



The use of enzymes in egg processing

Biocatalysts Ltd produces a complete range of enzymes for use in all aspects of egg processing.

Lipomod™ 699L	Improve emulsification properties by modifying yolk phospholipids
Lipomod™ 34P	Improve foaming properties of egg white by removing contaminating yolk lipids
Promod™ 194P	Improve foaming properties of egg white by modifying protein
Catalase C641L	Remove peroxide used to pasteurise egg
Glucose Oxidase 168L	Prevent browning by removing sugar

Eggs are extremely useful food ingredients and have a variety of functional properties including foaming, gelation, emulsification and texturisation. Eggs provide foaming properties in cakes and meringues; gelation in cakes and quiches; emulsifying components in batters and mayonnaise and improve the texture of baked goods. The main components of egg are proteins and lipids and these are responsible for the functional attributes. Other components are present in small quantities.

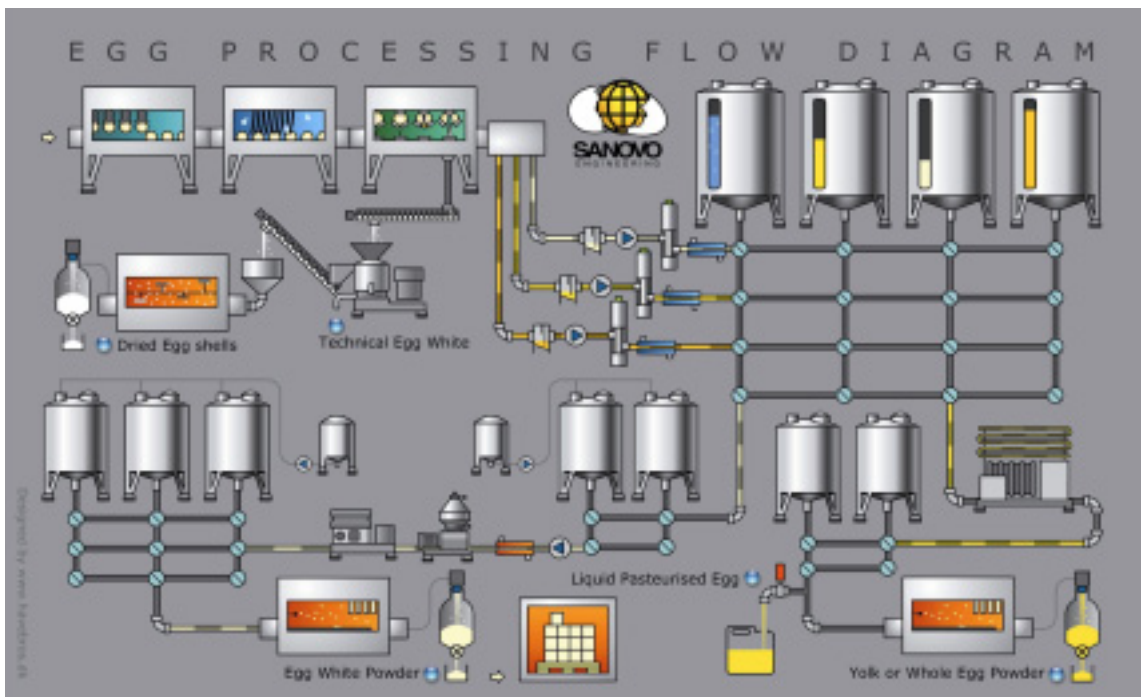
EGG PROCESSING OVERVIEW

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 liquid, frozen, dried, whole eggs, whites or yolks. It used to be that fresh 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 (see process diagram on Page 2). The entire process is best run in a chilled room to reduce to likelihood of microbial growth. Eggs are washed, cracked and the egg 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 if dried egg products are desired.





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, it may be valuable to add hydrogen peroxide to the liquid egg to prevent microbial growth. The hydrogen peroxide is then removed with Catalase 641L immediately prior to pasteurisation (see section on “preventing microbial spoilage with peroxide and catalase”).

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



Figure 1: A cracker / separator



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”).



Figure 2: Holding tanks for liquid egg products

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 all function at -10 °C but, if the tanks can be heated this may allow faster, more efficient reaction times. 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.

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. Lipomod™ 699L can be used to improve this functionality producing enzyme-modified yolk with enhanced emulsification characteristics or to manufacture speciality emulsifiers such as lyso-lecithin giving the benefit of less yolk being required to produce a firmer emulsion. Also the

emulsion is more stable and can be heated (e.g. during pasteurisation) without separating out.

