not foam.	Eliminate egg yolk lipids with Lipomod® 34P.
Pasteurisation destroys egg functional properties.	Sterilise with hydrogen peroxide and eliminate residual hydrogen peroxide with Catalase 929L.
Insufficient emulsion of egg yolk.	Addition of Lipomod <sup>®</sup> 699L or Lipomod <sup>®</sup> 833L2 will increase emulsification of egg yolk.



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If you would like to further discuss 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.