KLM3' – Study of Emulsification in Liver Sausages

ABSTRACT

The effect of acyltransferase KLM3' was examined in a liver/oil emulsion, first in a model system and secondly in application trials of liver sausages with a high water and a high fat content, respectively. The activity of KLM3' was also investigated by HPTLC by measuring the presence of phosphatidylcholine (phospholipid), phosphatidylethanolamine (phospholipid) and lysophospholipids (emulsifying agent).
The model system demonstrated improved oil emulsion stability with the presence of KLM3'. At a concentration of 0.07% KLM3', the liver emulsion had an oil stability of 94% compared to 51% in the control. In the application trial, the KLM3'-treated liver sausages with high fat had a lighter colour, harder texture and improved fat retention compared to the control and CITREM-treated liver sausage. The results from HPTLC confirmed the activity of KLM3' in liver emulsion and liver sausage by degradation of phospholipids and accumulation of lysophospholipids, and formation of cholesterol ester in the enzyme-treated emulsion compared to the control. A higher dosage of KLM3' correlated to an increased activity, which seemed to have the negative effect of further degradation of the lysophospholipids in liver sausages.

INTRODUCTION

In emulsified meat products with a considerable fat content, e.g. emulsified sausages and pâtés, it is desirable to have fat stability so that fat losses are minimized and the amount of visible fat is reduced. Additionally, it is desired that the loss of meat juice is low and that the taste, texture and appearance of the product are acceptable. Emulsifiers may be added to achieve these effects and some of the most commonly known are isolated protein or protein concentrates like soy protein or Na-caseinate. However, these proteins are characterised by being relatively expensive, and the level allowed in meat products is limited. Additives like mono- and di-glycerides and citric acid esters can also be used as emulsifiers, but their application is often unwanted due to price or labelling (no additives on the label of the meat product).

Thus, alternatives to these existing emulsifiers are most welcome in the meat business. Recently, an enzymatic method was applied as an emulsifier in food products. In a study by Lilbæk et al., 2006 where mozzarella cheese was treated with a phospholipase A, they observed reduced fat losses in whey and cooking water and moisture retention in the cheese curd. In emulsified meat products it could be interesting to test such an enzymatic method with KLM3'.

The objective of the present work was to evaluate the effect of KLM3' on the yield and texture of liver sausages, and to investigate the changes in phospholipids and cholesterol levels. Liver sausages usually have a low content of meat and a high content of non-mixable fat/water, and it is necessary to use emulsifiers to avoid separation of fat. GRINDSTED® CITREM N 12 Citric Acid Ester, which KLM3' was compared to, is a commercial product from Danisco normally used as an emulsifier in liver sausages.

TECHNOLOGY

Liver/oil emulsion

Initially, enzyme (Acyltransferase KLM3', Batch 1026295001, Activity 1128 LATU/g) was tested in a liver/oil emulsion by using a model system as described by Zorba & Kurt, 2007 with some modifications. 20g pork liver and 80g water were mixed in a Stephan cutter at 300-600rpm for 30 seconds, 1500rpm for 30 seconds and lastly 3000rpm for 3 minutes. 100g of the blend was added to a thermomixer (Vorwerk) and the enzyme was added while stirring at level 1 (100 rpm) for 15 seconds. 300ml rapeseed oil was slowly added to the thermomixer, first at level 1 for 15 seconds and then level 6 within 3 minutes.

