Afslutningsrapport

Optimering af smørfedt ved enzymkatalyseret omestring med planteolier

Mejeribrugets ForskningsFond

Rapport nr. 2006-76

Juni 2006

mejeriforeningen
danish dairy board

Slutrapport for "Mejeribrugets ForskningsFond"

Optimering af smørfedt ved enzymkatalyseret omestering med plantolier

Projektperiode: 1. Jan. 2003 – 31. Dec. 2005

Projekt deltagere: Danmark Teknisk Universitet
Danmarks Fiskeforskning Instituttet

Projektleder: Lektor Xuebing Xu.

Medarbejdere:

Seniorforsker	Charlotte Jacobsen
Lektor	Huiling Mu
	Trine Porsgaard
Forskningsadjunkt	Tiankui Yang
	Zheng Guo
	Hong Zhang
Forskingsassistant	Torben Rønne
	Tinghong Chang
Teknikere	Malene Leidecker
	Trang Vu
	Karen Jensen

Content

Résumé

Lipozyme TL IM blev ved hjælp af et enzymatisk screeningsstudie selekteret som et af de bedste enzymer til enzymatisk interesterificering af smørfedt. Enzymet er mindre kostbart og desuden anvendeligt i industriel sammenhæng til modifikation af fedt i stor skala. Specificiteten af Lipozyme TL IM afhænger til dels af typen af fedtsyrer. De interesterificerede produkter er produkter mellem blandede produkter og randomiserede produkter.

Interesterificeringsreaktionen blev påvirket af kolesterol- eller smørsyrekomponenter i smørfedt. Undersøgelsen viser at effekten af disse komponenter kunne minimeres ved langtidsbehandlingen, hvor vandindholdet i systemet er reduceret og stabiliseret. Processen blev skaleret op fra 2,6 til 100 kg/dag i en packed-bed reaktor. Trykfaldet for upstream eller downstream flow i packed-bed reaktoren var hhv. 1,1 og 0,4 bar/m ved en lineær flow rate på 0,5-4,0 cm/min hvilket understøtter processens industrielle potentiale.

Det var nødvendigt at deodorisere de interesterificerede produkter p.g.a. afgivelsen af korte fedtsyrekæder fra reaktionen. Den efter omstændighederne optimalt tilpassede deodoriseringstemperatur er på 100 til 120 ºC i 1-2 timer. Forskellige mængder smørfedt i blandingerne førte til store kemiske og fysiske forskelle i egenskaberne for de interesterificerede produkter. Særligt efter interesterificering blev indholdet af fast fedtstof forøget sammenlignet med en fysisk blanding af fedtstofferne, hvilket tyder på at større mængder planteolier kan tilsættes til de interesterificerede produkter.

Det smørbare produkt lavet fra interesterificeret fedtstof havde en hårdhed, som var sammenlignelig med Kærgårdens. Derimod var de smørbare produkter lavet fra fysisk blandet fedtstof tre til fire gange hårdere end det smørbare produkt fra interesterificeret fedtstof. Enzymmodificeret smørbare produkter havde mindre smag af smør end de fysisk blandede smørbare produkter. De smørbare produkter, der indeholdt antioxidanter til at forhindre fiskeolie i at blive oxideret udviklede en mere fiskeagtig smag, end de smørbare fiskeolieprodukter uden antioxidanter.

Et opfodringsforsøg i hamstre med 3 forskellige smørprodukter viste, at inkorporeringen af n-3 fedtsyrer var signifikant højere i lever, plasma og erythrocytter i den gruppe, der blev fodret med smørproduktet indeholdende fiskeolie sammenlignet med de andre grupper. Både erythrocytter og plasma er gode indikatorer for kostens ændringer i fedtsyrerne. Fedtsyrerne i leverens TAG og PL afspejlede plasma fedtsyreprofilerne. Opfodringsperioden (6 uger) var ikke lang nok til at kunne påvirke fedtsyresammensætningen af fedtvævet. Der var ingen forskel imellem de 2 smørprodukter baseret på smørfedt og rapsolie, hvilket betyder, at omestringen ikke havde indflydelse på de ernæringsmæssige effekter af smørprodukterne.

For mulig online kontrol under den enzymatiske interesterificeringsprocess blev der observeret høje korrelationer mellem NIR spectra/Ln(P ratio) og NIR spectra/SFC ved 5ºC i et spektrum fra 4513-5269 cm-1. Overordnet set kan FT-NIR bruges til at overvåge enzymatiske interesterificeringsprocesser af smørfedtsproduktion. Kort analysetid for FT-NIR (2,5 min) gør brugen af denne teknologi mulig til online kontrol.

1 Summary

Lipozyme TL IM was selected to be one of the best enzymes for enzymatic interestesterification from enzyme screening study. The enzyme is less expensive and suitable for industrial application for bulk fat modification. The specificity of Lipozyme TL IM partially depends on the types of fatty acids. The interesterified products are products between the blended products and randomized products.

The reaction was affected by components of cholesterol or butyric acid in butterfat. A follow-up study showed that the effect of these components was minimised in the long term operation where water content in the system is reduced and stabilised. The process was scaled up from 2.6 to 100 kg/day in a packed-bed reactor. The pressure drops for upstream or down stream flow in the packed-bed were 1.1 and 0.4 bar/m at the linear flow rate of 0.5-4.0 cm/min, indicating the feasibility of industrial scale operation.

The interesterified products have to be deodorised due to the release of short chain fatty acids during reaction. The compromised optimum deodorization temperature is 100 to 120 ºC in 1 to 2 h. Different amounts of butterfat in the blends led to the large differences in the chemical and physical properties of the interesterified products. After interesterification, in particular, the solid fat content was increased compared to the blend, indicating the possibility that more vegetable oils can be added to the interesterified products.

The butter spreads from interesterified fats had similar hardness as "Kærgaarden", However, the spread from the physically blended fat was three or four time harder than spreads from interesterified fats. Enzyme modified butter spreads had less butter flavour than the butter spread from physical blending. The spreads containing antioxidants to protect the fish oil against oxidation developed more fishy off-flavours than the fish oil spread without antioxidants.

A feeding study in hamster with three different butter products showed that the incorporation of n-3 fatty acids was significantly higher in liver, plasma and erythrocytes in the group fed the butter spread containing fish oil compared to the other groups. Both erythrocytes and plasma are good indicators for changes of fatty acids with diet. Fatty acids of liver TAG and PL reflected the fatty acids of plasma fatty acid profiles. The fatty acid composition of adipose tissue was not affected by the diet in a short period (6 weeks). There were no differences between the two butter spreads based on butterfat and rapeseed oil meaning that the interesterification process had no impact on nutritional effects of the butter products.

For the possibility of online control during the enzymatic interesterification process, high correlations were observed between NIR spectra/Ln(P ratio) and NIR spectra/SFC at 5°C in the spectra range of 4513-5269 cm^{-1}. Overall, FT-NIR can be used for monitoring enzymatic interesterification process for butter fat production. Short analytical time for FT-NIR (2.5min) makes it possible of using this technology for on-line control.

2 Purpose

This project focused on the development of enzyme processes for the modification of butterfat in order to optimise the butterfat performance with respect to consistency, flavour, as well as other physical, chemical, and nutritional characteristics.

3 Background and target

Butterfat, also called butter oil or milkfat, ranks third in the worldwide production of edible fats and oils. Butterfat comes from milk. In addition to being an important source of dietary fat, butterfat imparts excellent flavour and superior mouth feel to dairy products.

