KLM3' – Modification of Egg Yolk

ABSTRACT

Egg yolk contains 31% lipid, 30% of which is lecithin. Enzymatic conversion of lecithin to lyso-lecithin improves the emulsification properties of the egg yolk. When making sauces, dressing or mayonnaise, these improved emulsification properties are important in preventing separation of the product upon pasteurisation. Opposed to applying conventional phospholipases for modification of egg yolk KLM3', a glycerophospholipid cholesterol acyltransferase should give a lower level of free fatty acid due to the formation of cholesterol ester and hence a mayonnaise less prone to oxidation.

An in-house ^{31}P-NMR method was set up to monitor the lecithin, lyso-lecithin level in enzymatically modified egg yolk. Egg yolk was modified with KLM3' at the following parameters: pH (5.0 -10.0), enzyme dosage (0.5-5.0 LATU/g egg yolk), incubation temperatures (50-60°C), and incubation times (15-360 min). Kinetics were followed by analysis of amount of free fatty acid and cholesterol as well as by ^{31}P-NMR analysis of the lecithin and lyso-lecithin level. The lyso-phosphatidylcholine (LPC) activity of KLM3' caused accumulated LPC to be degraded resulting in a low phosphatidylcholine (PC) conversion degree and contemporary elevated free fatty acid (FFA) levels.

Pilot scale mayonnaises made with KLM3'modified egg yolk were on level with pilot scale mayonnaises made with egg yolk modified with phospholipases, Lysomax PLA2 (Genencor) or Lipomod 699L (Biocatalyst) and with mayonnaise made with HSEYP (Heat stable egg yolk powder, Sanovo) with respect to viscosity, particle size distribution and heat stability despite the lower PC conversion ratio for KLM3' (50% versus 75-95 %). Opposed to expected, mayonnaises made with KLM3' modified egg yolk did not show improved oxidation stability compared with mayonnaises made with Lipomod 699L or Lysomax modified egg yolk or with HSEYP, due to the elevated FFA level in KLM3' modified egg yolk caused by the LPC activity.

The trials made with pilot scale mayonnaises showed that the egg content can be reduced by 50% (from 5% to 2.5%) when using enzyme modified egg yolk instead of unmodified egg yolk, irrespective of the enzyme used for the modification.

ABRIVIATIONS

FFA: Free fatty Acid
GPC: Glycerophospholipd
HSEYP: Heat Stable Egg Yolk Powder
LPC: Lyso-phosphatidylcholine
LPE: Lyso-phosphatidylethanolamine
PC: Phosphatidylcholine
PE: Phosphatidylethanolamine
TPP: Triphenylphosphate

INTRODUCTION

Egg yolk is well known for use in the food industry due to its emulsification properties. Approximately 31% of egg yolk is lipid and 31% of the lipid is lecithin, see Figure 1. Lecithin contributes to egg yolk's emulsification properties. In many foods including mayonnaise, sauces, dressings, and cakes, the emulsification properties of egg yolk are exploited. For some food applications, however, the emulsification properties of egg yolk are not sufficient to obtain a homogenous product without separation. In mayonnaise for instance, pasteurisation of the product at high temperatures causes the product to separate. Changing the lecithin:lyso-lecithin ratio will improve the emulsifying properties of egg yolk because lyso-phospholipid is a better oil in water emulsifier than lecithin. Improved emulsifying properties of egg yolks are of importance when making a.o. heat stable mayonnaise

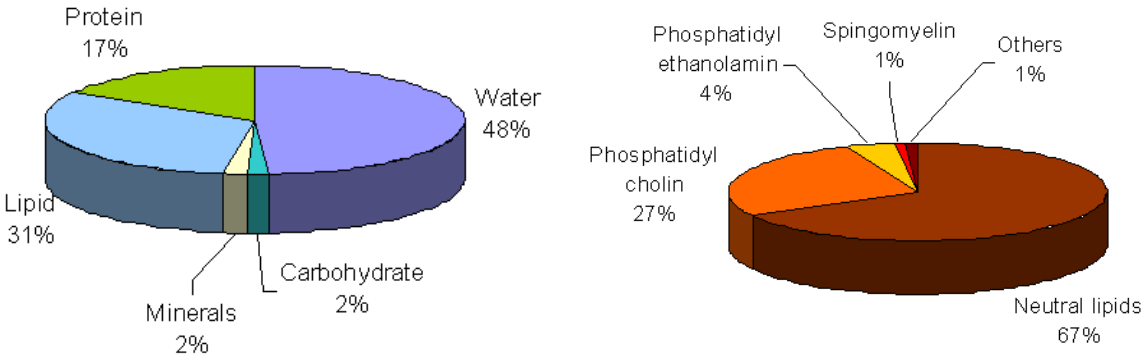

Figure 1: Egg yolk composition, and lipid distribution (www.aeg.org)

The acyltransferase KLM3' catalyses the conversion of the phospholipid and cholesterol to lyso-phospholipid and cholesterolester in egg yolk, see Figure 2.

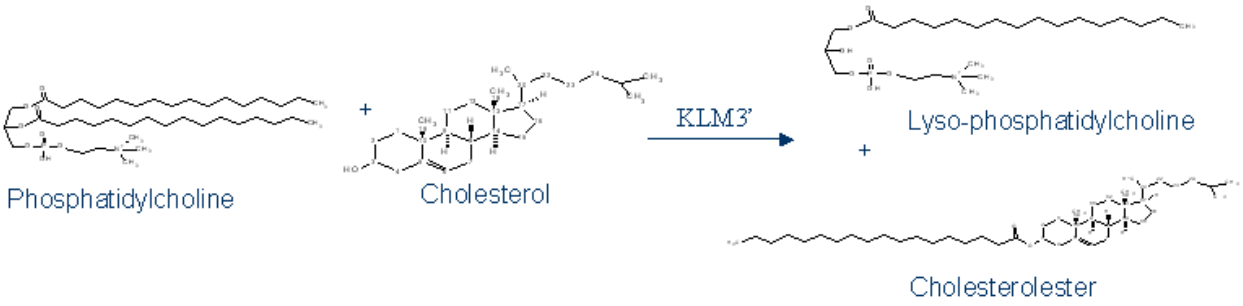

Figure 2: The acyltransferase KLM3' catalyses the conversion of phosphatidylcholine and cholesterol to lyso-phospahatidylcholine and cholesterolester, respectively.

The aim of this study was to examine the conversion of phospholipid in egg yolk based on a kinetic study with KLM3' in egg yolk. Effects of incubation time, temperature, and pH as well as the effect of KLM3' enzyme dosage were investigated. Furthermore, pilot scale mayonnaise production with KLM3' modified egg yolk was performed.

The end product was evaluated with respect to the following characteristics: viscosity, particle size distribution, heat stability, and oxidation stability. References used were Lysomax PLA2, Genencor and Lipomod 699L, Biocatalyst treated egg yolk as well as Heat Stable Egg Yolk Powder (HSEYP), Sanovo A/S, and untreated egg yolk.

MATERIALS AND METHODS

Enzyme:
K460: KLM3' acyltransferase from *Aeromonas salmonicida* expressed in *Bacillus licheniformis* from fermentation DW3206-49, formulated on 35% glycerol and 0.5% potassium sorbate at 1505 LATU/ml. Diluted to 150 LATU/ml in demineralised water before use.
#3395, FoodPro LysoMax PLA2, Genencor, lot 102-06067-001
#3332, Lipomod 699L, Biocatalyst, lot 10510125

Enzymatic treatment, kinetic study:

pH: Enzymatc treatment with a dosage of 2.0 LATU/g yolk at 50°C for 120 minutes was carried out at the following pH values: 5.0, 5.5, 6.0, 7.0, 8.0, 9.0, and 10.0. Egg yolk has a natural pH value of 6.0. Initial pH adjustment of pasteurised egg yolk with 8% NaCl from Sanovo A/S, Denmark, to the values listed above was made with citric acid or NaOH. 15 g of the pH adjusted egg yolk was transferred to a Wheaton tube and placed in a heating block controlled to 50°C. At time t=0, KLM3' enzyme stock solution was added to the egg yolk according to Table 1 to obtain an enzyme dosage of 2.0 LATU/g yolk and a control was included as well. At time t=120 minutes, 1.0 g samples were taken from the egg yolk/enzyme solutions. The enzymatic reaction in the samples was stopped by adding 7.5 ml organic solvent ($CHCl_3$:MeOH (2:1 (v/v)).

Temperature: Enzymatic treatment was carried out at the three following temperatures: 50°C, 55°C, and 60°C, each with the four following dosages: 1.5 LATU/g yolk, 2.0 LATU/g yolk, 3.0 LATU/g yolk, and 5.0 LATU/g yolk. In addition, at 55°C the three following dosages were tested: 0.5 LATU/g yolk, 1.0 LATU/g yolk, and 2.5 LATU/g yolk. At 60°C the dosage of 2.5 LATU/g yolk was also tested.

15 g pasteurised egg yolk with 8% NaCl from Sanovo A/S, Denmark, was transferred to a Wheaton tube and placed in a heating block controlled to the appropriate temperature. At time t=0, KLM3' enzyme stock solution was added to the egg yolk according to Table 1 to obtain the different enzyme dosages and a control was included as well. At time t=15 minutes, 30 minutes, 60 minutes, 120 minutes, 180 minutes, 240 minutes, and 360 minutes, 1.0 g samples were taken from the egg yolk/enzyme solutions. The enzymatic reaction in the samples was stopped by adding 7.5 ml organic solvent ($CHCl_3$:MeOH, (2:1 (v/v))).

Table 1: Amount of KLM3' enzyme stock solution (150 LATU/g yolk) added to egg yolk to obtain different enzyme dosages and a control was included as well. Demineralised water was added to 0.5 ml to eliminate any difference in volume upon adding different volumes of enzyme stock solution.

Sample	Amount egg yolk	Enzyme activity	Volume enzyme stock solution added	Volume dem. H_2O added
Control	15.0 g	0 LATU	0 ml	0.5 ml
0.5 LATU/g yolk	15.0 g	7.5 LATU	0.05 ml	0.45 ml
1.0 LATU/g yolk	15.0 g	15.0 LATU	0.10 ml	0.40 ml
1.5 LATU/g yolk	15.0 g	22.5 LATU	0.15 ml	0.35 ml
2.0 LATU/g yolk	15.0 g	30.0 LATU	0.20 ml	0.30 ml
2.5 LATU/g yolk	15.0 g	37.5 LATU	0.25 ml	0.25 ml
3.0 LATU/g yolk	15.0 g	45.0 LATU	0.30 ml	0.20 ml
5.0 LATU/g yolk	15.0 g	75.0 LATU	0.50 ml	0 ml

Lipid extraction:
Addition of 7.5 ml organic solvent ($CHCl_3$:MeOH, (2:1 (v/v))) to the sample did not only stop the enzyme reaction, but also extracted the lipids. Furthermore, 0.2 ml demineralised H_2O was added to the sample before it was dispersed using a Whirley mixer for 1 minute. The sample was then centrifuged for 10 minutes at 110 x g. Approximately 3 ml of the organic phase was transferred to a different tube and this extracted lipid was used for various analyses.

Free fatty acid analysis:
100 µl of the extracted lipid solution was evaporated under nitrogen at 50°C. 1.0 ml demineralised H_2O was added and the lipid was dispersed using a Whirley mixer. The amount of free fatty acid was determined on Konelab Autoanalyser (Thermo, Finland) using the NEFA C kit (WAKO GmbH, Germany). Assay was run at 37°C. 75 µl solution A and 15 µl redispersed extracted lipids were incubated for 10 minutes. 150 µl solution B was added and incubated for 10 minutes. The absorbance at 520 nm was measured. The amount of free fatty acid was determined, using the read absorbance and a standard curve based on oleic acid (0.05 mM to 1.0 mM).