2 x 10ml emulsion were weighed into a test tube and capped and immediately heated at 80°C in a water bath for 30 minutes. The tubes were centrifuged at 310 x g for 20 minutes and the amounts of water and oil separated were measured. Emulsion stability was calculated from the water and oil losses of the heat-treated liver emulsion using the following equations:

Emulsion stability (%): 100 – (mL of water separated x 10) + (mL of oil separated x d x 10) (d:specific gravity of oil)

Liver sausage production

Table 1: Liver sausage recipe

	Recipe	
	High water	**High fat**
Pork liver	14.8	14.8
Pork skin	14.8	29.6
Back fat	19.7	29.6
Water	49.3	24.6
Spices liver sausage	1	1
Nitrite curing salt	0.5	0.5

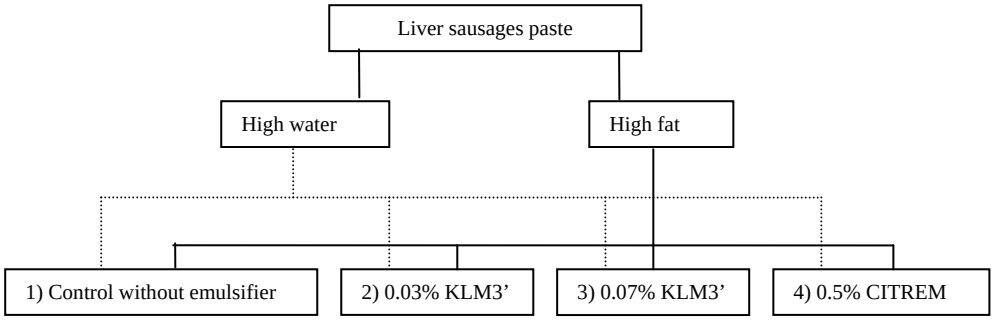

Figure 1: Experimental design of liver sausage production.

In total, 8 batches of 5kg liver sausages were produced. Half of the batches were based on a high-water recipe whereas the other half was based on a high-fat recipe (see table 1).

Each of the two recipes consisted of 4 batches: 1) a control without emulsifier, 2) 0.03% KLM3', 3) 0.07% KLM3' and 4) 0.5% CITREM (figure 1).

Pork skin (3mm), back fat (3mm), hot water (80°C), spices and salt were chopped in a bowl chopper and heated with steam until a temperature of 65°C was reached. The steam was turned off and when temperature was down to 50°C, the liver was added. In recipes 2 & 3, the enzyme was added at 60°C. In recipe 4, CITREM was added at the beginning together with the spices. The chopping was stopped when 40°C was reached, and the liver paste was stuffed into tins and F-plus cal. 60 casings. Total processing time was approximately 10 minutes. The tins were autoclaved at 120°C with a 2-minute holding time, and the liver sausages were cooked at 75°C to a core temperature of 72°C.

HPTLC analysis of phospholipids

Enzyme reaction and lipid extraction:

The sample with liver paste was frozen and lyophilized. The test sample was ground in a coffee mill.

0.5g dry meat powder was extracted with 7.5ml hexane:isopropanol 3:2 for 30 minutes.

The organic phase was isolated and analysed by HPTLC.

HPTLC was used to measure the contents of free cholesterol, phospholipids and lysophospholipids in the liver paste samples.

Applicator: CAMAG applicator AST4.

HPTLC plate: 20 x 10 cm (Merck no. 1.05641)

The plate was activated before use by drying in an oven at 160°C for 20-30 minutes.

Application: 6.0µl of extracted lipids dissolved in hexane:isopropanol 3:2 was applied to the HPTLC plate using an AST4 applicator.
0.1, 0.3, 0.5, 0.8, 1.5µl of a standard solution containing standard components (phosphatidylethanolamine, phosphatidylcholine and lysophospholipids) with known concentrations were also applied to the HPTLC plate

Running buffer 5: Hexane: MTBE (70:30)
Running buffer 6: Chloroform:1-propanol:Methylacetate:Methanol : 0.25% KCl in water 25:25:25:10:9
Elution length: 7cm
Developing fluid: 6% cupric acetate in 16% H_3PO_4

After elution, the plate was dried in an oven at 160°C for 10 minutes, cooled and immersed in the developing fluid (10 seconds) and then dried additionally for 6 minutes at 160°C. The plate was evaluated visually and scanned (Camag TLC scanner).

