The Use of Enzymes in Egg Processing

Eggs provide valuable ingredients to the food industry with a variety of functional properties including foaming in cakes and meringues; gelation in cakes and quiches; emulsifying components in batters and mayonnaise; and improved texture of baked goods. The main components of egg are proteins and lipids and these are responsible for the functional attributes.

Enzymes can make the quality of these functional ingredients even better. From ensuring dried egg white remains white to improving the emulsification properties of egg yolk. At Biocatalysts, all your enzyme requirements can be satisfied - we can be your one stop shop. This technical bulletin will take you through each enzyme available and detail the different ways which they can improve your egg process, 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 up 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 age of the eggs used can have a significant impact on the process. Generally, the fresher and cleaner the eggs used, the less the risk of microbial spoilage. The surface of the shells can carry a high load of debris, so it is important to remove as much of this as possible during washing.

Some processors use a mild sanitizing solution or hot water to reduce microbial contamination prior to cracking. If older eggs are used or there is a risk of debris contaminating the liquid egg, then
addition of hydrogen peroxide to the liquid egg will prevent microbial growth. The hydrogen peroxide is then removed with the use of an enzyme (Catalase 641L) immediately prior to pasteurisation.

After washing, the eggs are cracked and then strained to remove shell fragments. If whole egg is required the liquid egg is homogenised and pumped to a collecting tank. For separated egg white and egg yolk the eggs are cracked in a separator.

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™ 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 temperature, hydrogen peroxide may be added to prevent microbial growth. The hydrogen peroxide can then be removed with Catalase 641L 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.

**Biocatalysts’ Range of Enzymes for Egg Processing**

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<td>Improves emulsification properties by modifying yolk phospholipids.</td>
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**Improvement of Emulsifying Properties with Lipomod™ 699L**

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.

Using Lipomod™ 699L, a highly cost effective non-GMO phospholipase, gives the enzyme-modified yolk greatly improved emulsification properties so that less modified yolk is required to produce the same viscosity as normal yolk in mayonnaise and salad dressings.
A porcine phospholipase may convert up to 95% lecithin to lysolecithin whereas the microbial GMO alternatives generally achieve lower levels of conversion. This allows Lipomod™ 699L to provide even greater emulsification, or results in lower addition levels and therefore cost savings.

Another key benefit to using enzyme-modified yolk is that the mayonnaise is more heat stable and can now be pasteurised without separating.

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 approximately 80% is phosphatidylcholine, 12% is phosphatidylethanolamine 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™ 699L contains the enzyme phospholipase A2 derived from porcine pancreas. The enzyme cuts at the Sn-2 position on the glycerol backbone (see Fig 4) to produce new molecules with different and superior emulsifying properties. Other phospholipase enzymes such as phospholipase A1, phospholipase D and microbial phospholipase A2 are generally not as effective at improving the emulsification properties of egg yolk so porcine phospholipase A2 should be used for this application.

Lipomod™ 699L is an ideal enzyme to improve the emulsifying properties of liquid egg. As a guide, whole egg or a 65 - 80% w/v aqueous solution of egg yolk can be prepared. A salt concentration of 10-12% may be advisable to prevent microbial growth during the process. The enzyme is stimulated by the presence of calcium. There is usually sufficient calcium present in egg products but in some cases addition of extra calcium may increase the efficiency of the reaction.

Lipomod™ 699L can be dosed at 1,000 to 2,000 units (0.1 to 0.2 ml) per litre of egg product. No pH adjustment is necessary. The reaction takes 2-4 hours at 40 - 60°C with gentle mixing.

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

Following addition of Lipomod™ 699L, phospholipids are rapidly hydrolysed to produce lyso-phospholipids and free fatty acids. Titration methods can be used to estimate the end point of the reaction by measuring the concentration of free fatty acids released or 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.
Effect of temperature on activity of Lipomod™ 699L

Effect of pH on activity of Lipomod™ 699L

Heat Stability

Emulsions produced from egg yolk treated with Lipomod™ 699L show better stability after heat treatment. In contrast to the unmodified yolk the changes in the droplet size in emulsions made with hydrolysed yolk after heating up to 80°C are very small indicating a much better stability of the emulsions. The improved heat stability of the emulsion can be observed after 1 hour.

Fig 5. Droplet sizes of model emulsions before and after heat treatment made from hydrolysed and non-hydrolysed egg yolk.
Mayonnaise

Yolk treated with Lipomod™ 699L makes a superior mayonnaise that can be pasteurised without separating out. Enzyme–modified yolk is used in place of untreated egg yolk in a standard mayonnaise recipe. Because the enzyme-modified yolk has superior emulsifying properties, it is also possible to achieve better results using less yolk, saving money on expensive ingredients.

Note: These results originate from independent studies carried out at the German Institute of Food Technology (www.dil-ev.de).

Mayonnaise Recipe:

- Oil - 72.4%
- Vinegar (4.5% acetic acid) - 1.4%
- Egg yolk - 4.5%
- Sugar - 2.3%
- Salt - 1.4%
- Water - 18%

Egg yolk, water, salt sugar and vinegar are mixed and the oil is gradually beaten in.

Because the enzyme-modified yolk has superior emulsification properties, the resulting mayonnaise will be firmer than when using unmodified yolk as shown in Fig 6 below.

The mayonnaise should then be pasteurised. The temperatures used during pasteurisation can cause droplets in mayonnaise made with untreated egg yolk to combine and separate out. Using enzyme modified egg yolk allows the mayonnaise to be pasteurised at higher temperatures with no separation, loss of firmness or increase in droplet size.