Cholesterol analysis:
100 µl of the extracted lipid solution was evaporated under nitrogen at 50°C. 1.0 ml EtOH was added and the cholesterol was dispersed using a Whirley mixer. Analysis was carried out using a Konelab Autoanalyzer (Thermo, Finland) and a coupled enzymatic assay applying cholesterol oxidase and ABTS and peroxidase. The assay was run at 37°C. 100 µl substrate (1.25 U/ml cholesterol oxidase, 0.04% ABTS, 0.05% Peroxidase in 100 mM Tris/HCL, pH 6.6, 0.45% Triton) was incubated for ten minutes before 5µl redispersed extracted lipid solution was added. After five minutes incubation, OD 405 nm was read. The amount of cholesterol was determined using the read absorbance and a standard curve based on cholesterol (0.05 mg/ml to 0.40 mg/ml).

^{31}P NMR analysis:
NMR-SAMPLE PREPARATION:
Preparation of CsEDTA-solution: 0.2 M EDTA in MilliporeQ-water was titrated with 0.2M CsOH x1H_2O to a pH=10.5.
IS-solution: 250mg Triphenylphosphate (TPP) in 5 ml CDCl3/MeOH (2:1 (v/v))
2.0 ml of the extracted lipid solution was evaporated and re-dissolved in 2.0 ml $CDCl_3$/MeOH (2:1 (v/v)), washed with 2.0 ml CsEDTA(aq) and adjusted to pH=10.5. After centrifugation (4500rpm/5mins), 800 µl of the lower phase was placed in a 5 mm NMR-tube and 5mm NMR-tube and 25µl of the IS-solution (1.25mg TPP) was added.

NMR-CONDITIONS:
Spectra were recorded using a Varian Mercury 200vx (200MHz – ^{1}H resonance frequency) NMR-instrument at 81MHz (^{31}P resonance frequency) equipped with a liquid BB-probe. Parameters used: full proton decoupling, spectral width=2500Hz, temperature=28°C, pw=9.8µsec/52dB, d1= 10sec., number of scans =256.

Chemical shifts (PC at δ=-0.87ppm, 2-LPC at δ=-0.42ppm, 1-LPC at δ=-0.54ppm, PE at δ=-0.10ppm and LPE at δ=0.24ppm) were relative to an external standard (H_3PO_4 at δ=0.0ppm and checked using an internal standard TPP at δ=-17.84ppm).

Data were processed and integrated in Varian VNMR vers.1.6a (lb=1.0) and MestRe-C vers.4.9.9.6.

Enzymatic treatment, yolk for mayonnaise:

For the production of mayonnaise, pasteurized egg yolk with 8% NaCl from Sanovo A/S, Denmark, was enzymatically treated according to Table 2. Enzymatic treatment was carried out with slow agitation in either a heating block or a water bath at appropriate temperatures. To simulate pasteurization after treatment, the egg yolks were heated to 68-72°C with a holding time of 3 minutes applying a water bath at 85°C. Samples were taken and treated as under Lipid extraction.

Table 2: Enzymatic treatment of egg yolk from Sanovo A/S using KLM3', Lysomax PLA2 and Lipomod 699L, respectively. The KLM3' solution used had an activity of 150 LATU/ml, the Lysomax PLA2 had an activity of 473 U/ml, and the Lipomod 699L had an activity of 2000 U/ml.

Sample	**KLM3'-K460 Dosage/g yolk**	**Lysomax PLA2 Dosage/g yolk**	**Lipomod 699L Dosage/g yolk**	**Temperature (°C)**	**Time (min)**
2344-89-Control-60#	-	-	-	60	240
2344-89-K460#	2.0 LATU	-	-	60	240
2344-89-Lysomax#	-	5 U		45	180
2344-89-Lipomod#	-	-	15 U	45	180
2344-93-K460-60*	1.5 LATU	-	-	55	60
2344-93-K460-240*	1.5 LATU	-	-	55	240
2344-93-Lipomod*	-	-	15 U	45	180
2344-93-Control-45*	-	-	-	45	180
2344-94-K460#	1.5 LATU	-	-	50	20
2344-94-Lipomod#	-	-	15 U	45	180

#Enzymatic treatment in heating block.
*Enzymatic treatment in water bath.

Production of mayonnaise:

Mayonnaise with varying contents of egg yolk was produced using a FrymaKoruma mixer (Disho A15) (Table 3). Water, salt, sugar and potassium sorbate were mixed. For mayonnaises made with stabilizer, Grinsted FF 5105 was mixed 2:1 with oil before addition to the water phase. When all ingredients were dissolved, the egg yolk was added. Under vacuum, the oil was then emulsified into the water phase (stirring speed 3500 rph). Finally, vinegar and mustard were added.

Table 3: Ingredients used to produce mayonnaise with stabiliser.

Ingredient	**Mayonnaise 5% yolk**	**Mayonnaise 3% yolk**	**Mayonnaise 2.5% yolk**	**Mayonnaise 1.5 % yolk**	**Mayonnaise 2+1 % yolk***
Water	9.5 %	11.5 %	12 %	13.0 %	34.5 %
Oil	80.0 %	80.0 %	80.0 %	80.0 %	50.0 %
Modified yolk	5.0 %	3.0 %	2.5 %	1.5 %	2.0 %
Raw yolk	-	-	-	-	1.0 %
Salt	0.7 %	0.7 %	0.7 %	0.7 %	1.0 %
Sugar	1.0 %	1.0 %	1.0 %	1.0 %	3.0 %
Potassium Sorbate	0.1 %	0.1 %	0.1 %	0.1 %	0.1 %
Grindsted FF 5105	0.2 %	0.2 %	0.2 %	0.2 %	1.7 %
Vinegar 10%	3.00 %	3.00 %	3.00 %	3.00 %	4.00 %
Mustard	0.50 %	0.50 %	0.50 %	0.50 %	1.50 %
Sum	**100 %**	**100 %**	**100 %**	**100 %**	**100 %**

***2% modified egg yolk + 1% raw egg yolk used to give a total of 3.0% yolk**

Table 4: Ingredients used to produce mayonnaise without stabiliser.

Ingredient	**Mayonnaise 3% yolk**	**Mayonnaise 1.5 % yolk**
Water	11.7 %	13.2 %
Oil	80.0 %	80.0 %
Modified yolk	3.0 %	1.5 %
Raw yolk	-	-
Salt	0.7 %	0.7 %
Sugar	1.0 %	1.0 %
Potassium Sorbate	0.1 %	0.1 %
Grindsted FF 5105	-	-
Vinegar 10%	3.00 %	3.00 %
Mustard	0.50 %	0.50 %
Sum	**100 %**	**100 %**

Egg yolks modified as described under Enzymatic treatment, yolk for mayonnaise, were used to produce the mayonnaises listed in Table 5.

Table 5: Pilot scale mayonnaise produced with enzymatically modified egg yolk listed in Table 2. Red box outline indicates mayonnaise made without stabilizer.

Egg yolk used	Mayo 5% yolk	Mayo 3% yolk	Mayo 2.5% yolk	Mayo 2.0% yolk	Mayo 1.5 % yolk	Mayo 2+1 % yolk
2344-89-Control-60	FM06-31-1		FM06-31-2			
2344-89-K460	FM06-31-3		FM06-31-4			
2344-89-Lysomax	FM06-31-5		FM06-31-6			
2344-89-Lipomod	FM06-31-7		FM06-31-8			
2344-93-K460-60		FM06-31-13			FM06-31-14	
2344-93-K460-240		FM06-31-15			FM06-31-16	
2344-93-Lipomod		FM06-31-17			FM06-31-18	
2344-93-Control-45		FM06-31-19			FM06-31-20	
2344-94-K460	FM06-31-42	FM06-31-41			FM06-31-40	
2344-94-Lipomod		FM06-31-44			FM06-31-43	FM06-31-45
HSEYP, Sanovo	FM06-31-9	FM06-31-47	FM06-31-10		FM06-31-46	
		FM06-31-21/31	FM06-31-33	FM06-31-32	FM06-31-22/34	
Raw yolk, Sanovo						FM06-31-45

Shake test

An accelerated stability study was performed as a shake test. Mayonnaise was shaken at 300 rpm at room temperature for 72 hours.

Heat stability

To evaluate the heat stability of the mayonnaise, it was placed in a 90°C water bath for an hour. Afterwards, the degree of oiling-out was judged visually.

Viscosity measurement

Viscosity of the mayonnaise was measured on a Brookfield, DV-II, with a Helipat spindle type T-D at 2 rpm. See procedure A100.

Oxidation stability

The mayonnaise is stressed oxidatively by heating. The breakdown of the product will induce oxygen consumption, which is registered as change in pressure by a pressure transducer. The induction period (hour), which is a measure of the oxidation stability of the product, is the time that elapses before a change in pressure is observed.

Using a ML OXIPRES (MicroLab, Århus, Denmark), a 5.2 bar oxygen pressure was applied to 20 g mayonnaise. Analysis temperature was 100°C. Induction period was read as the time that elapsed from start to the intercept between the two tangents.

Particle size determination in mayonnaise

Particle size distribution can be determined by exploiting that laser light is spread in different angels depending on the size of the particle. Small particles spread laser light in large angels, whereas large particles spread lights in small angels. The particle size distribution is based on volume, but results are expressed in particle diameters. 2.0 g mayonnaise sample was dissolved in 10 ml demineralised water. The particle size distribution was then measured on a Malvern Mastersizer S.

RESULTS AND DISCUSSION

The temperatures and pH were chosen based on the temperature and pH optima and stability anticipated. KLM3' showed optimum activity at 50-65°C and pH 7.0-10.0. Optimum stability of KLM3' was observed at temperatures of 50°C or below and at pH between 6.0 and 10.0. The enzyme dosages chosen were based on initial experiments. In Table 6, the seven pH values, the three temperatures, and the seven enzyme dosages included in the kinetic study are listed.

Table 6: Parameters evaluated in the kinetic study with KLM3' in egg yolk.

Parameter	Values
pH	5.0, 5.5, 6.0, 7.0, 8.0, 9.0 and 10.0
Temperature	50, 55 and 60°C
Enzyme dosage	0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 5.0 LATU/g yolk
Incubation time	0, 15, 30, 60, 120, 180, 240 and 360 minutes

^{31}P NMR was used to follow the change in phospholipid distribution in egg yolk as enzymatic treatment progressed over time. The molar percentage of each type of phospholipid present (Phosphatidylcholine (PC), phosphatidylethanolamine (PE), 1-Lyso-phosphatidylcholine (1-LPC), 2-Lyso-phosphatidylcholine (2-LPC), and Lyso-phosphatidylethanolamine (LPE)), as well as the internal standard Triphenylphosphate (TPP) was calculated based on integration of raw data, see Figure 3, Figure 4, Figure 5 and Table 7. Both a full NMR spectrum and a zoomed spectrum are needed to integrate the amount of TPP and the small peak of 1-LPC. The correction of the TPP integral ($TPP^* = TPP_{raw}/PC_{raw} * PC$) is performed to match PC in the zoomed spectrum.