RESULTS

Model system with liver/oil emulsion

KLM3'-treated liver/oil emulsion Control liver/oil emulsion

Figure 2: Pictures of raw liver emulsion
left: KLM3'-treated liver/oil emulsion right: Control liver/oil emulsion

From a visual inspection of the raw liver/oil emulsion presented in figure 2, it was observed that the control had so poor emulsion stability that it separated, whereas the KLM3'-treated liver/oil emulsion had a nice, homogeneous emulsion. Also a much lighter colour was observed in the enzyme-treated liver/oil emulsion compared to the control, which demonstrated a better emulsion.

Table 2: Oil stability in liver/oil emulsion added KLM3' and a control without enzyme

	Control	0.03% KLM3'	0.07% KLM3'
Oil stability	**51%**	**90%**	**94%**

The specific gravity of the liver/oil emulsion was determined to 900kg · m^{-3} at 4°C at a normal pressure of 1 atm.

The results show that the emulsion stability is improved with the presence of KLM3'. At a concentration of 0.07% KLM3', the liver/oil emulsion had an oil stability of 94% compared to 51% in the control.

Application trial on liver sausages

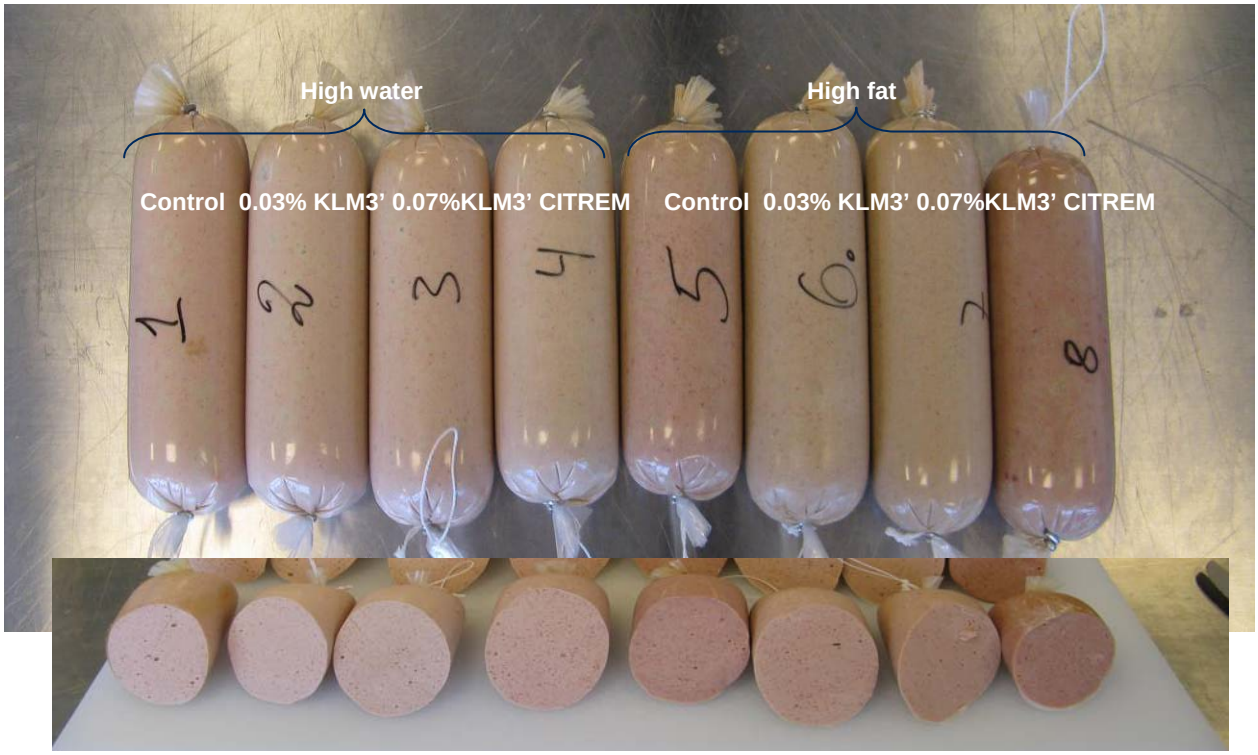

Figure 3: Pictures of heat-treated liver sausages

A difference among the samples was first observed during processing where the enzyme-treated meat batter had a thicker consistency compared to the control and the batch with CITREM N 12.