For a number of years, however, questions have been raised as to the health value of butterfat and it has often been claimed as hypercholesterolemic. The hypercholesterolemic effect of butterfat in human diets is associated mainly with lauric, myristic, and palmitic acids (Ney D.M., 1991, Potentials for enhancing the nutritional properties of milkfat, J. Dairy Sci. 74, 4002-4012). These questions have brought out the concerns of arterioscerosis and ultimately for cerebrovascular catastrophe and heart attack. At the same time, butterfat has difficluties to be easily spread immediately after taking it from the refrigerator. Therefore, an innovative use of butterfat is needed.

Studies (Glaeser H. and Keane M., 1992, Cholesterol-reduced dairy products- healthy or harmful? Dairy Ind. Int. 57, 39-42) show that stearic acid and oleic acid are conversely effective in lowering plasma cholesterol levels when replacing palmitic acid in the diet. Short chain fatty acids do not apparently raise the cholesterol level. Polyunsaturated fatty acids lower the HDL and LDL cholesterol levels. From these considerations, selective modification of the fatty acid residue structure of the triacylglycerol molecules of butterfat is a potentially suitable approach for developing a healthier butterfat.

In order to change the structure of butterfat, lipase-catalysed interesterification was considered. In previous research, only high value-added oil and fat products have so far been processed in industry, such as cocoa butter substitutes, human milk fat substitutes, nutrients-enriched marine oils, and functional 1, 3-diacylglycerides. However, new developments in enzyme production and immobilisation technology have made it possible for interesterification applications to be used in daily foods.

Mohamed *et al.* (*Fat Sci Technol* 95:428-431, 1993) studied the blends of refined cottonseed oil and fully hydrogenated soybean oil by using *Rhizomucor miehei* (Lipozyme RM IM) and *Candida antartica* (Novozym 435) lipases. They observed the changes induced in triglyceride structure and the formation of hydrolysis by-products after lipase-catalysed interesterification. Rousseau and Marangoni (J. Agric. Food Chem. 46, 2368-2374, 1998) used sn-1,3 specific lipase from *Rhizopus arrhizus.* They found that the interesterification reaction was optimal at 0.35% water content. The enzymatic interesterification of single butterfat has been conducted by Kalo et al. (Fat Sci. Technol. 91, 276-280, 1898) and De Greyt and Huyghebaert (Lipid Techol. 1, 10-12, 1995). The physical changes have been observed. We have studied margarine fat modification from palm stearin and coconut oil in pilot scale

batch reactors. Two commercial lipase preparations were used including Lipozyme TL IM, a new developed cheaper commercial lipase and also Lipozyme RM IM, a previously developed lipase. It was found that Lipozyme TL IM was very active (*Eur. J. Lipid Sci. Technol.* 102, 411-418, 2000. and *J. Am. Oil Chem. Soc*. 78, 57-64, 2001). It is more promising that Lipozyme TL IM needed no additional water during operation. By-products are therefore minimised and yields are largely improved. Some other studies have been also conducted with little consideration of process technology, mostly having little value for scale-up evaluation. All above investigations are based on lab flasks and batch reactors. No paper has been so far published on packed bed reactors for the modification of butterfats by lipase-catalysed interesterification. The latter is more promising for industrial operations.

4 Objectives

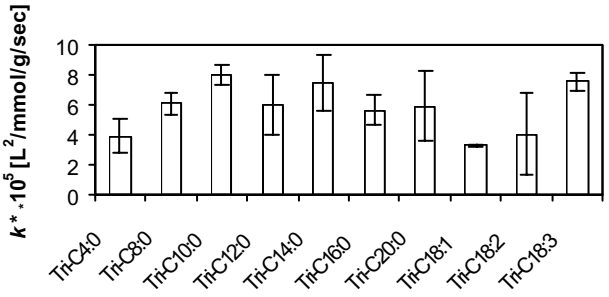
The target is to provide the knowledge of process technology and the understanding of the product properties in order to give guidance for industrial application in butterfat modification. The relationship between process technology and product properties will be established theoretically and practically. The main contents of this project are to investigate the process technology for the enzymatic modification of butterfat and the properties of the products therefrom. The joint effort will be made to provide a more mature process technology and well-characterized products in terms of suitable physical and flavour properties and improved nutritional potentials.

5 Results

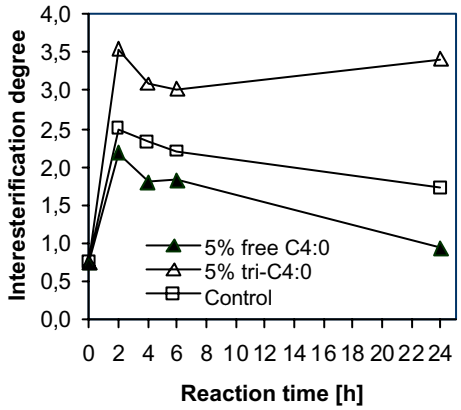
5.1 Enzyme screening

Lipozyme TL IM, Lipozyme TL lab-immobilized, Lipozyme RM IM, and Novozym 435, Lipase AK-20, and Lipase PS-D-I were used for enzyme screening study. Lipase PS-D-I, Lipozyme TL IM and Lipozyme RM IM seems to give a faster reaction with a higher final interesterification degree. Since Lipozyme TL IM is less expensive and more suitable for industrial application for bulk fat modification. Therefore, Lipozyme TL IM was chosen for further investigation and pilot production of interesterified fats.

The triglyceride selectivity of the immobilized lipase was investigated in the lipase-catalyzed interesterification reactions between two mono-acid triglycerides. None of the methods employed showed any significant differences between the performances of the lipase on the different triglycerides, indicating that Lipozyme TL IM is non-selective towards fatty acid or triglycerides in the system used (**Figure below**).



5.2 Effect of minor components on enzymatic interesterification

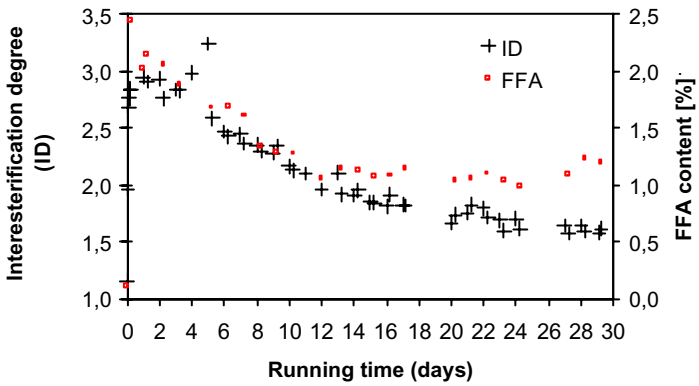


Short-chain fatty acids, such as butyric acid, which butterfat contains (10.8%), are more hydrophilic and they lower the pH of the microaqueous layer surrounding the enzyme, which may lead to enzyme deactivation in the free forms (**Left Figure**). Therefore, water content should be minimised to lower the hydrolysis reaction in the system. The effect was observed to be minimised in long-term operations where water content is reduced and stabilised.