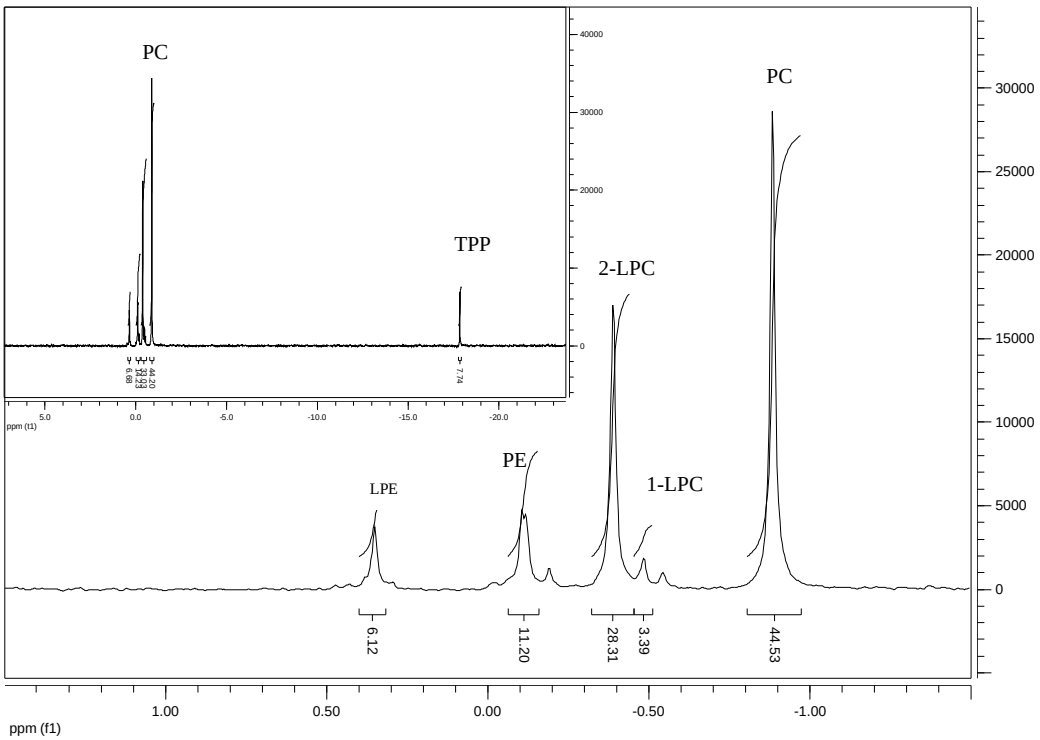

Figure 3: ^{31}P NMR spectrum of sample H2771-9 – LBJ#2344-85-4 30. The zoomed spectrum [-1.5 – 1.5ppm] shows the chemical shifts of the phospholipids as well as the integrals. The inserted spectrum is the full spectrum [-23.7 – 7.2ppm] with the internal standard TPP* at δ= -17.84ppm (corrected integral value 7.74).Nomenclature: 2-LPC: 1-acyl-sn-glycero-3-phosphorylcholine.

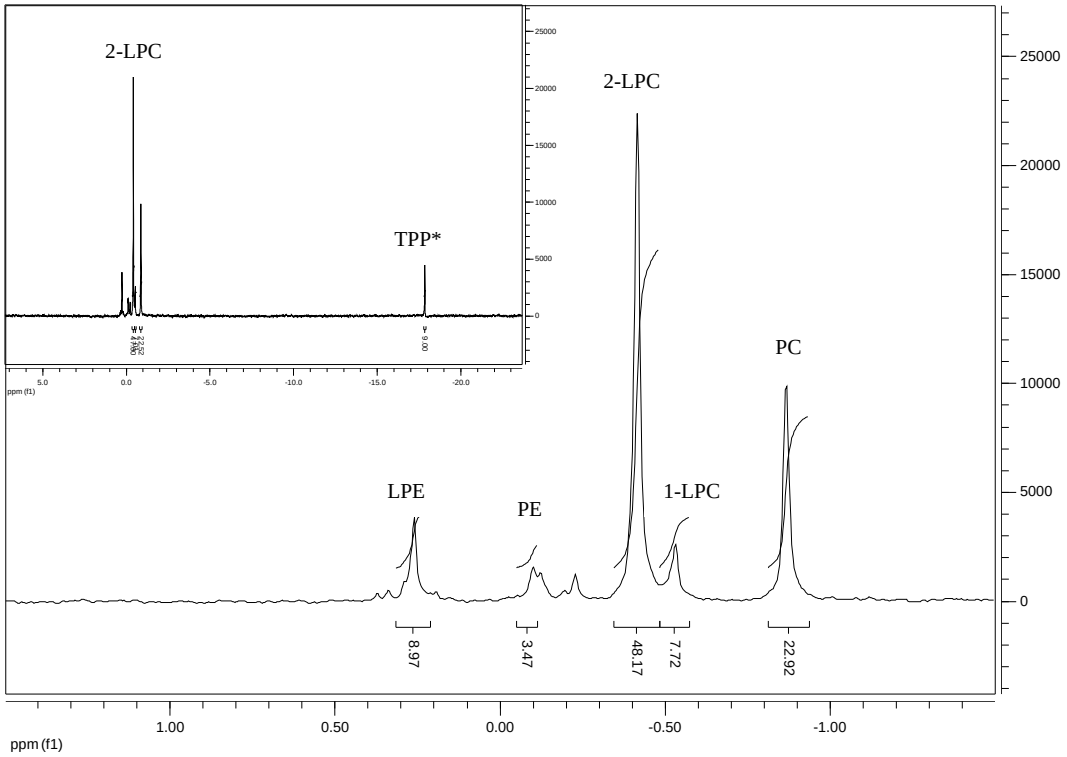

Figure 4: ^{31}P NMR spectrum of sample H2771-11 – LBJ#2344-85-4 120. The zoomed spectrum [-1.5 – 1.5ppm] shows the chemical shifts of the phospholipids as well as the integrals. The inserted spectrum is the full spectrum [-23.7 – 7.2ppm] with the internal standard TPP at δ= -17.84ppm (corr. integral value 9.00).

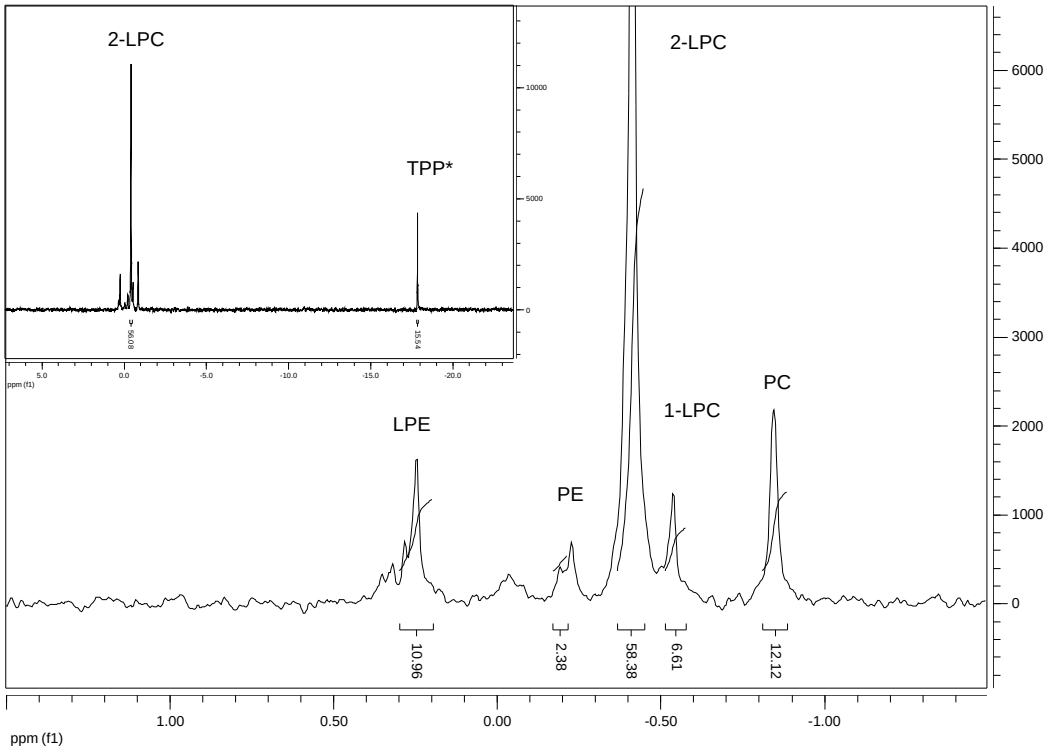

Figure 5: ^{31}P NMR spectrum of sample H2771-14 – LBJ#2344-85-4 360. The zoomed spectrum [-1.5 – 1.5ppm] shows the chemical shifts of the phospholipids as well as the integrals. The inserted spectrum is the full spectrum [-23.7 – 7.2ppm] with the internal standard TPP at δ= -17.84ppm (corr. integral value 15.54).

Table 7: Percentage molar distribution of phospholipid from enzymatic treatment of egg yolk with KLM3' dosed at 2.0 LATU/-g yolk at 50°C. Amount internal standard TPP was 3.9 µmol.

Sample ID	Incubation time	g yolk	TPP*	PC	1-LPC	2-LPC	PE	LPE
2344-85-4-0	0 min	1.030	7.93	73.71	0.0	2.44	17.49	1.27
2344-85-4-30	30 min	1.067	7.74	44.53	3.39	28.31	11.20	6.12
2344-85-4-120	120 min	1.052	9.0	22.92	7.72	48.17	3.47	8.97
2344-85-4-360	360 min	0.996	15.54	12.12	6.61	58.38	2.38	10.96

*The value listed for TPP is the corrected integral value.

The increase in molar percentage of the internal standard TPP over time (from 7.93 to 15.54 for series 2344-85-4) indicated loss of phosphorous from the system. In the egg yolk system studied, loss of phosphor was most likely caused by the formation of glycerophospholipid (GPC). Besides being an acyltransferease, see Figure 2, KLM3' was demonstrated to also to have lyso-phospholipid activity thereby hydrolysing LPC to GPC, see Figure 6. Being water-soluble, GPC was not detectable in the organic phase analysed by ^{31}P NMR. Presenting conversion degree of phospholipid to lyso-phospholipd (Sum of percentage LPC+LPE at time t=x divided by the sum of percentage PC+PE at time t=0) based on relative molar distribution gave a wrong indication, since the conversion degree was seen to increase over time, see Figure 7. Because part of the lyso-phosholipid formed by the acyltranseferase reaction was degraded by the lyso-phospholipid reaction, the conversion degree based on absolute data (Sum of µmol LPC+LPE at time t=x

divided by sum of µmol PC+PE at time t=0) first increased and then decreased as incubation time elapsed, see Figure 7.

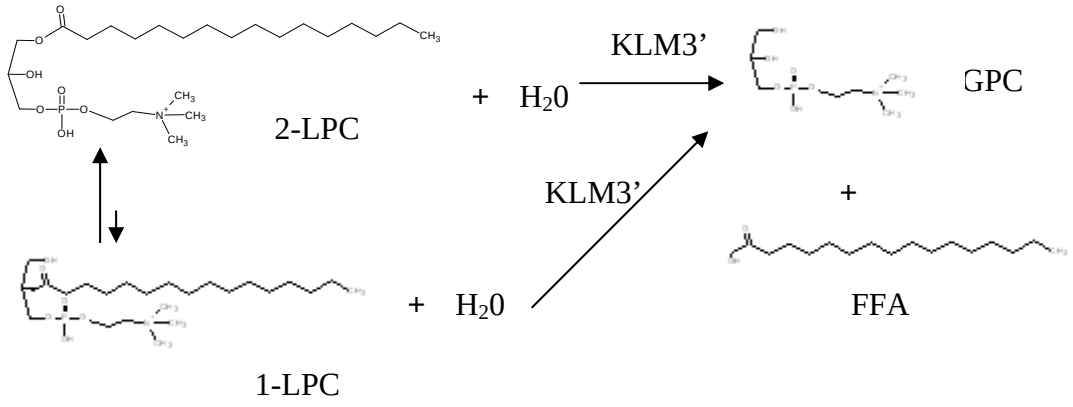

Figure 6: The acyltransferase KLM3' also has lyso-phospholipid activity thereby degrading LPC to GPC with contaminant formation of a free fatty acid.