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 sphingomyline 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 Figure 4 on page 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 not 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. It is often advisable to add 10 - 12 % salt 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 might be dosed at 10,000 to 20,000 units (1 to 2 ml) per litre of egg product. The pH should be checked and adjusted to pH 8.0 if necessary. The reaction takes 2-4 hours at 40 - 60°C with gentle mixing.

The activity of Lipomod™ 699L is optimal at pH 10.0 and at temperatures above 60°C but these harsh conditions may damage egg proteins. To prevent damage to the egg, some processors prefer to incubate the reaction at lower temperatures (25°C) for longer periods (overnight). Because the enzyme is not stable for long periods at higher pHs, it may be necessary to adjust the pH below pH 7.0 to maintain activity during longer incubation periods.



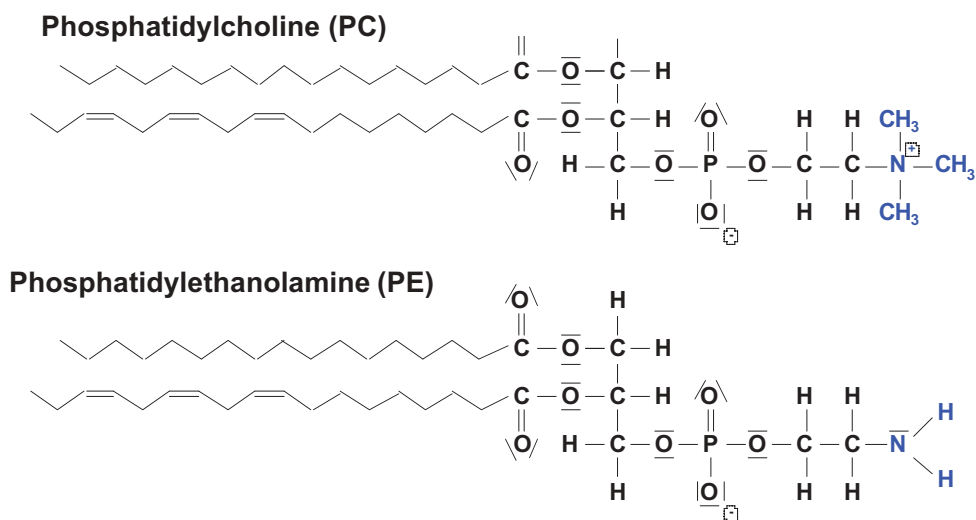


Figure 3: Structures of the main phospholipids in egg yolk

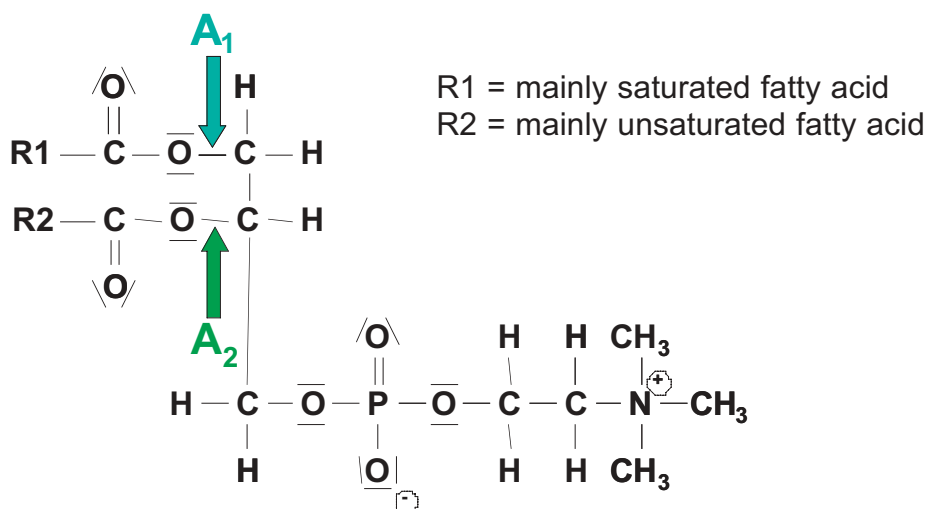


Figure 4: Phospholipase A classes and their specificities

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 may be measured by Nuclear Magnetic Resonance.

Under optimum conditions, over 80% of the phospholipids will be hydrolysed within the first hour.

Once the reaction is complete, the modified egg yolk can be pasteurised.