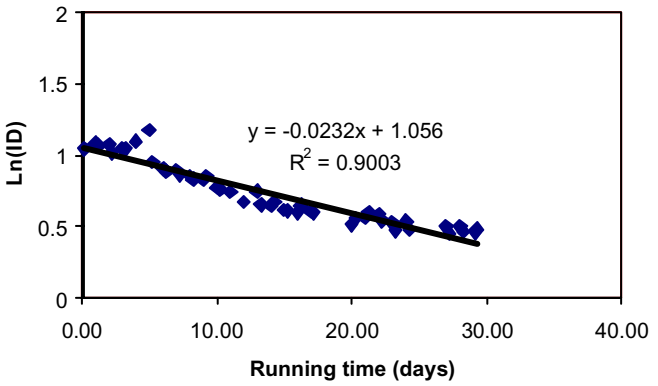
Adding cholesterol (0.1-0.5%) to the blend seems, in general, leads to a faster loss in activity compared to the control. When operated in long-term operations, this effect is minimised. It is expected that an accumulation of cholesterol in the enzyme surface will change the polarity and so as to be removed in long-term flow.

5.3 Enzyme stability study

The activity de-creased a bit faster until day fifteen where it reached a plateau (**Figure right**). This level was more or less kept for the remainder of the test period. From the profile of FFA, the fast reduction of activity may be affected by the FFA content as early discussed.



The above stability profile follows the first order decay (**Figure right**), which can be described by $ID = ID_0 * e^{-kt}$. Therefore, the reaction rate k was 0.0232 for this reaction. The enzyme half-life is about 29.9 days calculated by $t_{1/2} = Ln2/k$, where $t_{1/2}$ is the enzyme half-life, k is deactivation constant.



5.4 Scaling-up of enzyme interesterification process

The continuous enzymatic interesterification system has been scaled-up to a 450cm^2 packed bed reactor (PBR, 500 * 34 mm). A flow rate on 11.7 mL/min is required to get a residence time of 30 min, which in small scale was shown to give a conversion of 80%. At this flow rate it was possible to produce more than 15 kg of interesterified product per day. Based on the results obtained from the long-term operational stability study, the same enzyme can be used for 5 days without any change in the reaction conditions.

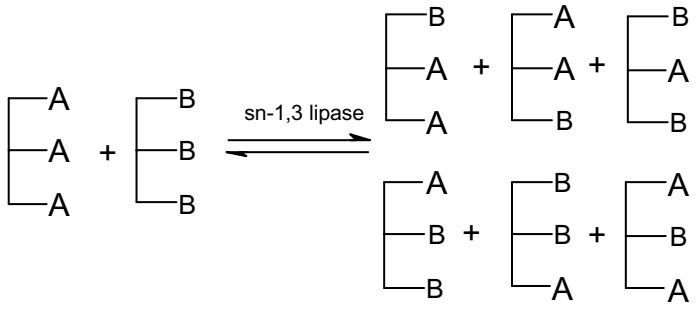
In order to look into the engineering aspects of the process, a further larger scale (PBR, 1000*50mm) was used (**Right picture**). The pressure drop across the column under different flow rates had been measured. The pressure drop had a linear relationship with flow rates (or linear flow rates).

$$\text{Linear velocity (m/h)} = \frac{Flow\ rate\ (g/\min) * 60\ \min/\ hour}{Blend\ \ density\ (g/ml) * PBR\ \ cross\ \sec tion\ (cm^2) * 100\ cm/m}$$

An average pressure drop can be calculated as 1.1 bar/m column per unit of linear flow rate within a range of 0.5-4.0 cm/min for upward flowing, whereas the pressure drop was 0.4 for downward flowing.

5.5 Regio-specificity of interesterified products

When an sn-1,3 specific lipase is used as a catalyst for interesterification reaction, the distribution of fatty acids at sn-2 position will remain unchanged during reaction at an ideal reaction condition (A, B represent two different type of fatty acids).



A problem often encountered for a selective lipid modification is acyl migration, which leads to non-specific distribution of fatty acids. The acyl migration is a non-enzymatic reaction. It can be catalyzed by acids, bases, ion exchange resin (carrier) or elevated by heat and reaction time. In our experiments, the feedstocks for butter spread production were produced in a packed-bed reactor (47×500 mm, residence time ˜ 50 min) catalyzed by Lipozyme TL IM at 60 °C. Acyl migration was expected to be avoided due to using short reaction time.

To check the specificity of Lipozyme TL IM-catalyzed interesterification, fatty acid distributions at sn-2 position both for blends (Before) and interesterified products (After) were analysed (**Table below**). It shows that Lipozyme TL IM had certain

	Butterfat /rapeseed oil/fish oil, (BF/RO/FO, w/w/w) mole %											
	70/30/0				50/50/0				70/25.5/4.5			
	Before EI		After EI		Before EI		After EI		Before EI		After EI	
FA	FAC	sn-2	FAC	sn-2	FAC	sn-2	FAC	sn-2	FAC	sn-2	FAC	sn-2
C4:0	3,11	0,00	3,02	0,00	2,30	0,00	2,26	0,00	2,95	0,00	3,18	0,00
C6:0	2,84	0,00	2,77	0,00	2,08	0,00	2,07	0,00	2,79	0,00	2,88	0,00
C8:0	1,69	0,00	1,66	0,00	1,24	0,00	1,24	0,00	1,71	0,00	1,68	0,00
C10:0	3,18	1,09	3,13	1,28	2,34	0,86	2,32	0,74	3,24	1,34	3,11	1,57
C11:0	0,25	0,00	0,24	0,00	0,18	0,00	0,09	0,00	0,25	0,00	0,24	0,00
C12:0	2,98	3,16	2,92	2,56	2,19	2,35	2,16	1,64	3,02	3,45	2,91	2,94
C14:0	8,23	13,55	8,02	8,69	6,00	9,63	5,92	6,23	8,50	13,66	8,22	10,32
C16:0	22,87	27,94	22,24	25,06	17,92	19,73	17,72	18,59	23,23	27,64	22,62	25,59
C16:1	1,48	2,04	1,44	1,56	1,14	1,50	1,12	1,23	1,82	2,28	1,75	1,98
C18:0	6,93	3,74	6,73	6,56	5,47	2,73	5,38	5,02	6,95	3,73	6,77	5,92
C18:1n-12	1,59	1,19	1,54	1,38	1,19	0,80	1,16	1,01	1,62	1,21	1,55	1,80
C18:1n-9	29,03	23,77	29,04	30,60	36,41	30,61	36,68	38,00	27,72	22,73	27,07	27,03
C18:1n-7	1,39	0,49	1,31	1,06	1,92	0,62	1,82	1,48	1,47	0,53	1,43	1,01
C18:2n-6	6,61	9,98	6,46	8,05	10,17	16,25	10,22	12,72	5,95	8,94	5,92	7,57
C18:3n-3	3,47	5,01	3,07	3,57	5,39	8,37	4,88	5,67	3,17	4,42	2,92	3,44
CLA C9-T11	0,50	0,26	0,47	0,41	0,39	0,20	0,35	0,31	0,52	0,28	0,49	0,42
C20:5 n-3 (EPA)	0,00	0,03	0,00	0,02	0,00	0,02	0,00	0,02	0,39	0,35	0,38	0,37
C22:5 (DPA)	0,00	0,07	0,00	0,07	0,00	0,06	0,00	0,05	0,00	0,14	0,00	0,13
C22:6 n-3 (DHA)	0,00	0,00	0,00	0,00	0,00	0,01	0,00	0,00	0,41	0,62	0,39	0,63

specificities for different chain lengths and saturations. When 4.5% fish oil was added into a blend (BF/RO/FO, 70/25.5/4.5), the distribution of fatty acids at sn-2 position, such as EPA, DPA, DHA, significantly remained unchanged after interesterification. In general, Lipozyme TL IM-catalyzed interesterification showed selectivity for shorter chain length and polyunsaturated fatty acids in a solvent free system.

This indicates that the enzymatically interesterified products are products between the blended products and randomized products. This gives different speculations for physical properties and nutritional complications based on the results from this study and previous work.