Improve foaming properties of egg white by removing contaminating yolk lipids with Lipomod™ 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 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™ 34P (L034P). This enzyme breaks down the lipid complexes and ensures the egg white maintains full foaming capacity.

The enzyme may be added to the liquid egg white. As an initial guide Lipomod™ 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.
The effect of temperature on Lipomod™ 34P

The effect of pH on Lipomod™34P

Fig 7. Improved foaming properties of egg white by removing contaminating yolk lipids with Lipomod™ 34P
Improve foaming properties of egg white by modifying protein with Promod™ 194SP

Foaming ability (volume and speed of formation) can be improved by a minor modification of the egg white proteins. Some processors incubate dried egg white in a hot box for several days to produce high whip egg white powders. The heat treatment partially denatures the egg proteins, improving their whipping ability and resulting in greater foam height. However, if the hydrolysis goes too far then a dramatic decrease in foam stability will be seen. There is an optimum hydrolysis point whereby foam volume is increased without adversely affecting the foam stability; this is at quite a low degree of hydrolysis (DH). This improvement can be achieved much faster in liquid egg by the use of Biocatalysts' Promod™ 194SP protease to modify the egg proteins. Promod™ 194SP is added at a dose of 0.2 to 1% based on protein weight (0.02-0.2% of liquid egg). After incubation the egg white can be pasteurised and, if desired, spray dried. (see Fig 8).

**Fig 8. Improved foaming properties of egg white by modifying protein with Promod™ 194SP**

The effect of temperature on Promod™ 194SP
The effect of pH on Promod™ 194SP

Preventing microbial spoilage using peroxide and Catalase 641L

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 egg proteins changing 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 641L should then be used to remove residual peroxide, breaking it into harmless water and oxygen.

To sterilize 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 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 peroxide to kill vegetative micro-organisms. The peroxide can be left for longer. For example, low levels of peroxide can be used to prevent growth during long incubations in holding tanks. Once sterilization is complete, residual peroxide must be eliminated with Catalase 641L. As a guide, 100 - 150 ml of Catalase 641L is added per tonne of liquid egg and mixed. The mixture may bubble for a while as the 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 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 peroxide and heat pasteurisation achieves a much greater reduction in microbial numbers than either technique can independently. Interestingly, the peroxide treatment sensitises sporeformers making them much easier to kill by heating.
The effect of temperature on Catalase 641L

The effect of 

The effect of pH on Catalase 641L

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.

Prevent browning by removing sugar with Glucose Oxidase 789L

Another problem occurring during the heat treatment of eggs is browning caused by the Maillard reaction. This occurs as a result of small amounts of glucose in the egg whites reacting with amino acids. This can be problematic for dried egg whites if the product is traditionally pasteurised after drying in a hot room, for an extended period of time. Biocatalysts’ Glucose Oxidase 789L (G789L) is able to break down the glucose to products which do not cause browning.

Glucose Oxidase requires the presence of dissolved oxygen to function so it is important to ensure the liquid egg is well aerated. Desugaring is generally best carried out in combination with the same catalase used to remove peroxide from egg producing water and oxygen, ensuring there is an excess of dissolved oxygen available for the reaction.

Glucose Oxidase 789L can be used to remove sugar from whole liquid eggs, egg yolk or liquid egg white. The reaction is normally carried out in a heated stirred tank, though lower temperatures may also be used. First, check the pH of the egg, and if necessary adjust to pH 7.0 or below by the slow addition of a 10% solution of citric acid. Gently warm the egg to 35°C and slowly add 3–4 litres of hydrogen peroxide (35%) to avoid denaturing the egg. Take care as the solution may bubble and
foam as oxygen is released. Add 100-200ml of Glucose Oxidase 789L per tonne of liquid egg (0.01-0.02%). An increased amount of enzyme may be required if the reaction is performed at lower temperatures. Continue to slowly stir the egg and maintain the desired temperature throughout the incubation period. After 6 hours take a sample and test for glucose. If the sample tests positive for glucose, add another 3-4 litres of hydrogen peroxide and incubate for a further 2 to 4 hours, then retest. The egg can then be pasteurised as normal and if desired dried without browning.

**Flavorpro™ 786P 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™ 786P 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™ 786P 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™ 786P 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.

**Application and dosage recommendations for Flavorpro™ 786P**

Flavorpro™ 786P is supplied as a standardised powder. Please contact Biocatalysts Ltd via sales@biocats.com or +44 (0)1443 843712 for further application data, advice on how to produce egg white protein hydrolysates using Flavorpro™ 786P or for a sample of egg white hydrolysate produced using Flavorpro™ 786P.

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.

All of the aforementioned enzymes are food grade and have been traditionally available within Biocatalysts’ product range for use as food processing aids and ingredients. Biocatalysts duce a range of enzymes originating from various organisms with similar but not always identical functionality. If one of the above does not prove suitable for a particular process Biocatalysts would be happy to advise where possible and assist with offering samples of alternative products for trialling.
### Common Problems Encountered in Egg Processing

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<th>Enzyme Solution</th>
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<td>Egg white goes brown during pasteurisation and drying.</td>
<td>De-sugar with Glucose Oxidase 789L to prevent this.</td>
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<tr>
<td>Egg white does not foam.</td>
<td>Eliminate egg yolk lipids with Lipomod™ 34P.</td>
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<tr>
<td>Pasteurisation destroys egg functional properties.</td>
<td>Sterilise with hydrogen peroxide and eliminate residual hydrogen peroxide with Catalase 641L.</td>
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<tr>
<td>Insufficient emulsion of egg yolk.</td>
<td>Addition of Lipomod™ 699L will increase emulsification of egg yolk.</td>
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### Contact Us

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.

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