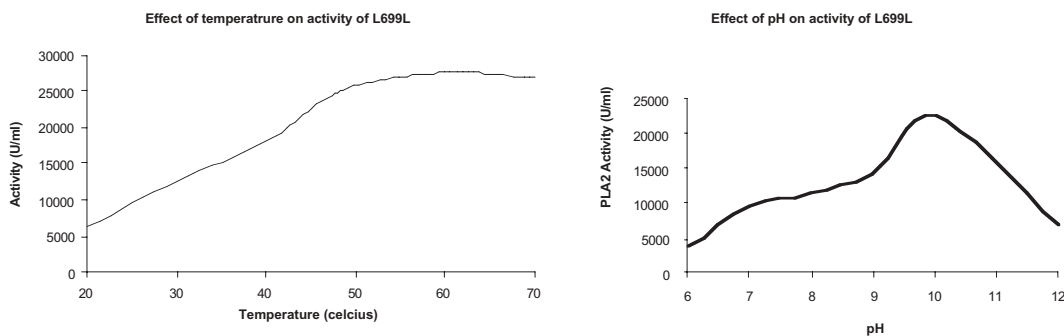


Figure 5: Effect of temperature and pH on L699L

HEAT STABILITY**

Emulsions produced from egg yolk treated with Lipomod™ 699L shows better stability after heat treatment. In contrast to the unmodified yolk, the changes in the droplet size in emulsions made with hydrolysed yolk after the 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 although the further increase of free fatty acids indicated that the hydrolysis is not completed.

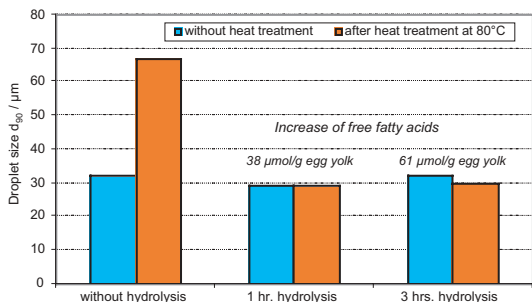


Figure 6: Droplet sizes of model emulsions before and after heat treatment made from hydrolysed and non-hydrolysed egg yolk.

MAYONNAISE**

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

** 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, 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 the diagram below**.

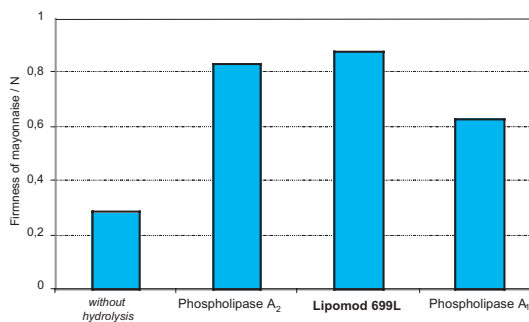


Figure 7: Firmness of mayonnaise made from non hydrolysed egg yolk and egg yolk hydrolysed by different phospholipases.

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, whole egg or yolk. As an initial guide, Lipomod™ 34P might be dosed at 10 - 30 mg per kg of egg. 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.

IMPROVE FOAMING PROPERTIES OF EGG WHITE BY MODIFYING PROTEIN WITH PROMOD™ 194P

Foaming ability 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. This improvement can be achieved much faster in liquid egg by the use of the Biocatalysts protease Promod™ 194P (P194P) to modify the egg proteins. Promod™ 194P is added at a dose of 0.5 to 2% to liquid egg white and incubated with gentle mixing for 2 to 4 hours at 40°C. After incubation, the egg white can be pasteurised and if desired, spray dried. (See Figure 10 on Page 7).