Assuming the amount of internal standard (TPP) and the phospholipid that is removed during the CsEDTA wash was the same in all samples, the amount of µmol of each phospholipid type in the 800 µl $CDCl_3$/MeOH extract was calculated by relating the known amount of internal standard added to the molar percentage of the internal standard in a given sample, see Equation 1. No calculation back to the original sample was made due to unknown distribution between organic and water phases at numerous extractions during sample preparation. Assuming though that distribution between the two phases was the same in all samples, the amount µmol in 800 µl $CDCl_3$/MeOH extract was adjusted for the exact amount of sample withdrawn from the egg yolk, see Equation 1.

Results are expressed as percentage phospholipid conversion based on µmol 1-LPC+2-LPC+LPE at a given time relative to µmol PC+PE at time t=0 min present in 800µl $CDCl_3$/MeOH NMR sample adjusted for the exact amount sample withdrawn from the egg yolk, see Equation 2.

Equation 1: Calculation of µmol of a given component.

$$\mu mol\ X = \frac{\mu mol\ TPP \cdot}{TPP_{molar\ \%}} \cdot X_{molar\ \%}$$

Equation 2: Calculation of % phospholipid conversion based on absolute data. Percentage molar distribution data are taken from Table 7.

$$\%Phospholipid\ conversion = \frac{\mu mol\ TPP_{t=x} \cdot \frac{1-LPC_{molar\%\ at\ t=x} + 2-LPC_{molar\%\ at\ t=x} + LPE_{molar\%\ at\ t=x}}{TPP_{molar\%\ at\ t=x} \cdot g\ yolk_{t=x}}}{\mu mol\ TPP_{t=0} \cdot \frac{PC_{molar\%\ at\ t=0} + PE_{molar\%\ at\ t=0}}{TPP_{molar\%\ at\ t=0} \cdot g\ yolk_{t=0}}} \cdot 100\%$$

Internal standard TPP = 3.9 µmol

$$\%Phospholipid\ conversion\ 30\ \text{min} = \frac{3.9\ \mu mol \cdot \frac{3.39+28.31+6.12}{7.74 \cdot 1.067 g}}{3.9\ \mu mol \cdot \frac{73.41+17.49}{7.93 \cdot 1.030 g}} \cdot 100 = 41.1\%$$

$$\%Phospholipid\ conversion\ 120\ \text{min} = \frac{3.9\ \mu mol \cdot \frac{7.72+48.17+8.97}{9.00 \cdot 1.052 g}}{3.9\ \mu mol \cdot \frac{73.41+17.49}{7.93 \cdot 1.030 g}} \cdot 100 = 61.6\%$$

$$\%Phospholipid\ conversion\ 360\ \text{min} = \frac{3.9\ \mu mol \cdot \frac{6.61+58.38+10.96}{15.54 \cdot 0.996 g}}{3.9\ \mu mol \cdot \frac{73.41+17.49}{7.93 \cdot 1.030 g}} \cdot 100 = 44.0\%$$

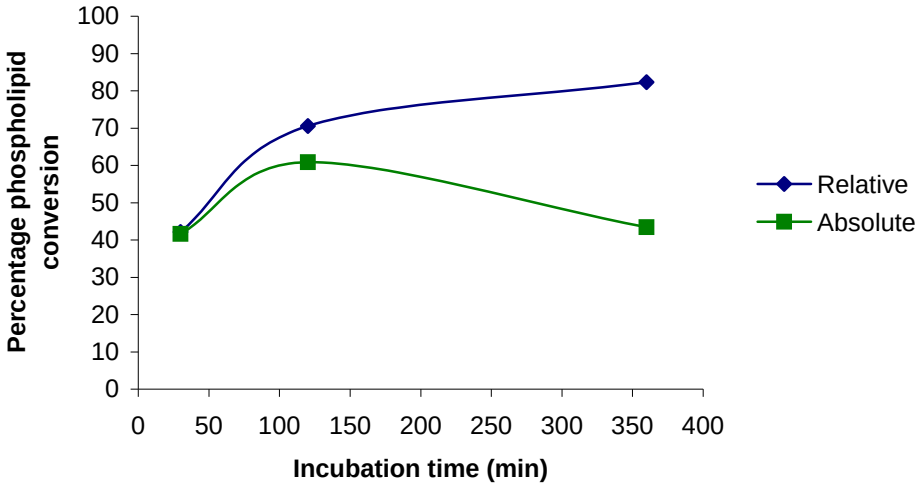

Figure 7: Percentage phospholipid conversion based on relative (percentage molar distribution) and absolute (µmol) data, respectively.

The first parameter evaluated was pH. Egg yolk has a natural pH of approximately 6.0 and the optimum pH for KLM3' was found to be between 7.0 and 10.0, peaking at pH 8.0. Therefore, an initial pH adjustment of the egg yolk to a more alkaline pH value could give a higher conversion of phospholipid to lysophospholipid applying the same enzyme dosage. Effect of pH on phospholipid conversion in egg yolk can be seen in Figure 8. The enzyme dosage used was 2.0 LATU/g yolk and incubation time was 2 hours at 50°C.

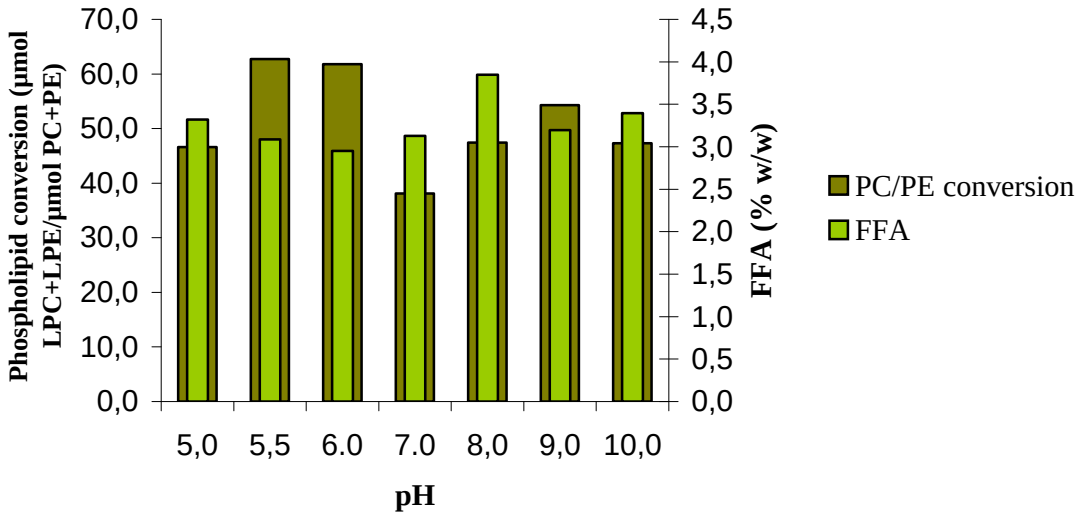

Figure 8: Effect of pH on phospholipid conversion in egg yolk with KLM3' dosed at 2.0 LATU/g yolk. Incubation temperature was 50°C and incubation time was 2 hours.

As seen in Figure 8, the optimum conversion of phospholipid is obtained at pH 6.0, the natural pH of egg yolk. At pH 6.0, the ratio between free fatty acid and the phospholipid conversion is the lowest. This is preferable, since free fatty acid contributes to the oxidation instability of the end product, mayonnaise. Based on these observations, natural egg pH, 6.0, was chosen as the pH value for the remaining part of the kinetic study. From an industrial point of view, running

the enzymatic treatment of egg yolk at its natural pH value is also preferable, as a pH adjustment would add another process step.

Next, the effect of temperature in combination with enzyme dosage as well as incubation time was evaluated. KLM3' has a temperature optimum of 65°C but as heat inactivation of the enzyme at temperatures of 60°C and above are pronounced and enzymatic treatments ran for up to six hours, enzymatic treatments were run at 50°C, 55°C and 60°C. The high temperature (65°C) at long holding times (six hours) could also very well affect the egg yolk.

For enzymatic treatments run at 50°C, the percentage phospholipid conversion as well as percentage FFA and cholesterol as a function of enzyme dosage are seen in Figure 9. Irrespective of the enzyme dosage used or the incubation time elapsed, the maximum percentage phospholipid conversion reached was approximately 60%. The percentage FFA increased steadily with increasing enzyme dosage and longer incubation time. Within the first 60 minutes incubation, 90% of the cholesterol was eliminated for all enzyme dosages. At incubation times longer than 60 minutes, no further change in cholesterol level was observed for any enzyme dosage. The 90% reduction in cholesterol level within the first 60 minutes of incubation showed the acyltransferase reaction of KLM3' to be fast and efficient as long as a certain level of cholesterol was still present.

For the lowest dosage, 1.5 LATU/g yolk tested at 50°C, a plateau close to 60% phospholipid conversion was observed at incubation times between 120 and 360 minutes. During the same period of time a steady increase in FFA was observed. This indicated a steady state at which the amount of LPC formed by hydrolysis of PC equalled the amount of LPC degraded by the lyso-phospholipid reaction. At the two highest enzyme dosages, 3.0 and 5.0 LATU/g yolk tested at 50°C, a significant decrease in percentage phospholipid conversion was observed at long incubation times. At these dosages and times, the lyso-phospholipid reaction exceeded the phospholipid hydrolysis reaction leading to a reduction in the amount of LPC present.

In Figure 10, the percentage phospholipid conversion as well as percentage FFA and cholesterol as a function of enzyme dosage for enzymatic treatments run at 55°C can be seen. As was the case for enzymatic treatments run at 50°C, the maximum percentage conversion of phospholipid obtained at 55°C was 60% regardless of the enzyme dosage used or the incubation time elapsed. Percentage FFA increased with increasing enzyme dosage and longer incubation times. At 55°C, 90% of the cholesterol was eliminated within the first 30 minutes of incubation as opposed to 60 minutes at 50°C; hence the acyltransferase reaction of KLM3' is even faster at 55°C than at 50°C.

For enzymatic treatments run at 55°C, the three lowest dosages tested, 0.5, 1.0, and 1.5 LATU/g yolk, a plateau close to 60% phospholipid conversion was observed for incubation times between 60 and 360 minutes. During the same time interval, a steady increase in FFA was observed. In Figure 12, the amount of µmol PC and LPC is seen for the enzymatic treatment with KLM3' dosed at 0.5 LATU/g yolk. PC was steadily degraded for the first 240 minutes incubation time, whereas the accumulation of LPC ceased after 60 minutes incubation time, showing that the LPC formed by hydrolysis of PC equalled the amount of LPC degraded by the lyso-phospholipid reaction for incubation times between 60 and 240 minutes. During this period of time no change in the total amount of LPC was observed, but as the total amount of PC decreased with time, so did the total emulsification capacity of the egg yolk, because PC as opposed to GPC is an emulsifier.

At the higher dosages tested at 55°C, reduction in phospholipid conversion was observed at prolonged incubation times, indicating the lyso-phospholipid reaction to exceed the phospholipid reaction. This reduction was not as pronounced at 55°C as at 50°C, presumably because the enzyme was partly inactivated at the higher temperature at long holding times.

In Figure 11, the percentage phospholipid conversion as well as percentage FFA and cholesterol as a function of enzyme dosage for enzymatic treatments run at 60°C is seen. As for enzymatic treatments run at 50°C and 55°C, the maximum percentage conversion of phospholipid obtained at 60°C was 60% irrespective of the enzyme dosage used or the incubation time elapsed. For all dosages tested at 60°C, the phospholipid conversion peaked within the first 60 minutes of incubation time. At incubation times longer than 120 minutes, no further change was observed in percentage phospholipid conversion within each dosage tested. Percentage FFA increased for the first 120 minutes incubation time and then ceased. This combined with the plateau of percentage phospholipid conversion for each dosage at incubation times above 120 minutes indicated pronounced heat inactivation of the enzyme. At 60°C, 90% of the cholesterol was eliminated within the first 30 minutes of incubation as was the case for enzymatic treatments at 55°C.