As shown in figure 3, the colour of the sausages varied from batch to batch. In batches 1 to 4 which contained high water in the recipe, all the colours were light and it seemed that sample 4, the one with CITREM, was a bit lighter that the others. As mentioned previously, a lighter colour may indicate a better emulsion.

In the liver sausages with a high amount of fat (5-8), it seemed that a better emulsion was obtained in both enzyme-treated sausages (6 and 7); they were clearly lighter in colour compared to both the control (5) and the CITREM (8) batch. The colour of the liver sausage treated with CITREM was very dark, and a heavy separation of fat appeared on the surface.

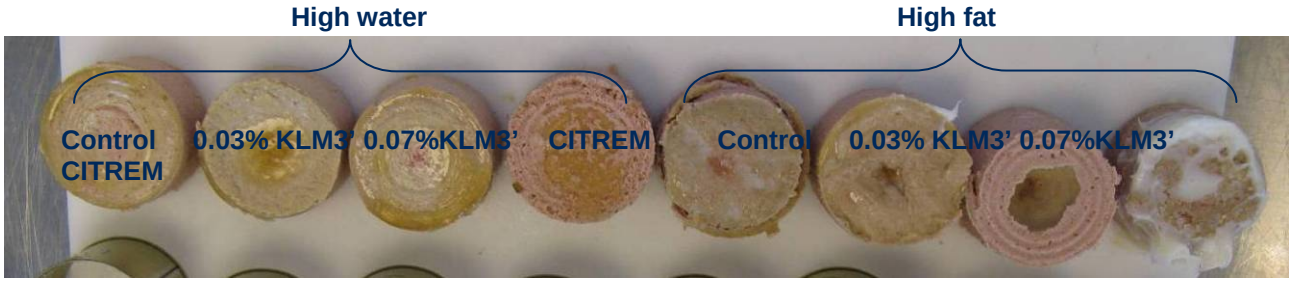

Figure 4: Picture of pasteurised liver pastes

The autoclaved samples of liver paste shown in figure 4 very well illustrate the fat and jelly separation. The samples of liver paste with a high content of water had jelly separation to a similar extent. However, there was a clear difference among the samples of liver paste with a high content of fat in which the fat separation was an indicator of poor emulsion stability. The sample with CITREM had an extreme fat separation compared to the samples treated with enzyme. Also, but to a much lower extent, the control showed some fat separation.

It seemed from the results presented that the formed lysophospholipids exhibit a strong lipophilic group within the molecule as it worked very well in an emulsion with a high fat content. On the other hand, the emulsifier CITREM had a negative impact on the liver sausages, especially the ones with a high content of fat. Most probably the content of fat in the recipe exceeded the emulsion capability of CITREM.