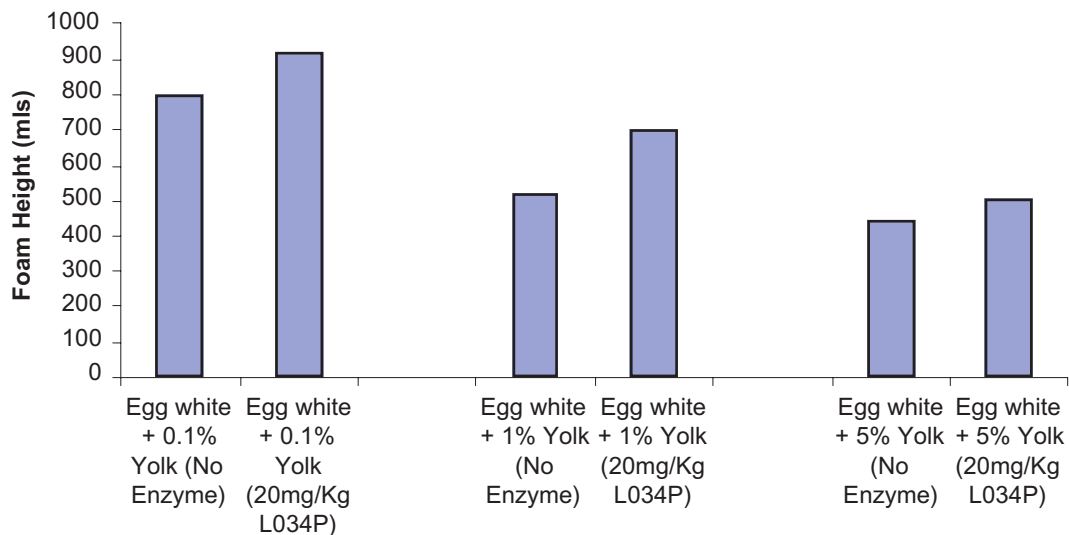


Figure 8: Improved Foaming Properties of Egg White by removing contaminating yolk lipids with Lipomod™ 34P

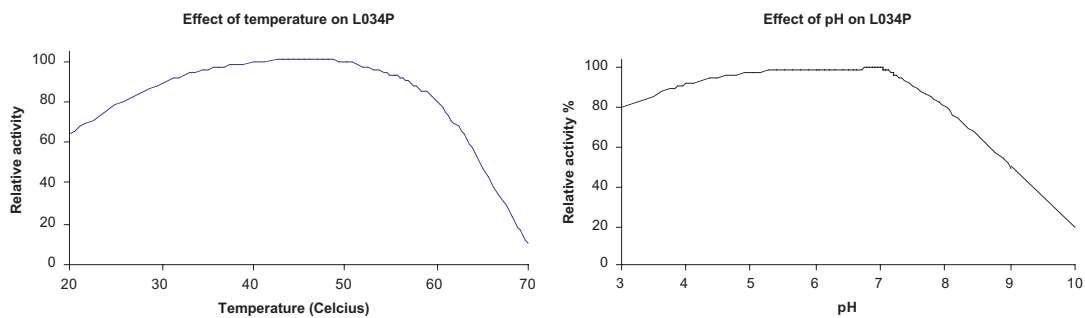


Figure 9: Graphs showing the effect of temperature and pH on LO34P



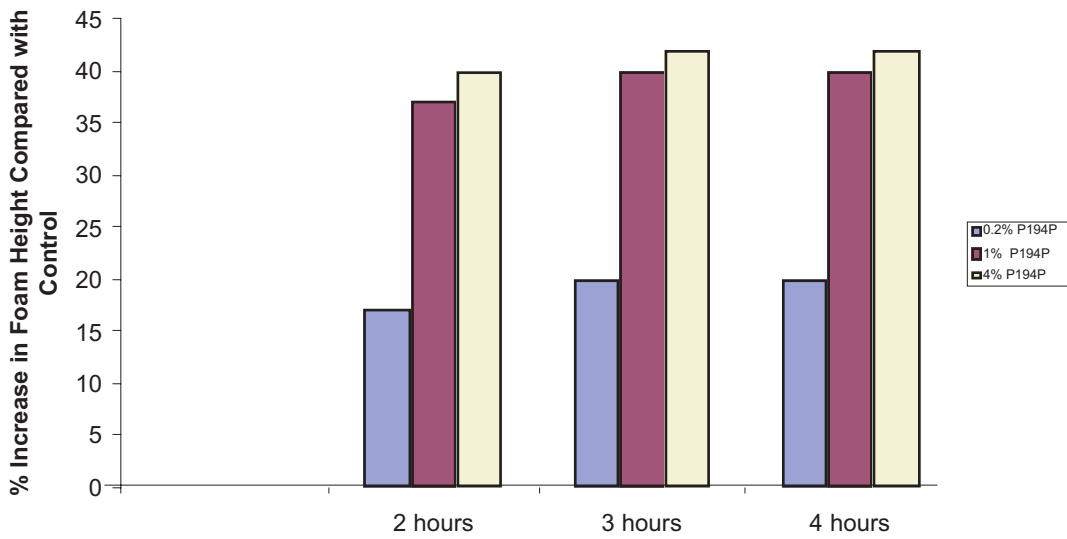


Figure 10: Improved foaming properties of Egg White by modifying protein with Promod™ 194P