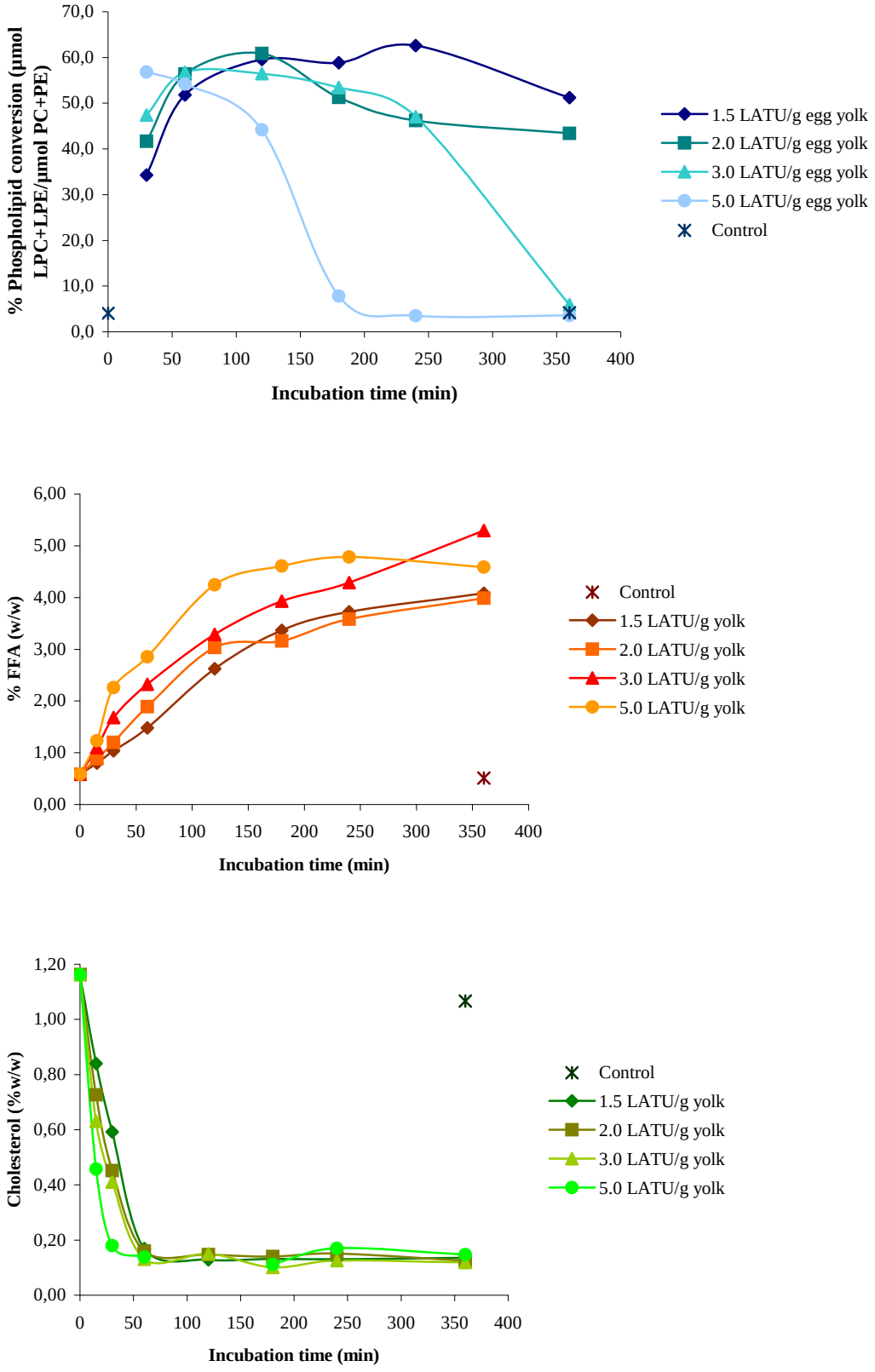

Figure 9: Enzymatic modification of egg yolk with KLM3' acyltransferase at 50°C and natural egg pH. Phospholipid conversion is expressed at µmol LPC+LPE at a given time relative to µmol PC+PE at time t=0 present in 800 µl CDCl3/MeOH NMR sample. Free fatty acid and cholesterol are expressed as percentage of egg yolk.

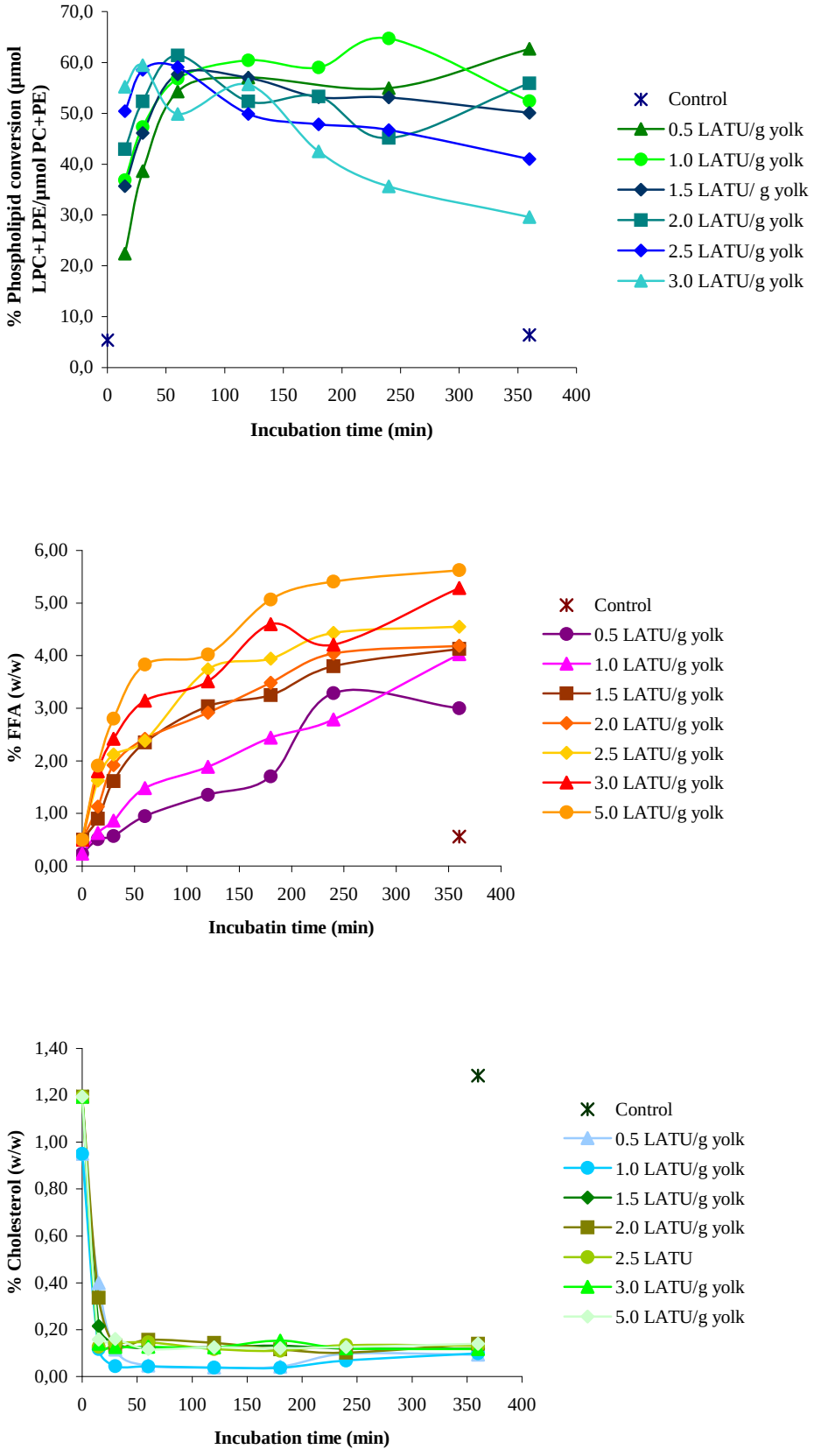

Figure 10: Enzymatic modification of egg yolk with KLM3' acyltransferase at 55°C and natural egg pH. Phospholipid conversion is expressed at µmol LPC+LPE at a given time relative to µmol PC+PE at time t=0 present in 800 µl CDCl3/MeOH NMR sample. Free fatty acid and cholesterol are expressed as percentage of egg yolk.

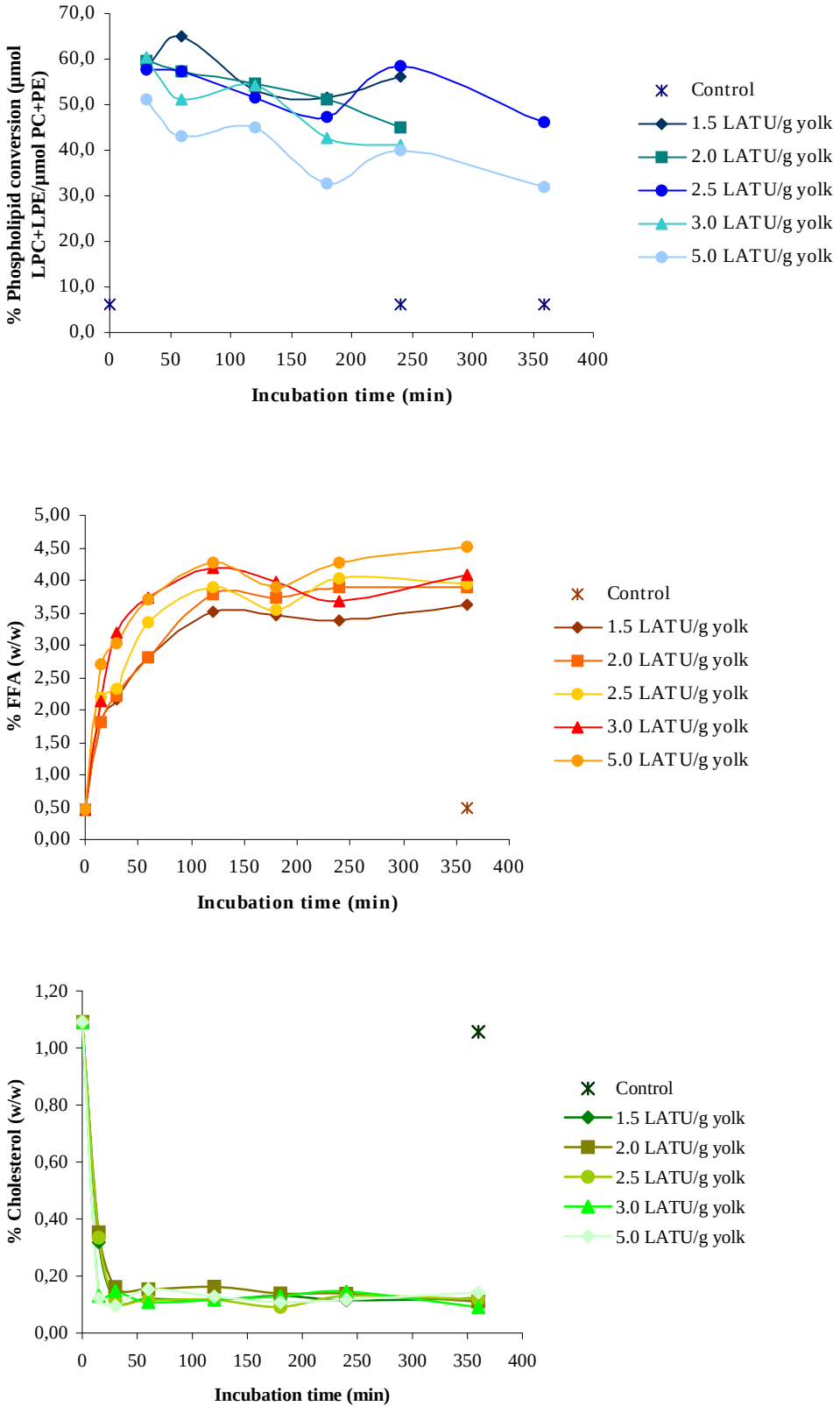

Figure 11: Enzymatic modification of egg yolk with KLM3' acyltransferase at 60°C and natural egg pH. Phospholipid conversion is expressed at µmol LPC+LPE at a given time relative to µmol PC+PE at time t=0 present in 800 µl CDCl3/MeOH NMR sample. Free fatty acid and cholesterol are expressed as percentage of egg yolk.