5.6 Optimization of deodorization for enzymatically interesterified products

A preliminary sensory evaluation indicated that the off-flavour of interesterified products is related to the content of free fatty acid (FFA). In order to obtain a product with an acceptable smell and taste, FFA has to be removed by deodorization. On the other hand, butter flavour should be kept as much as possible. Therefore, an optimisation is required.

The interesterified mixture was deodorized at 5 temperatures (60 to 180ºC) and three time periods (1, 2, and 3 h) in a pilot deodorizer. The operation was monitored by free

fatty acid content (FFA), peroxide value (PV), volatiles, and the sensory evaluation of the samples with respect to flavor and odor (most importantly the butter flavor and odor and the off-flavor and odor from butyric acid). ANOVA partial least squares regression analysis showed that deodorization time and especially deodorization temperature significantly affected the sensory properties and level of volatiles, FFA and peroxides in the samples. The best compromise between removing undesirable off-flavors while maintaining the desirable butter flavor seemed to be obtained by using a deodorization temperature of 120ºC for 2 h. Response surface methodology analysis showed a significant effect of deodorization temperature and to a lesser extent deodorization time. The butter flavor and odor had an optimum at a deodorization temperature of approximately 100 to 120 ºC for 1 to 2 h. These conditions are therefore recommended in order to remove the off-flavor and odor, while maintaining as much as possible of the attractive butter flavor and odor.

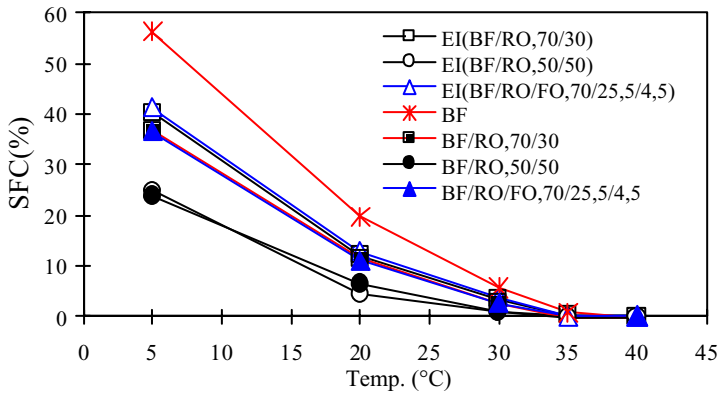
5.7 Feedstock formulation and production for butter spread

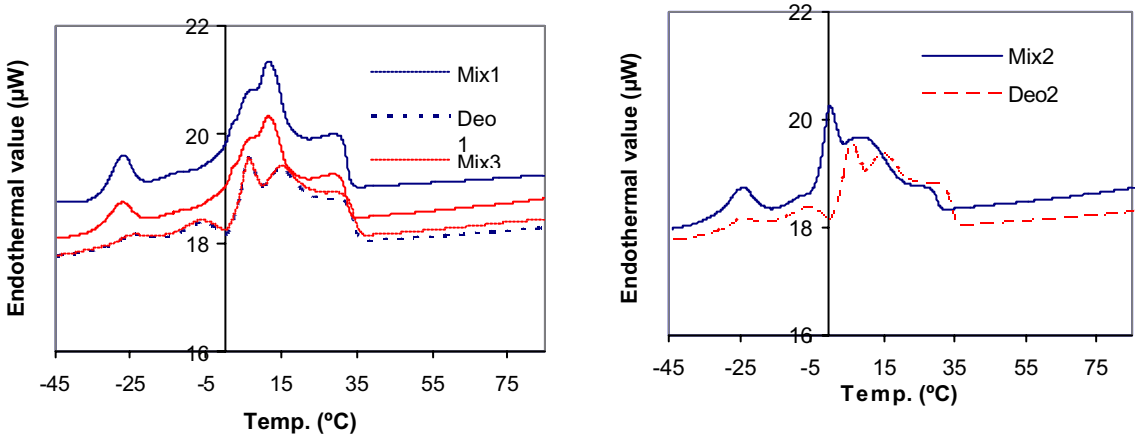
Three basic mixtures (**Right Table**) were used for butterfat feedstock production. The physical properties of the mixtures were totally related to the content of butterfat in the mixtures, which was observed both from NMR and DSC measurements. The slight differences of TAG distribution between mix1 and mix3 due to adding 4.5% of fish oil did not cause significant differences based on the physical properties.

Mixtures	Butterfat (BF)	Rapeseed oil (RO)	Fish oil (FO)
Mix1	70	30	0
Mix2	50	50	0
Mix3	70	25.5	4.5

Based on these mixtures, enzymatic interesterifications were carried out in a packed-bed column (34.7mm×500mm, 380-400g of Lipozyme TL IM). The reaction was continuously run for about 110 h at 60 °C with flow rate 13.7 mL min^{-1}. The collection for the interesterified products began after the first hour. The resulting lipids were deodorized using previously optimized conditions, namely 120 °C, vacuum 5 mbar and 2 h. The deodorized lipids have a free fatty acid content 0.45-0.55%, in accordance with the optimized results.

The interesterified fats after deodorization (Deo) were characterized by SFC (**Right figure**), DSC (**Figures below**), and HPLC.





From SFC results, it can be seen that the changes of SFC at 5 °C after interesterification were increased and more pronounced for higher content of BF in the blends. The melting behaviours of different blends (**Figure above**) and interesterified products (**Table below**) were affected by amount of BF in the blends (Mix1 and Mix3 were close to each other).

Blend ratio	Onset (°C)		Offset (°C)	
	Before EI	After EI	Before EI	After EI
BF/RO, 70/30	14,5 ± 0,1	16,4 ± 0,9	33,5 ± 0,0	36,0 ± 0,3
BF/RO, 50/50	11,9 ± 0,0	9,7 ± 0,0	31,2 ± 0,1	30,9 ± 0,1
BF/RO/FO, 70/25.5/4.5	15,2 ± 0,5	16,0 ± 0,6	33,8 ± 0,4	35,8 ± 0,0

The changes of physical property were due to the changes of triglyceride (TAG) composition after interesterification. In general, ECN32, 34, and ECN50 to 56 were increased and ECN36 to 46 were decreased after enzymatic interesterification. The large differences of TAG composition between interesterified products were also caused by different amount of BF in the blends. Peroxide values (PVs), FFAs, diacylglycerol (DAG) and cholesterol contents for interesterified products after deodorization were generally lower than 0.65meq/kg, 0.55%, 2.1%, and 1.7mg/g, respectively.

The butter spreads (**Table below**) were produced using a margarine production unit, which included pasteurization, three scraped-surface heat exchangers and one pin machine worker for storage stability study.