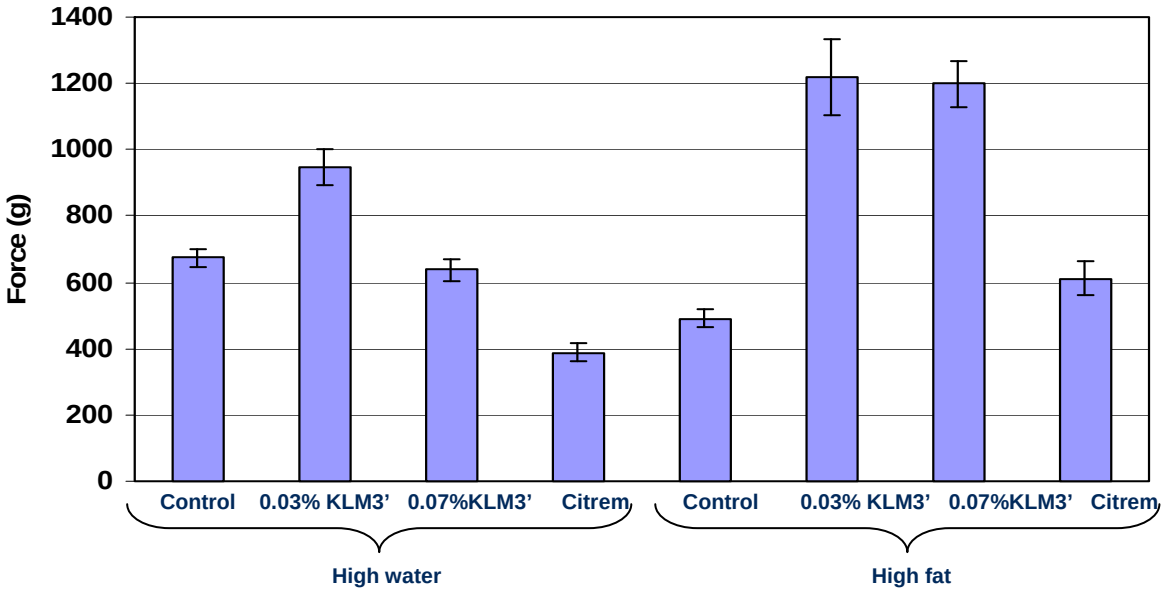

Figure 5: Texture analysis of cooked liver sausages.

From the results presented in figure 5, it seemed that the liver sausages treated with enzyme compared to the control and the CITREM-treated sausages, except from 0.07% KLM3’ in a high water recipe, had a harder texture, which may be correlated to a better emulsion.