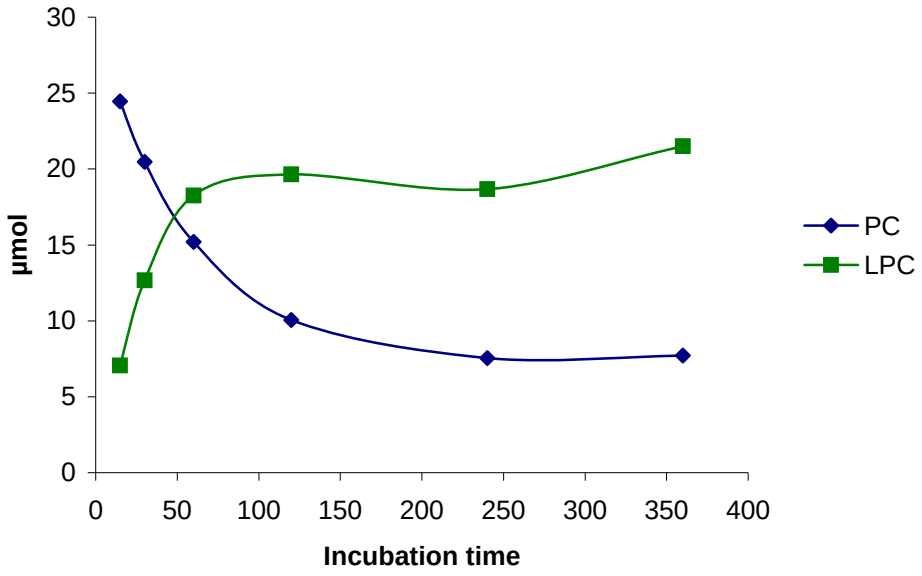

Figure 12: µmol PC and LPC as a function of incubation time for enzymatic treatment at 55°C with KLM3' dosed at 0.5 LATU/g yolk. PC was degraded for incubation times until 240 minutes, whereas the accumulation of LPC ceased after 60 minutes incubation time, showing the lyso-phopholipid reaction to be equal to the phospholipid reaction at incubation times between 60 and 240 minutes.

Enzymatic conversion of lecithin to lyso-lecithin improves the emulsification properties of egg yolk. These improved emulsification properties of egg yolk are of importance when making a.o. mayonnaise by preventing separation of the product upon pasteurisation. To evaluate the effect of enzymatic modification of egg yolk, pilot scale mayonnaises were made. Four sets of pilot scale mayonnaises were made using egg yolks treated with varying enzymes, dosages, and incubation times, and temperatures, see Table 2 and Table 5. Full fat mayonnaise made with unmodified egg yolk has an egg yolk content of 5.0%. Within each of the four sets of mayonnaises, 2% of egg yolk was tested to evaluate to which level the egg content in mayonnaise can be reduced by using enzymatically modified egg yolk. By measuring viscosity and particle size distribution of the pilot scale mayonnaises, the emulsification property of the enzyme treated egg yolk used was monitored, see Modification of egg yolk with KLM3'

. Particle size distribution data was also used for evaluation of the heat stability of the mayonnaises as well as visual judgement of oiling- out upon heat treatment. As opposed to applying conventional phospholipases for modification of egg yolk, KLM3', will give a lower level of free fatty acid due to the formation of cholesterol ester. Therefore the correlation between amount FFA present in enzyme treated egg yolk and oxidation stability of the pilot scale made mayonnaises was also evaluated, see Modification of egg yolk with KLM3'

.

Mayonnaise ID	Enzyme used	% egg yolk	% PC conversion	% FFA	Particle size (% particles with diameter below 10 µm)			Viscosity (cp)	Oxidat stabil (h)
					No treatment	Shaken	Heated		
FM06-31-1	None	5.0	5	0.4	81.4	68.3	nd	369000	10.3
FM06-31-2	None	2.5	5	0.4	51.6	53.8	nd	225000	11.9
FM06-31-3	KLM3'	5.0	54	4.1	87.8	73.6	nd	-	7.0
FM06-31-4	KLM3'	2.5	54	4.1	58.6	43.6	nd	788000	8.4
FM06-31-5	Lysomax PLA2	5.0	75	2.5	90.3	77.0	nd	-	8.1
FM06-31-6	Lysomax PLA2	2.5	75	2.5	85.0	63.6	nd	706000	8.9
FM06-31-7	Lipomod 699L	5.0	94	2.0	92.6	89.3	nd	-	6.0
FM06-31-8	Lipomod 699L	2.5	94	2.0	85.5	82.1	nd	850000	7.4
FM06-31-9	HSEYP	5.0	77	nd	80.4	85.6	nd	441000	7.3
FM06-31-10	HSEYP	2.5	77	nd	64.5	56.0	nd	758000	7.8
FM06-31-13	KLM3'	3.0	25	2.5	81.9	60.7	nd	407000	8.8
FM06-31-14	KLM3	1.5	25	2.5	43.5	32.2	nd	347000	9.4
FM06-31-15	KLM3'	3.0	19	4.2	77.6	55.0	nd	255000	8.6
FM06-31-16	KLM3	1.5	19	4.2	45.3	29.4	nd	233000	8.2
FM06-31-17	Lipomod 699L	3.0	94	2.2	81.6	42.9	nd	407000	8.5
FM06-31-18	Lipomod 699L	1.5	94	2.2	54.2	42.1	nd	282000	8.8
FM06-31-19	None	3.0	5	0.5	72.9	60.8	nd	176000	12.0
FM06-31-20	None	1.5	5	0.5	27.0	23.3	nd	66000	nd
FM06-31-21	HSEYP	3.0	77	nd	nd	nd	nd	375000	nd
FM06-31-22	HSEYP	1.5	77	nd	nd	nd	nd	280000	nd
FM06-31-31	HSEYP	1.5	77	nd	48.4	31.1	nd	259000	8.3
FM06-31-32	HSEYP	2.0	77	nd	nd	nd	nd	255000	nd
FM06-31-32	HSEYP	2.5	77	nd	nd	nd	nd	273000	nd
FM06-31-34	HSEYP	3.0	77	nd	74.1	57.3	nd	302000	8.8
FM06-31-40	KLM3'	1.5	57	1.6	64.5	61.9	54.0	499000	8.6
FM06-31-41	KLM3'	3.0	57	1.6	94.0	93.5	89.3	880000	8.0
FM06-31-42	KLM3'	5.0	57	1.6	98.7	98.8	nd	-	7.6
FM06-31-43	Lipomod 699L	1.5	>95	3.0	74.3	76.1	73.0	555000	9.0
FM06-31-44	Lipomod 699L	3.0	>95	3.0	95.9	96.7	95.2	934000	7.8
FM06-31-45	Lipomod 699L+none	2.0+1.0	60*	2.2	95.1	95.8	95.6	854000	7.5
FM06-31-46	HSEYP	1.5	77	nd	53.4	59.5	58.1	519000	8.9
FM06-31-47	HSEYP	3.0	77	nd	93.7	92.6	91.8	790000	7.9

Table 8: Pilot scale made mayonnaises

*Estimated based on 67% Lipomod 699L treated egg yolk with >95% PC conversion plus 33% raw egg yolk.

Besides KLM3', two other enzymes, Lipomod 699L (commercial phospholipase A_2 from Biocatalyst sold to the egg industry) and Lysomax PLA2 (commercial phospholipase A_2 from Genencor sold to the egg industry) were tested in the first set of mayonnaises made. As reference/market standard, HSEYP was used, a commercial enzymatically modified egg yolk product from Sanovo A/S. The first set of mayonnaises was made with 2.5% and 5.0% egg yolk. For the mayonnaises made with 5% enzyme treated egg yolk, the viscosity was above the upper detection limit and hence the mayonnaises were too viscous. In comparison, the mayonnaise made with 5% untreated egg yolk had a viscosity close to one third of the viscosity of the mayonnaises made with 5% enzymatically treated egg yolk (369000 cp vs.>999999 cp). Mayonnaise made with 2.5% unmodified egg yolk had a viscosity of 225000 cp. Mayonnaises made with 2.5% egg yolk modified with either KLM3', Lipomod 699L or Lysomax PLA2 corresponding to a PC conversion of 54%, 94% and 75%, respectively, had viscosities ranging from 700000 to 850000cp, see Figure 13. Based on mayonnaises made with 2.5% enzymatically modified egg yolk, no significant change in viscosity was observed for PC conversion ratios between 50 and 100%. Looking at particle size distribution for mayonnaises made with 2.5% egg yolk, no clear trend between this and the PC conversion ratio of the egg yolk seems obvious, see Figure 14. The change towards a lower percentage of particles with a diameter below 10 µm upon shaking the mayonnaises was most pronounced for PC conversions between 50% and 80%, but within this range no correlation between the drop in percentages particles with diameter below 10 µm and the degree of PC conversion was seen. For the high PC conversion of 94%, only a negligible drop in percentages particle with diameter below 10 µm was seen. Upon heat treatment, weak oiling-out was observed for mayonnaise made with unmodified egg yolk whereas no oiling-out was observed for any of the mayonnaises made with enzymatically modified egg yolk. Thus, mayonnaise with 2.5% KLM3' modified egg yolk is heat stable and viscosity-wise on level with market standard 2.5% HSEYP mayonnaise as well as with Lipomod 699L and Lysomax PLA2 mayonnaise, and particle size-wise on level with market standard HSEYP mayonnaise as well as with Lysomax PLA2 mayonnaise.

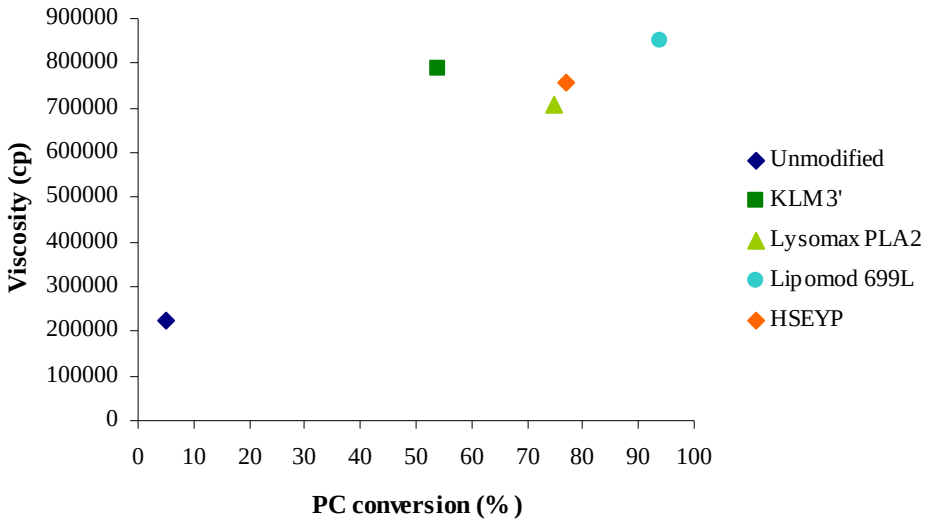

Figure 13: Viscosity of pilot scale mayonnaises as a function of PC conversion of the enzyme modified egg yolk. Mayonnaises were made with 2.5% egg yolk (data from set 1).