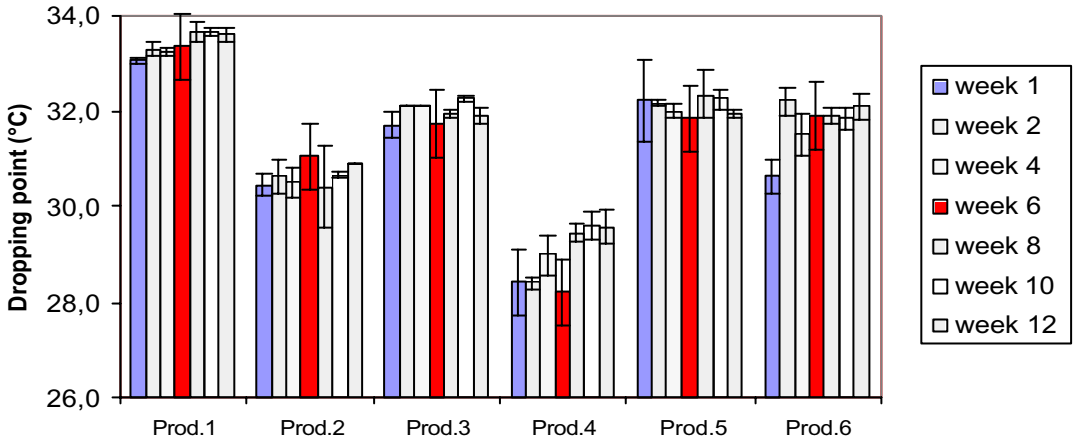
	Products	Interesterification			BF (%)	Antioxidants
		BF (%)	RO (%)	FO (%)		
Kærgården	Prod. 1					
Blend	Prod. 2		30*		70*	* only physical blending
Enzymatically interesterified products	Prod. 3	70	30			
	Prod. 4	30	30		40*	
	Prod. 5	70	25.5	4.5		
	Prod. 6	70	25.5	4.5		+

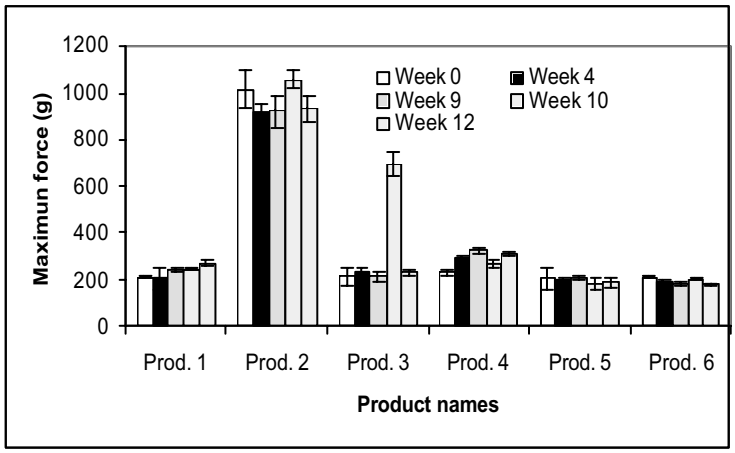
5.8 Storage stability study of butter spreads

A storage stability study was carried out for butter spreads stored at 5 °C in 12 weeks. The changes in physical (dropping point, hardness, crystal form), chemical properties (FFA, PV,volitiles, tocopherol), and sensory properties for the products were measured in during storage.

5.8.1 Physical properties

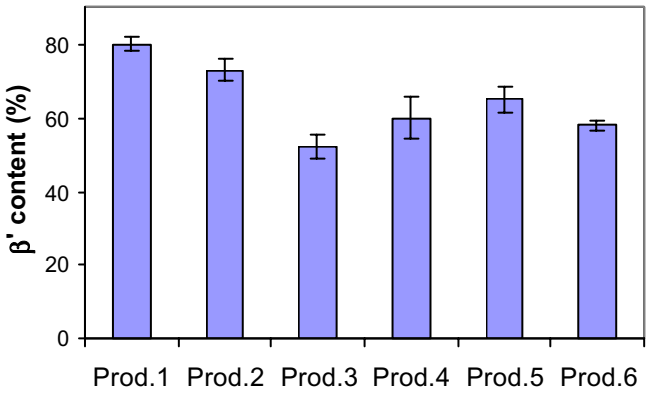
During storage, the changes of dropping point (DP) were insignificant for the same butter spread products (**Figure below**). However, differences were observed between the physical blending and interesterified products. The products which had the lowest DP was made of enzymatically interesterified BF/RO (50/50) blended with 40% BF, even though the total ratio of BF/RO was still 70/30 compared to Prod. 3 or Prod. 2. There were no significant differences for DPs by using 4.5% FO instead of RO. The butter spreads produced from enzymatically interesterified fat (Prod. 3, 5, 6) had higher DPs than the blend (Prod. 2). Overall, adding liquid oil for the butter production leads to decreasing DPs compared to the commercial butter spread.





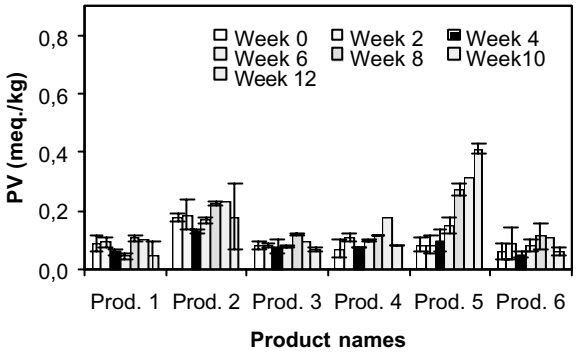
For the hardness measured by texture analyzer (**Left figure**), the differences between products were significant between the blend (Prod. 2) and interesterified mixtures (Prod. 3 to 6). The physical blend (Prod. 2) was five times harder than the enzymatically interesterified products, even for partially blended mix-ture (Prod. 4 : BF/EI(BF/RO), 40/30/30). The hardness of butter spreads produced from the interesterified products was similar to tthat of he commercial product (Kærgården).

The crystal form for butter spread products has been measured by X-ray analysis at room temperature. The crystal form was β'for all the initial products (data not shown here). After 12 weeks storage at 5 °C (**Right figure**), the commercial butter spread and the physical mixtures had the highest β' contents. The content of β' for the interesterified products were generally decreased faster than the physical blend.

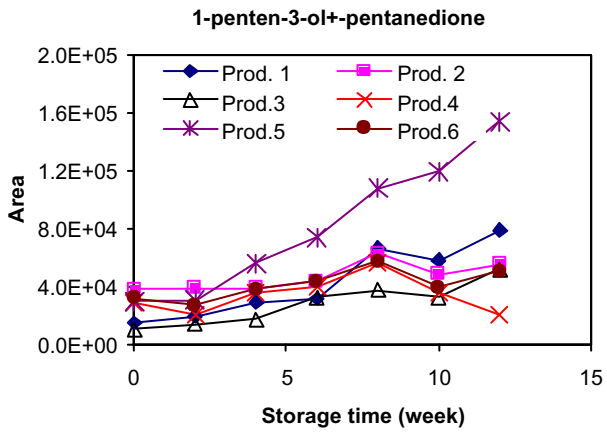


5.8.2 Chemical properties

FFAs were stable and no hydrolysis occurred during the storage at 5°C for 12 weeks. Higher FFAs (0.4-0.6%) were observed for butter spreads produced from purely interesterified fats compared to FFAs for butter spread produced from physical blending (0.2%) and Kærgården (0.3%). The contents of tocopherols remained constant during storage for all butter spreads.



Left figure shows the change of PV during 12-week storage at 5 °C. The butter spread produced from enzymatically interesterified mixture (Prod. 3) had lower PV than the physical blend (Prod. 2). This effect was also seen with partially blending enzymatically-interesterified product into the mixture (Prod. 4). It indicated that butter spread produced from enzymatic interesterification (Prod. 3 & 4) had better oxidative stability during storage. Based on PV, adding fish oil has major effects on product oxidation stability during storage. PV for the butter spread containing fish oil without adding antioxidant (Prod. 5) after 8 weeks storage was twice as high compared with the other products.