HPTLC analysis

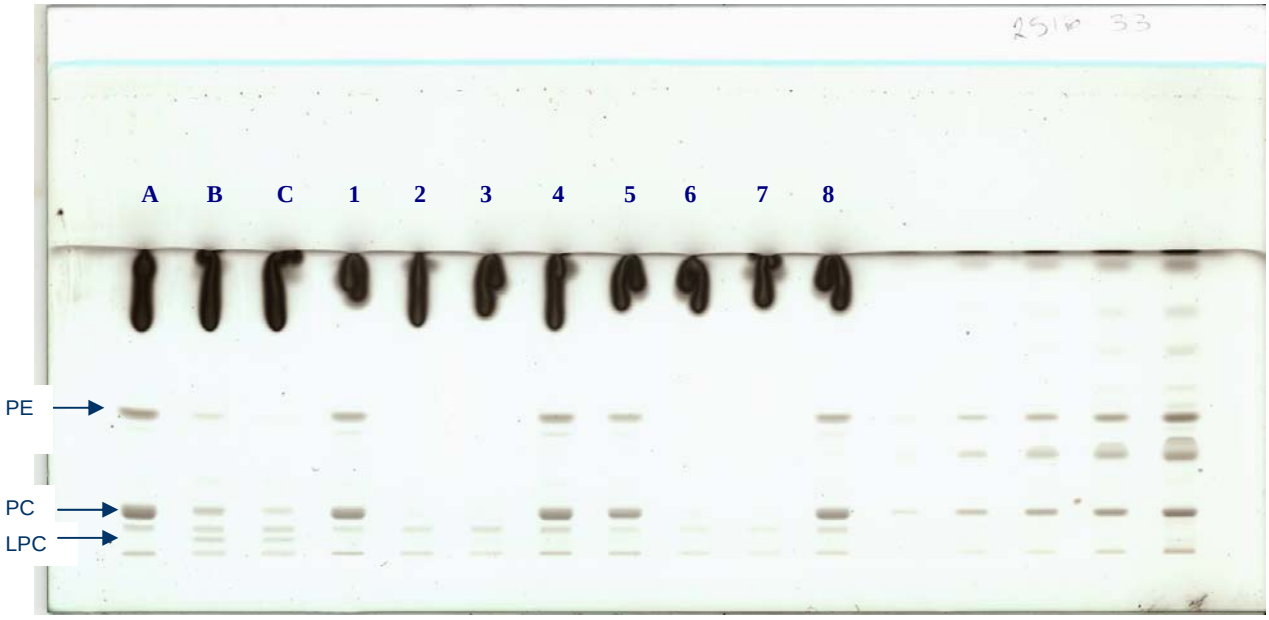

Figure 6: HPTLC analysis (running buffer 6) of phospholipids (PC) and (PE) and lysophospholipids (LPC) of liver emulsion with A) Control B) 0.03% KLM3' C) 0.067% KLM3' & of cooked low -fat liver sausages with 1) Control 2) 0.03% KLM3' 3) 0.07% KLM3' 4) CITREM and high-fat liver sausage 5) Control 6) 0.03% KLM3' 7) 0.07 %KLM3' 8) CITREM

The results from figure 6 confirm the activity of KLM3' in liver emulsions and liver sausages.

The activity of KLM3' caused a larger accumulation of lysophospholipids (LPC) in enzyme- treated emulsions B & C compared to the control A, illustrated by the appearance of LPC bands. The HPTLC analysis of liver sausages showed vague bands of lysophospholipids at both enzyme concentrations which could indicate an overdose of the enzyme in such a way that the lysophospholipids were further degraded.

The bands of phospholipids phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were quantified:

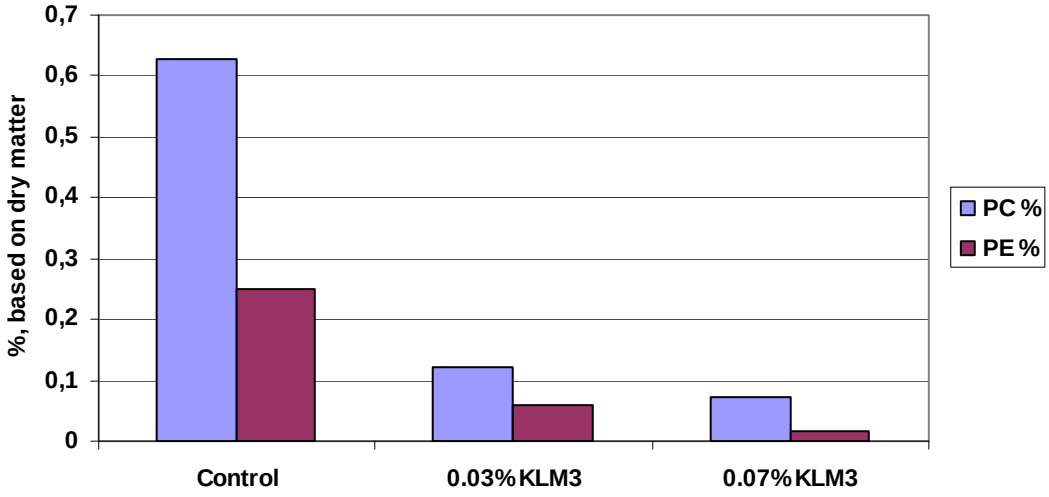

Figure 7: HPTLC analysis (running buffer 6) of phospholipids (PC) and (PE) from cooked liver/oil emulsion.

From the quantification of the primary bands presented in figure 7 it was shown that the activity of KLM3' on phospholipids caused a degradation of phospholipids PC and PE which correlated to increased concentration of enzyme in the liver/oil emulsion.