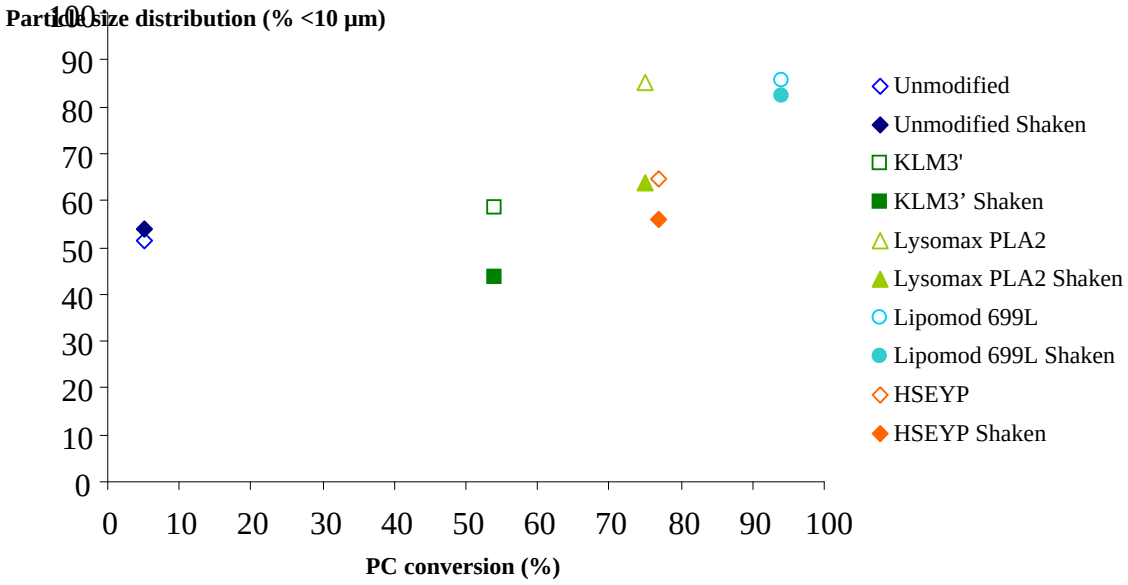

Figure 14: Particle size distribution of pilot scale mayonnaises as a function of PC conversion of the enzyme modified egg yolk. Mayonnaises were made with 2.5% egg yolk. Mayonnaise kept at 4-8°C for 72 hours as well as mayonnaise shaken at 300 rpm at room temperature for 72 hours was measured (Data from set 1).

The second set of mayonnaises was made without stabilizer and with 1.5% and 3.0% egg yolk. The enzyme modification of the egg yolks used in the second set of mayonnaises was carried out in a water bath as one batch for each enzyme, whereas the egg yolks used for the other three sets of mayonnaises were modified in a heating block as several batches for each enzyme and then pooled before use. The modification of egg yolk with KLM3' (1.5 LATU/g yolk) for both 60 minutes and 240 minutes caused LPC degradation due to the LPC activity of KLM3' and hence the PC conversion was reduced to 25% and 19%, respectively. The free fatty acid content of these egg yolks also reflected the LPC degradation as the egg yolk modified with KLM3' for 60 minutes contained 2.5% FFA and the 240 minutes contained 4.2% FFA even though the latter had the lowest PC conversion of the two. Difference in the FFA content of egg yolk modified with KLM3' for 60 or 240 minutes was not reflected in the oxidation stability of mayonnaises made with 3% egg yolk, as the oxidation stability of the two mayonnaises was the same despite the fact that one contained almost twice as much FFA as the other, see Figure 15. Possibly, the difference in FFA content was masked by antioxidant in the oil used for mayonnaise. Due to the lack of stabilizers, the viscosity of the mayonnaises with 3.0% egg yolk was halved compared to the mayonnaises made with 2.5% egg in the first set of mayonnaises (approximately 400000 cp versus approximately 800000 cp). As for the first set of mayonnaises, no significant change in viscosity was observed for a broad PC conversion interval (25% to 94% PC conversion), see

.

The third set of mayonnaises was also made without stabilizer and with 1.5%, 2.0%, 2.5%, and 3.0% HSEYP egg yolk in order to set the egg content for future pilot scale mayonnaise

productions. In all cases, the viscosity of the four mayonnaises was within a range of 255000 to 302000cp. Mayonnaise with an egg content of 1.5% and 3.0% was chosen for further study.

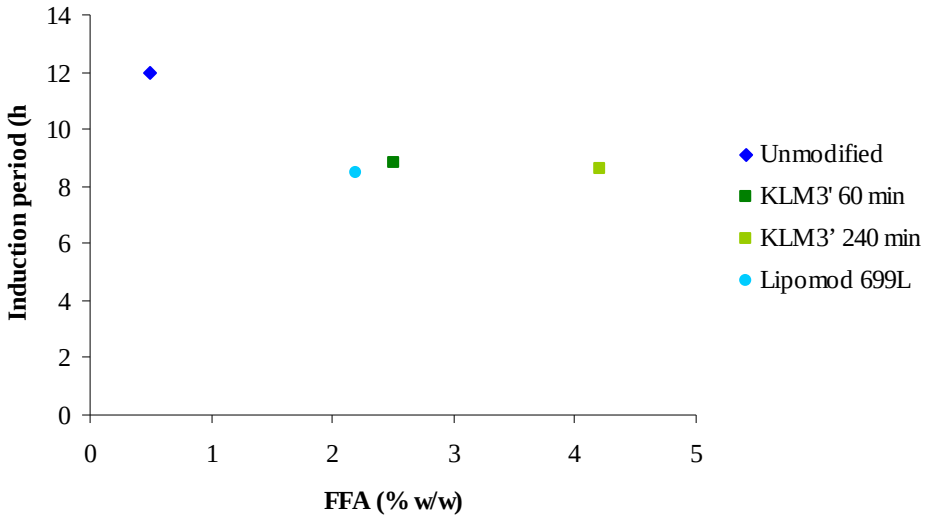

Figure 15: Oxidation stability of mayonnaise as a function of percentage FFA in egg yolk. Mayonnaises were made with 3.0% egg yolk and no stabilizer (Data from set 2).

In the fourth set of mayonnaises, egg yolks modified with KLM3' or Lipomod 699L were used as well as HSEYP. Also, two part Lipomod 699L modified egg yolk blended with one part unmodified egg yolk was used for pilot scale mayonnaise production. The viscosity of the four mayonnaises made with 3.0% egg yolk was in the range 790000 cp to 934000 cp, see Figure 16, which was on level with the mayonnaises made with 2.5% egg yolk in the first set (700000cp to 850000 cp). As was the case for mayonnaises made with 2.5% enzymatically modified egg yolk, no significant change in viscosity was observed for PC conversion ratios between 50 and 100% for the mayonnaises made with 3.0% modified egg yolk. As expected, the viscosity of mayonnaises made with 1.5% egg yolk was lower than the viscosity of mayonnaises made with 3.0% egg yolk, 499000 cp to 555000 cp versus 790000 cP to 934000 cP, see Figure 17. For mayonnaises made with 1.5% egg yolk, a gentle increase in viscosity was seen with the PC conversion ratio ascending from 57% to >95%, see Figure 17. Still the overall increase in viscosity does not exceed 10%.

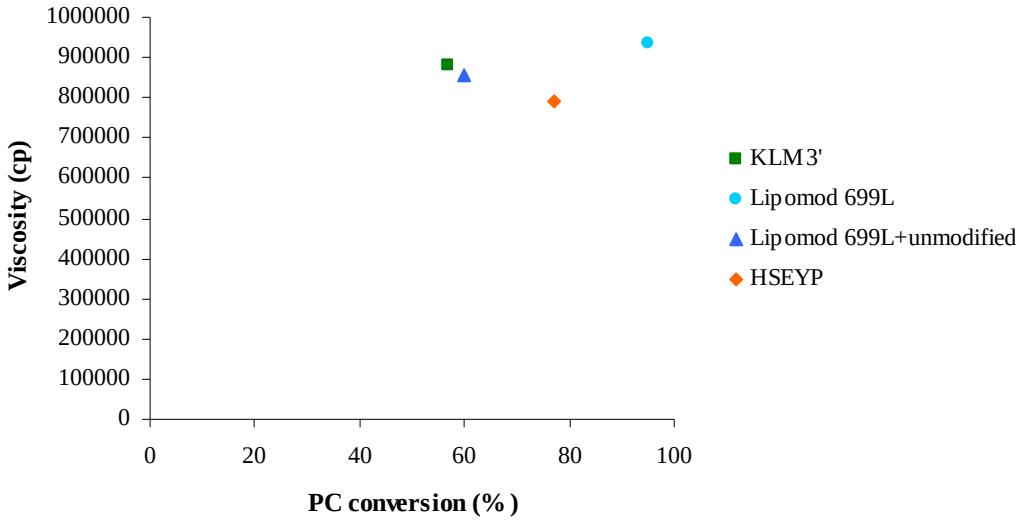

Figure 16: Viscosity of pilot scale mayonnaises as a function of PC conversion of the enzymaticaly modified egg yolk. Mayonnaises were made with 3.0% egg yolk (Data from set 4).

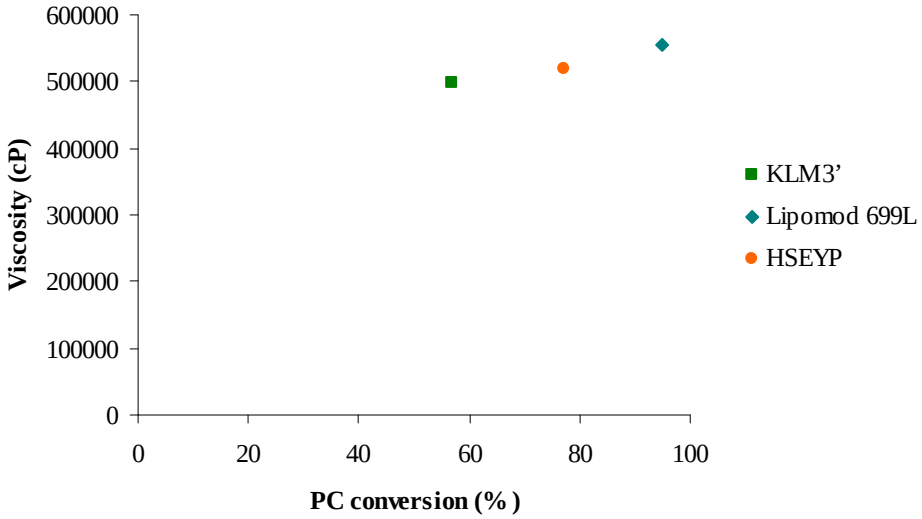

Figure 17: Viscosity of pilot scale mayonnaises as a function of PC conversion of the enzyme modified egg yolk. Mayonnaises were made with 1.5% egg yolk (Data from set 4).