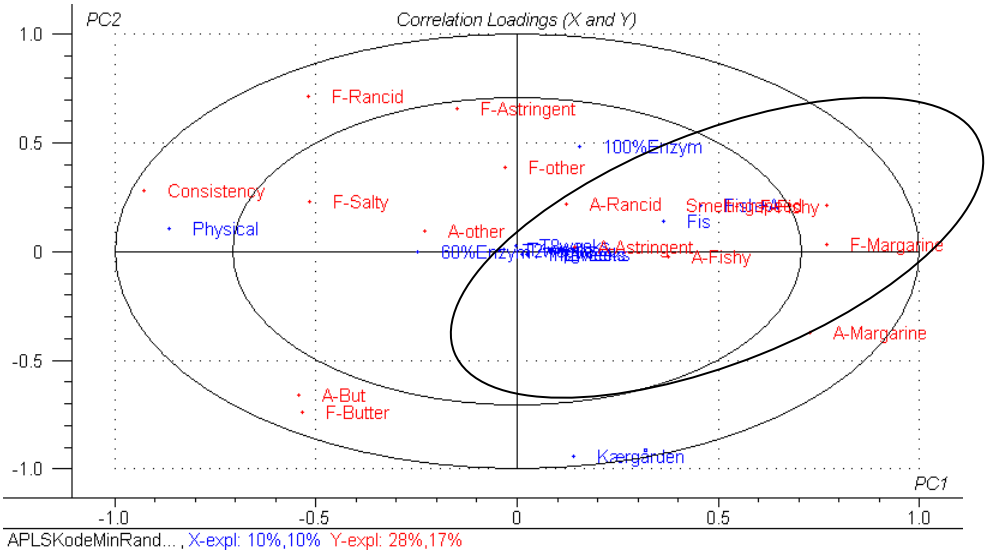


The secondary oxidation products were evaluated by the content of volatiles. The content of 1-penten-3-ol + pentanedione correlated well with PV (**Right figure**).

5.8.3 Sensory evaluation

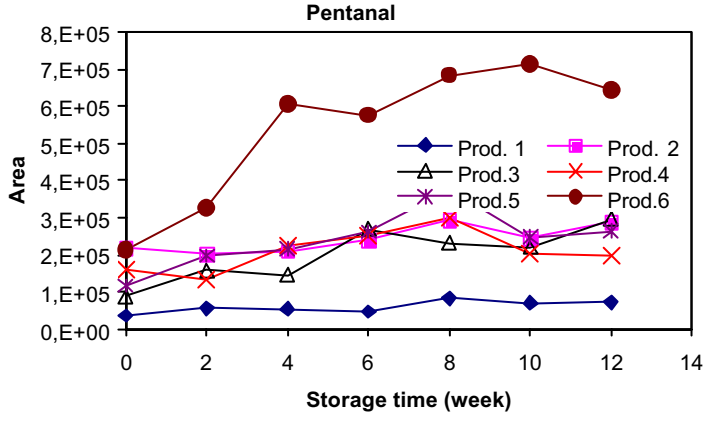
A trained sensory panel evaluated the smell, taste, consistency and melting speed of the butter spreads. The following attributes were evaluated for smell: butter, rancid, fishy, astringent, margarine and other. The same attributes were also evaluated for taste. The intensity of these attributes was evaluated on a scale from 0 to 9.

An ANOVA PLS analysis (**Figure below**) on the sensory data showed that butter spreads based on enzyme-modified lipids had less butter flavour and taste compared to Kærgården. As expected this was particularly the case for butter spread with 100 % modified butterfat (Prod.3), whereas the product containing a mixture of modified lipids and butterfat (Prod.4) had more butter taste.



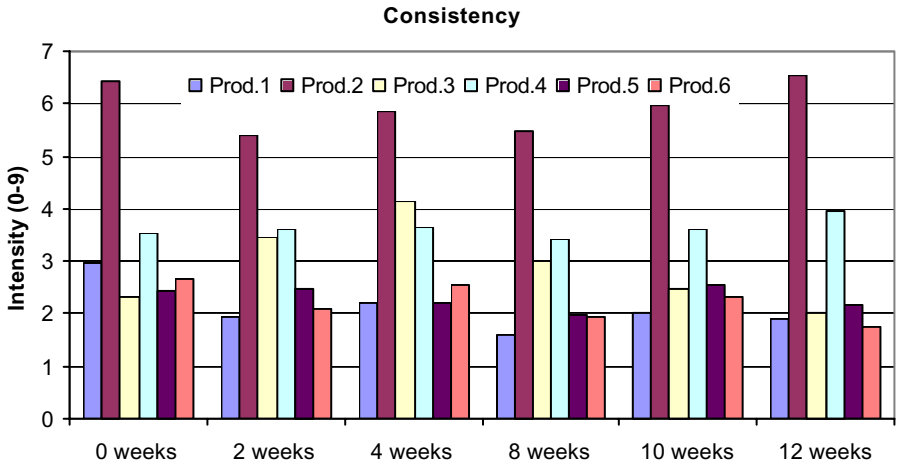
Kærgården developed significantly less rancid off-flavours during storage than the other products followed by butter spreads produced from enzyme modified lipids and with the butter spread containing a physical mixture of lipids developing the most rancid off-flavours. However, none of the spreads developed very rancid off-flavours during 12 weeks of storage.

As expected fishy off-flavours only developed in spreads containing fish oil. Interestingly, spreads containing antioxidants to protect the fish oil against oxidation developed more fishy off-flavours than the fish oil spread without antioxidants. These findings were in good agreement with pentanal development during storage (**Figure below**).



Margarine taste was significantly lowest in the spreads produced from the physical mixture of lipids, while the other products including Kærgården had similar intensities of margarine flavour (Figure from ANOVA analysis).

Another important finding was that the consistency of the spreads (**Figure below**) produced from a physical mixture of lipids was significantly harder than the other butterfat spreads. Kærgården and the spreads containing fish oil had very similar soft consistencies while the enzyme modified spreads without fish oil were slightly harder. In accordance with these findings, spreads produced from the physical mixture melted significantly slower than the other products with the fish oil spreads having the fastest melting rate.



5.9 Nutritional study of butter spreads

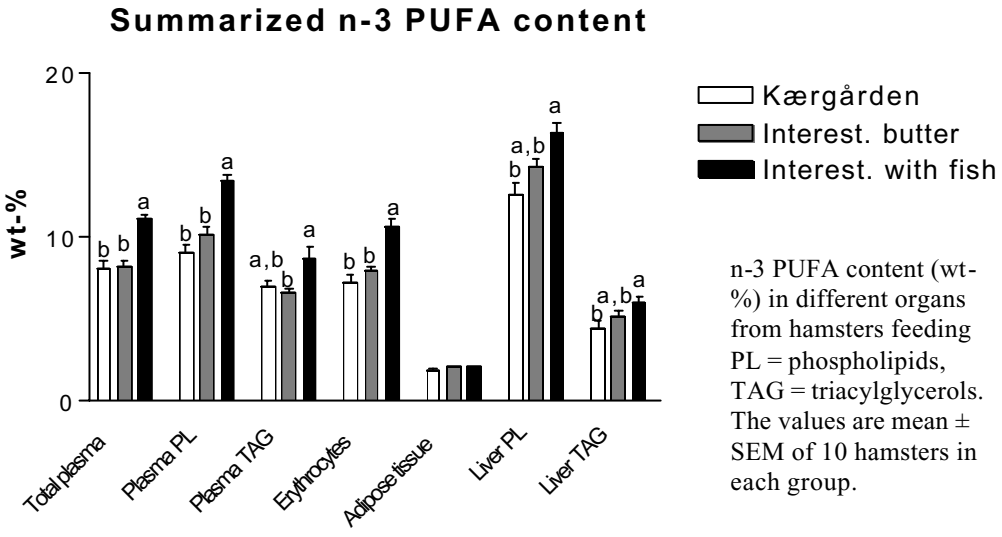
5.9.1 Single meal nutritional study

The nutritional effects of an interesterified butter spread containing fish oil were compared with the effects of Kærgården in a single meal study in healthy men. Twelve men aged 25-34 years (mean age 27.7 years) with BMI's from 19.6-27.0 (mean BMI 23.3) were randomized to receive either Kærgården or the interesterified butter spread on two different occasions. The butter spreads were ingested after an overnight fast as spread on three slices of bread with ^{13}C-labelled cholesterol, marmalade and coffee/tea. A fasting blood sample was collected before the meal and blood samples were collected every hour until 7 hours after the meal. A light fat-free lunch was served after the 4 h sample. On the following morning a single fasting blood sample was collected from the subjects to get a final value for the cholesterol absorption measured by the ingested ^{13}C-labelled cholesterol. No difference was observed in cholesterol absorption.