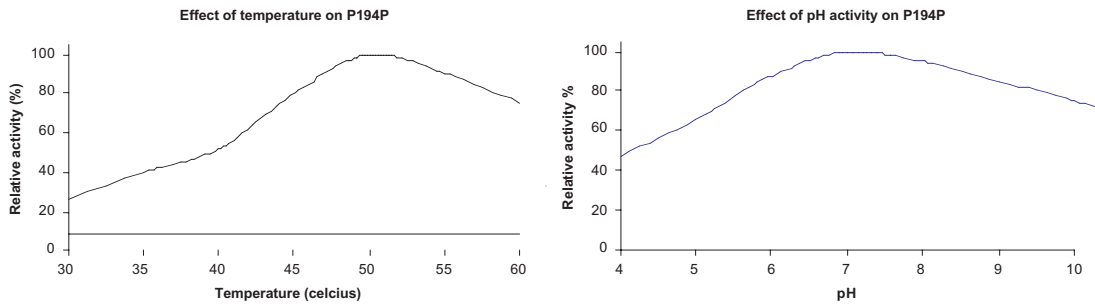


Figure 11: Graphs showing the effect of temperature and pH on P184P

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.



Figure 12: A pasteuriser used for liquid egg



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 solution egg 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 sensitise sporeformers 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.

PREVENT BROWNING BY REMOVING SUGAR WITH GLUCOSE OXIDASE 168L

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 an hot room, for an extended period of time. Biocatalysts' Glucose Oxidase (G168L) 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.

In desugarisation of egg ingredients, i.e. whole egg, egg yolks or egg white, Glucose Oxidase 168L is added at a dose of 100 - 150 ml per tonne of liquid egg product. Generally this process can be carried out at low temperatures e.g. 7-13°C, to prevent possible heat damage or microbial growth. The mixture is incubated for at least two hours or until tests show the desired reduction in glucose has been achieved. The egg can then be pasteurised normally. Because the glucose oxidase has eliminated the glucose, there will be much less Maillard reaction products formed during pasteurisation resulting in a brighter looking product with no browning.

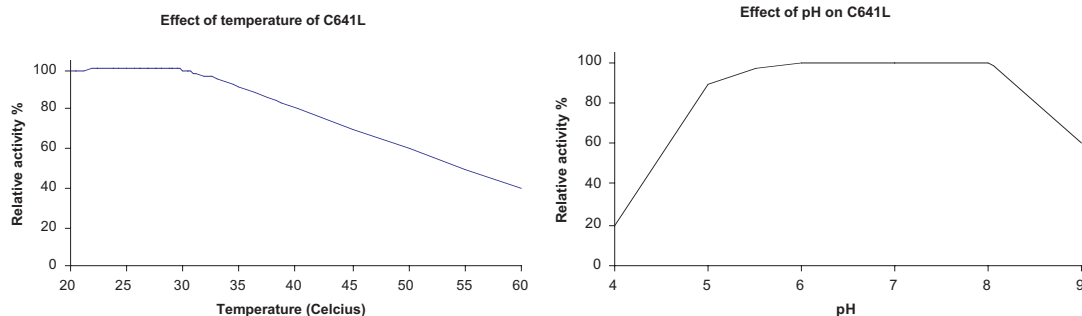


Figure 13: Graphs showing the effect of temperature and pH on C641L



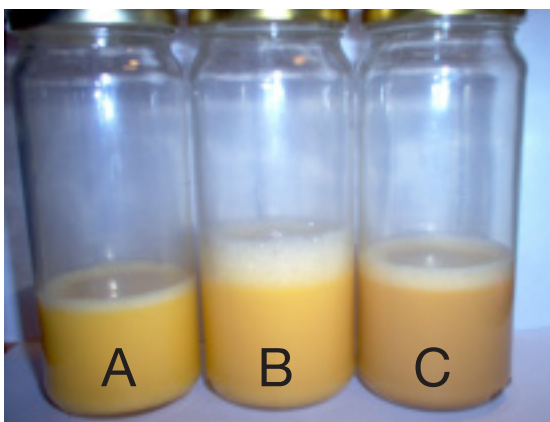


Figure 14:
 A) Egg prior to pasteurisation
 B) Glucose Oxidase treated egg after pasteurisation
 C) Untreated egg after pasteurisation

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 trials. 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 the Biocatalyst's product range for use as food processing aids and ingredients. Biocatalysts produce 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 trialing.

ACKNOWLEDGEMENTS

We would like to thank Sanovo Engineering for provision of the diagram of egg processing and photographs of equipment used in this technical bulletin.

COMMON PROBLEMS ENCOUNTERED IN EGG PROCESSING	
Problem	Enzyme Solution
Egg white goes brown during pasteurisation and drying	Desugar with Glucose Oxidase 168L to prevent this
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 641L
Insufficient emulsion of egg yolk	Addition of Lipomod™ 699L will increase emulsification of egg yolk