The heat stability of the mayonnaises in the fourth set was tested by measuring particle size distribution before and after heat treatment (1 hour at 90°C), see Figure 18. Before heat treatment all mayonnaises made with 3.0% egg yolk had more than 93% particles with a diameter below 10 µm, indicating a fine/delicate emulsion also reflected by the high viscosities of these mayonnaises. Upon heating, only minor drops in percentage particles with a diameter below 10 µm was observed; hence the mayonnaises made with modified egg yolk were heat stable. Mayonnaise made with KLM3' treated egg yolk had the most pronounced drop in percentage particles with a diameter below 10 µm (94% to 89.3%). Disregarding data from the mayonnaise made with Lipomod 699L treated egg yolk blended with unmodified egg yolk, it seemed that the lower the PC conversion ratio, the larger the drop in percentage particles with a diameter below 10 µm upon heating. The observations made for mayonnaises made with 3% egg yolk became

more evident for mayonnaises made with 1.5% egg yolk. The lower viscosities of these 1.5% egg yolk mayonnaises and hence more coarse emulsions were reflected in the lower percentage particles with a diameter below 10 µm (less than 75% particles with a diameter below 10 µm for mayonnaise made with 1.5% egg yolk as opposed to at least 93% particles with a diameter below 10 µm for mayonnaises made with 3.0% egg yolk), see Figure 19. Mayonnaise made with 1.5% KLM3' modified egg yolk showed a significant drop in percentage particles with a diameter below 10 µm upon heating, whereas the mayonnaise made with Lipomod 699L modified egg yolk only showed a minor drop, see Figure 19. Hence for mayonnaise with low egg yolk content, a PC conversion ratio of 50% seemed on the low edge regarding heat stability. Still no oiling-out was observed upon heat treatment for any of the mayonnaises made with enzymatically modified egg yolk.

For mayonnaises made with HSEYP, the percentage particles with a diameter below 10 µm seemed low for the untreated mayonnaise compared to the percentage of the other untreated mayonnaises. Also heating the HSEYP mayonnaise gave an increase in percentage particles with a diameter below 10 µm. This was seen as an indication of the measurement of the untreated HSEYP mayonnaise is incorrect.

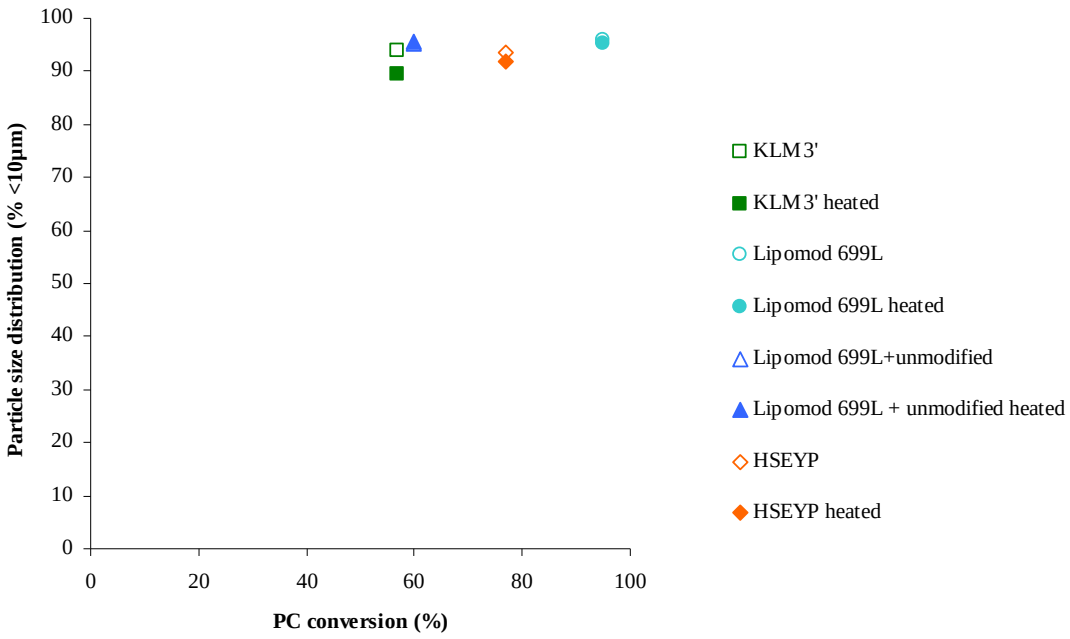

Figure 18: Particle size distribution of pilot scale mayonnaises as a function of PC conversion of the enzyme modified egg yolk. Mayonnaises were made with 3.0% egg yolk (Data from set 4). Mayonnaise kept at 4-8°C for 72 hours as well as mayonnaise kept at 90°C for one hour was measured.

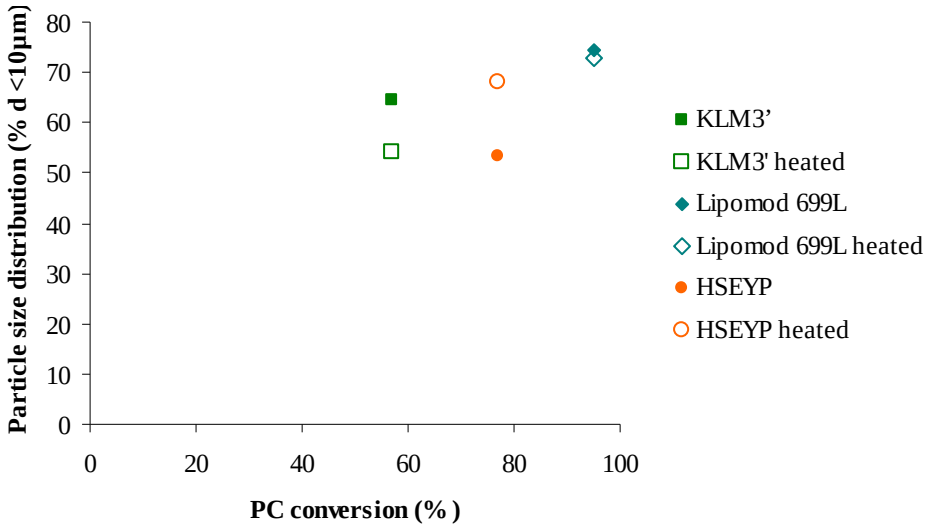

Figure 19: Particle size distribution of pilot scale mayonnaises as a function of PC conversion of the enzymatically modified egg yolk. Mayonnaises were made with 1.5% egg yolk (Data from set 4). Mayonnaise kept at 4-8°C for 72 hours as well as mayonnaise kept at 90°C for one hour was measured.

The amount of FFA in egg yolk modified with KLM3' was close to half the amount present in egg yolk modified with Lipomod 699L (corresponding to 57% PC conversion and >95% PC conversion respectively). However, this difference was not reflected in the oxidation stability of the mayonnaises, see Figure 20, as the induction period was approximately 8 hours for FFA contents between 1.6 and 3.0%. Again, as described for the second set of mayonnaises, the FFA content differences were most likely masked by antioxidant in the oil used for mayonnaise production. Based on the evaluation of the fourth set of mayonnaises, KLM3' modified egg yolk used to produce mayonnaise with an egg content of 3.0% gave viscosity and particle size distribution prior to heat treatment on level with the market standard HSEYP and Lipomod 699L modified egg yolk. With respect to heat stability, mayonnaise with an egg content of 3.0% made with Lipomod 699L modified egg yolk showed slightly better results than mayonnaise made with KLM3' modified egg yolk or HSEYP. This was more pronounced for mayonnaises made with an egg content of 1.5%.

The pilot scale mayonnaise trials have shown that the egg content in mayonnaise can be reduced from 5% as in a full fat mayonnaise to 2.5-3.0% by using enzyme modified egg yolk. Mayonnaise made with 2.5-3.0% enzymatically modified egg yolk had a higher viscosity as well as a larger amount of particles with a diameter below 10 µm both indicating a more delicate emulsion than mayonnaise made with unmodified egg yolk. Also the mayonnaises made with enzymatically modified egg yolk were heat stable as opposed to mayonnaise made with unmodified egg yolk that showed oiling-out upon heat treatment. The mayonnaises made with egg yolk modified with KLM3' were on level with mayonnaises made with egg yolk modified with Lysomax PLA2 or Lipomod 699L and HSEYP with respect to viscosity, particle size distribution, oxidation stability, and heat stability. However, mayonnaises made with an egg content of 1.5%, Lipomod 699L showed slightly better results than KLM3' regarding heat stability.

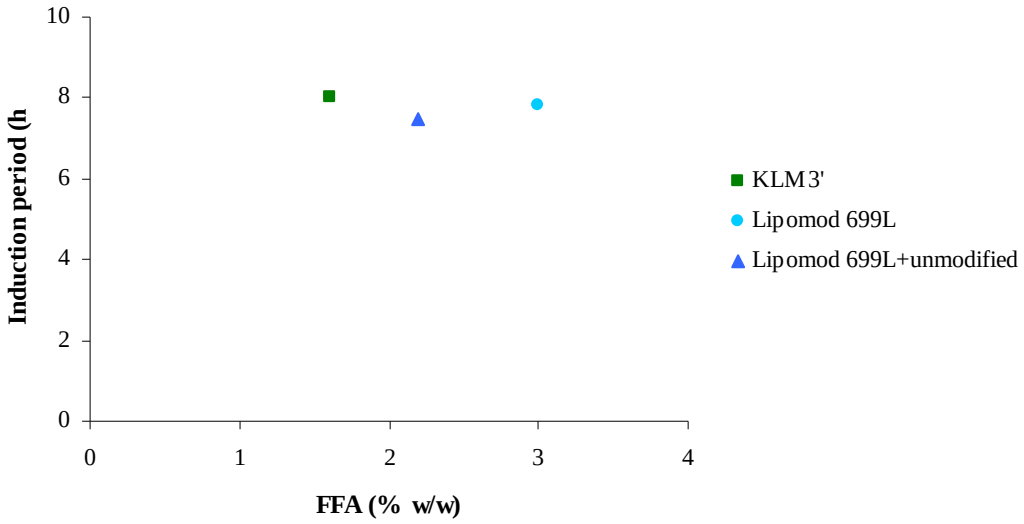

Figure 20: Oxidation stability of mayonnaise as a function of percentage FFA in egg yolk. Mayonnaises were made with 3.0% egg yolk (Data from set 4).

At the onset of the egg modifying project, the target was to develop an acyltransferase that was able to modify egg yolk on level with or better than Lecitase Ultra (competitor's product). The conversion of lecithin to lyso-lecithin makes it possible to produce heat stable mayonnaise. The ability of the acyltransferase to transfer the fatty acid moiety to cholesterol should give rise to a product less prone to oxidation, because of a reduced level of FFA. Based on a few application trials comparing mayonnaise made with KLM3' wild type and Lecitase Ultra, the KLM3' wild type met the above target.

Running the kinetic study to define the optimal conditions for PC conversion, it became evident that the maximum PC conversion degree obtainable with KLM3' regardless of the dosage, temperature and time was 60%. This level of PC conversion was demonstrated to be caused by KLM3' having LPC activity, which lead to degradation of lysolecithin.

CONCLUSION

A kinetic study with KLM3' in egg yolk was conducted to set the optimal parameters for PC conversion. Seven pH values in the range pH 5.0-10.0, three temperatures 50, 55, and 60°C, and seven enzyme dosages in the range 0.5 to 5.0 LATU/ g egg yolk were tested. Kinetics was followed by analysis of free fatty acid and cholesterol as well as by ^{31}P-NMR to monitor the PC/LPC level during modification. In all instances, cholesterol was eliminated within the first 30 minutes incubation time. Irrespective of the enzyme dosage, incubation temperature or time used, the maximum obtained PC conversion degree with KLM3' was 60%. KLM3' was demonstrated to have LPC activity causing degradation of already accumulated LPC and hence a low PC conversion ratio. The LPC activity of KLM3' was also reflected by elevated FFA amounts at low PC conversion degrees.

Pilot scale mayonnaise trials showed that KLM3' modified egg yolk was on level with mayonnaise made with Lysomax PLA2 or Lipomod 699L modified egg yolk, and with mayonnaise made with HSEYP with regard to viscosity, particle size distribution, heat stability,

and oxidation stability. Hence KLM3' modified egg yolk did not give a mayonnaise less prone to oxidation than mayonnaises made with conventional phospholipase modified egg yolk.
By using enzymatically modified egg yolk instead of unmodified egg yolk, the egg content in mayonnaise can be reduced by 50% from 5.0% to 2.5%.