The lipoprotein cholesterol levels were measured in the fasting blood samples to check if any of the subjects suffered from hypercholesterolemia but they were all within the normal range. Incorporation of n-3 fatty acids into plasma TAG was observed after the diet containing butter spread with fish oil, so it is possible to improve the level of n-3 fatty acids by consuming such a product.

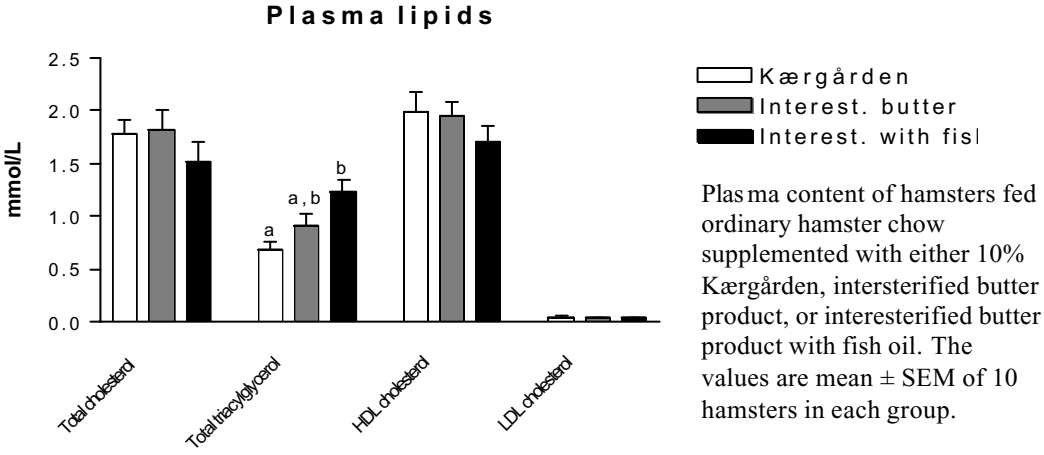
5.9.2 Animal feeding study

A feeding study in hamsters was conducted to investigate the longer-term effects of the interesterified butter spreads. Three groups fed ordinary hamster chow supplemented with 10% butter spreads were included: Kærgården, interesterified butter spread with butter fat and rapeseed oil (70/30), and interesterified butter spread with butter fat, rapeseed and fish oil (70/25.5/4.5). The hamsters were fed the diets for six weeks. Then they were bled by cardiac puncture and organs were removed.
Fatty acid composition of total plasma, plasma PL and TAG, isolated erythrocytes, liver and adipose tissue was determined. The incorporation of n-3 fatty acids was significantly higher in liver, total plasma, plasma PL and erythrocytes in the group fed the butter product containing fish oil compared with the other groups (**Figure below**). The changes in fatty acid composition of both total plasma and erythrocytes are good indicators for the change of diet. The change of liver PL and TAG reflected the change of plasma PL and TAG. The fatty acid composition of adipose tissue was not affected by the diet in a period of 6 weeks.



n-3 PUFA content (wt-%) in different organs from hamsters feeding PL = phospholipids, TAG = triacylglycerols. The values are mean ± SEM of 10 hamsters in each group.

No differences were observed in total plasma cholesterol and in lipoprotein cholesterol levels between the three groups (**Figure below**). There was no difference in the ratio between LDL and HDL cholesterol for the 3 groups. Total plasma TAG



Plasma content of hamsters fed ordinary hamster chow supplemented with either 10% Kærgården, intersterified butter product, or interesterified butter product with fish oil. The values are mean ± SEM of 10 hamsters in each group.

levels were significantly higher in the group fed interesterified butter product with fish oil than the group fed Kærgården. We have previously observed a higher plasma TAG level in rats fed fish oil in comparison with ordinary rat chow (Porsgaard, T., Xu, X. and Mu, H. (2005) Effects of dietary triacylglycerol structure on plasma and liver lipid levels in rats fed low-fat diets containing n-3 polyunsaturated fatty acids of marine origin. In *Seafood research from fish to dish: Quality, safety and processing of wild and farmed fish*, eds. Luten, J., Jacobsen, C., Bekaert, K., Sæbø, A. and Oehlenschlager, J. Accepted.)

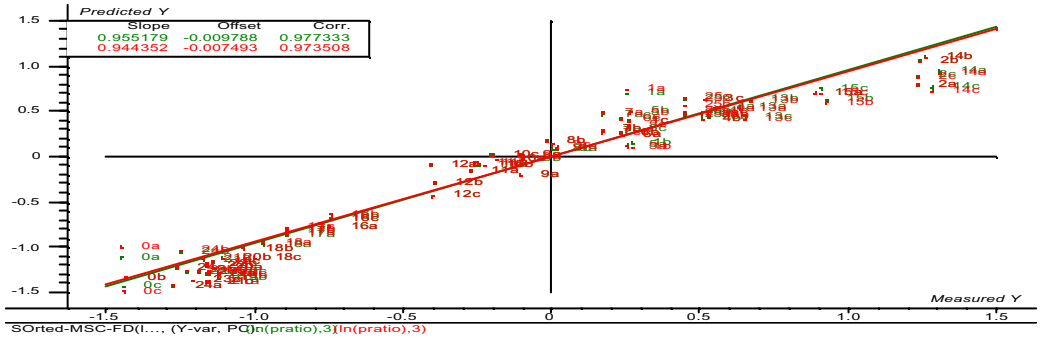
5.10 Monitoring the enzymatic interesterification using FT-NIR

A Bomem FT-NIR spectrometer (FTLA 2000-154, Bomem Inc.) was used. A sample-handling accessory for NIR analysis was a temperature-controllable multivial-holding block capable of 8-mm transparent glass vials with a volume of 1 mL.

The study was started with a margarine fat model without butterfat to simplify the system (palm stearin and coconut oil). The blends and interesterified fats samples in liquid form were measured by attenuated total reflectance (ATR) based FT-IR (spectra region: 1516-781 cm^{-1}) and transmission mode based FT-NIR (spectra region: 5369-4752 cm^{-1}) with temperature both controlled at 70°C. The samples in solid form were also measured by reflectance based FT-NIR (spectra region: 7037-6039 & 5995-5612 cm^{-1}) at room temperature. Calibrations of FT-IR and FT-NIR for conversion degrees (evaluated by triglyceride profile), solid fat contents (SFC), and dropping points (DP) of interesterified products were carried out by using partial least squares (PLS) regression. High correlations ($r > 0.96$) were obtained from cross validations of the data estimated by FT-IR, FT-NIR and above-mentioned conventional analytical methods, except for correlations ($r = 0.90$-$0,95$) between FT-IR and SFC profiles. Overall, FT-NIR spectroscopy coupled with transmission mode measured at 70 °C had the highest correlations. These sampling conditions are similar to real process conditions, indicating a big potential to implement as online control for monitoring enzymatic interesterification process.