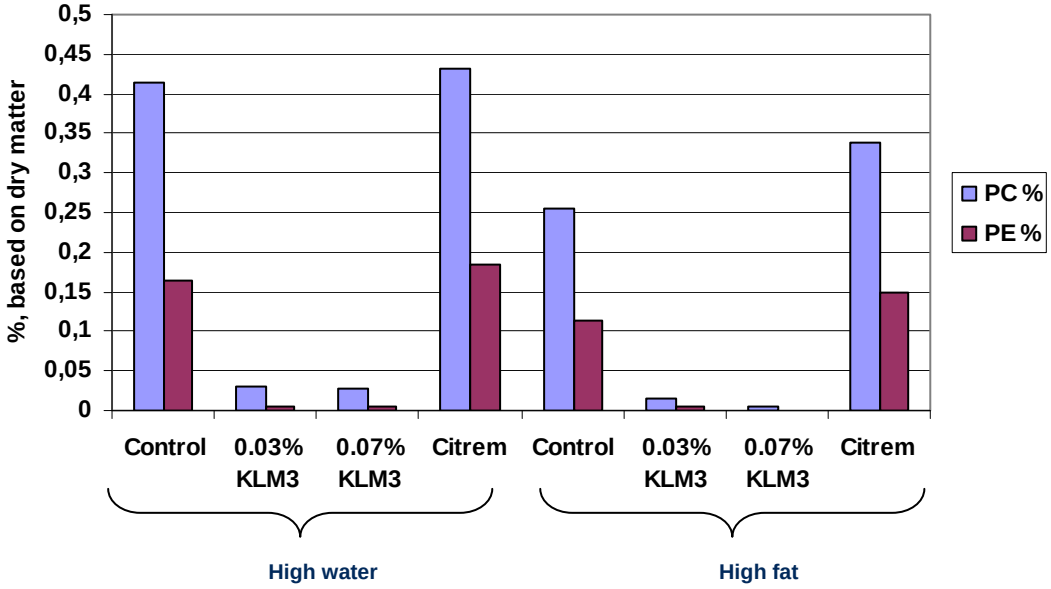

Figure 8: HPTLC analysis (running buffer 6) of phospholipids (PC) and (PE) in liver sausages

From the quantification of the primary bands presented in figure 7 it was shown that the activity of KLM3' on phospholipids caused a degradation of phospholipids, PC and PE, which correlated to increased enzyme concentration in the liver sausages.

Table 3: HPTLC analysis (running buffer 5) of cholesterol in liver sausage samples. % based on dry weight

	Control	**0,03% KLM3’**	**Citrem N12**
% Cholesterol	0.277	0.067	0.264
% cholesterol reduction	0	76	5

From a previous experiment with liver sausages/high water recipe, the effect of KLM3’ on cholesterol was examined. The results in table 3 show that cholesterol was significantly reduced in the KLM3’ treated liver sausages compared to the control and citrem treated sausages. This observation confirms acyltransferases ability as a converter of cholesterol.

CONCLUSION

- Results from the model system show that emulsion stability is improved with the presence of KLM3’. At a concentration of 0.07% KLM3’, the liver emulsion had an oil stability of 94% compared to 51% in the control.
- The KLM3’-treated liver sausages with a high fat content had a lighter colour and improved fat retention compared to the control and CITREM-treated liver sausages. It seems that KLM3’ works very well in high-fat recipes.
- Texture was much harder in the KLM3’-treated sausages with a high fat content compared to the control and the liver sausages with CITREM. To some extent, high water and 0.03% KLM3’ also resulted in texture hardening.
- The results from HPTLC confirmed the activity of KLM3’ in liver emulsions and liver sausages by an accumulation of lysophospholipids, and a degradation of phospholipids and conversion of free cholesterol to cholesterol ester in enzyme-treated emulsions compared to the control. A higher dosage of KLM3’ correlated to an increased activity which may have the negative effect by further degradation of the lysophospholipids.

REFERENCES

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Lilbæk, H. M., Broe, M. L., Høier, E., Fatum, T. M., Ipsen, R. and Sørensen, N.K., 2006: Improving the yield of Mozzaella cheese by phospholipase. J. dairy Sci. 89, 4114-4125.