Based on the work done using the margarine fat model, a model reaction system using butterfat and rapeseed oil (70/30, w/w) was used. Butterfat is much more complicated than other common fats in terms of fatty acid composition. We expect it could be more difficult to monitor the changes in the reaction system.

The best prediction was achieved at spectra range of 4513-5269 cm^{-1}. The correlations for calibration and validation models were only 0.88, 0.86 respectively, indicating the non-linear relationship between predicted and measured values. Therefore, pre-treatment of reference data was conducted by applying logarithm for the P ratio, an indicator of reaction degrees (Data range: -1.45~1.31).



An improved validation model (**Figure above**) was obtained with coefficient of 0.938 at spectra range of 4513-12019 cm^{-1}. By narrowing down the spectra range of 4513-5269 cm^{-1}, coefficients were 0.977 and 0.974 for calibration and validation models, respectively. The standard error for the validation model was 0.186.

For SFC, variations of SFC between the initial and final products when the reaction reached the equilibrium can be observed only for SFC measured at 5 °C (35.7-40.9). Therefore, SFC data at 5 °C were used for prediction. Relationships between different spectra range and effects of data treatment for SFC as references were listed in the **Table below**. It can be seen that correlations were improved by limiting the spectra range at 4513-5269 cm^{-1}.

	Calibration			Validation		
Spectra range (cm^{-1})	RMSEC	r	PLS factor	RMSEP	R	PLS factor
4513 – 12019	0.252	0.988	5	0.846	0.871	5
4513 – 5369	0.588	0.935	3	0.624	0.927	3
4513 – 5369 / Ln(SFC) values as reference	0.015	0.939	3	0.016	0.931	3

High correlations were observed between NIR spectra/Ln(P ratio) and NIR spectra/SFC at 5°C for using spectra range of 4513-5269 cm^{-1}. Overall, FT-NIR can be used for monitoring enzymatic interesterification process for butter-spread production as well. Short analytical time for FT-NIR (2.5min) makes it possible of using this technology for on-line control.

6 List of publications

6.1 Peer-reviewed papers

1. Torben H. Rønne, Charlotte Jacobsen and Xuebing Xu, Deodorization of lipase-interesterified butterfat and rapeseed oil blends in a pilot deodorizer, *Eur. J. Lipid Sci. Technol.* 108, 182-192, 2006.
2. Torben H. Rønne, Lars S. Pedersen, Xuebing Xu, Triglyceride selectivity of immobilized *Thermomyces lanuginosa* lipase in interesterification, *J. Am. Oil Chem. Soc*. 82, 737-743, 2005.
3. Tinghong Chang, Xuxin Lai, Hong Zhang, Ib Søndergaard, Xuebing Xu, Monitoring lipase-catalyzed interesterification for bulky fats modification with FT-IR/NIR spectroscopy, J. Agric. Food Chem. *53* (26) 9841 – 9847, 2005.
4. Torben H. Rønne, Tiankui Yang, Huiling Mu, Charlotte Jacobsen and Xuebing Xu, Enzymatic Interesterification of butterfat with rapeseed oil in a continuous packed bed reactor, *J. Agric. Food Chem*. 53, 5617-5624, 2005.

6.2 Papers in preparation

1. Jacobsen C. et al., Storage stability study of butter spreads produced from enzymatic interesterification
2. Zhang H., et al., Monitoring lipase-catalyzed interesterification for butterfat modification by Fourier transform infrared spectroscopy
3. Overgaard, J. et al., Nutritional effects of an interesterified butter product containing fish oil fed to healthy men in a single meal

4. Mu, H., et al., Nutritional effects of interesterified butter products during feeding in hamsters

6.3 Book chapter and proceedings papers

1. Xuebing Xu, Enzymatic processing of oils, fats, and other lipids: a brief progress update, in: *Proceedings of International Palm Oil Congress: Nutraceutical, Nutrition and Functional Foods*, MPOB, Kuala Lumpur 2005, pp. 38-46.
2. Xuebing Xu, Zheng Guo, Hong Zhang, Anders F. Vikbjerg, and Marianne L. Damstrup, Chemical and enzymatic interesterification in lipid modification, in: Modifying Lipids for Use in Food, ed. Frank Gunstone, Woodhead Press, Cambridge 2006 (in press).

6.4 Popular paper

- X. Xu, C. Jacobsen, H. Mu, J. Adler-Nissen, Forbedrede smørbare fedtprodukter via enzymatisk modifikation af smørfedt, *Mælkeritidende (17), 408-411, 2004.*

6.5 Conference presentations

1. Xuebing Xu, Tiankui Yang, Torben Rønne, Charlotte Jacobsen, and Huiling Mu, Enzymatic modification of butterfat: Effects of parameters and minor components on interesterification, oral presentation at the 95th AOCS Annual Meeting & Expo, May 9-12, 2004, Cincinnati, USA.
2. Xuebing Xu, Enzyme technology in lipid processing: an update, International Biotechnology conference, August 19-20, 2004, Shanghai, China.
3. Xuebing Xu, New Developments in Enzymatic Application in Oils and Fats, Plenary lecture at the Palm Oil International Congress (PIPOC 2005), September 25-29, 2005, Kuala Lumpur, Malaysia.
4. Torben H. Rønne, Tiankui Yang, Huiling Mu, Charlotte Jacobsen and Xuebing Xu, Optimization of Butterfat via enzymatic Interesterification with rapeseed oil, poster presentation at the Lipidforum seminar: Enzymes in Lipid Technology, February 26-28, 2005, Lyngby, Denmark.
5. Hong Zhang, Tinghong Chang, Huiling Mu, Xuebing Xu, Monitoring lipase-catalyzed interesterification for bulky fat modification and butter spread production with FT-IR/NIR spectroscopy, ISF meeting, October 1-4, 2006, Madrid, Spain.

7 Student projects

1. DuPont Gladys, Physical properties of products and evaluation of lipase-catalysed interesterification, Exchange master student project, 2003 (Socrates programme).
2. Torben Rønne, Enzyme-catalyzed interesterification of simple structured triglycerides: specificity characterization of Lipozyme TL IM, 30 ECTs, master final examination project, 2004.
3. Mette Behrmann & Anne Louise Krogh (20 points): Influence on organ fatty acid composition after feeding different butter products in hamsters, 2005.
4. Anne Julie Overgaard (60 point) Short- and long-term effects of Kærgården and a new interesterified butter product containing n-3 fatty acids (2005-2006)

8 Collaborations

1. Term meetings with Arla Foods were made every six months, one in Aarhus, one in Holsterbro, and three in Lyngby.
2. Pilot production of butter was conducted in Arla Foods at Holsterbro pilot plant with Henrik K. Frederiksen, Arla Foods.
3. Collaboration with Ib Søndegård, BioCentrum-DTU, on FTNIR uses for enzyme reaction monitoring.
4. Collaboration with Lars S. Pedersen, Novozymes A/S, was made concerning a student project (Torben Rønne) for Lipozyme TL IM specificity study during interesterification reaction.

Mejeriforeningen • Danish Dairy Board • Frederiks Allé 22 • DK-8000 Århus C
Telefon +45 8731 2000 • Fax +45 8731 2001 • ddb@mejeri.dk • www.mejeri.dk