Preparation, characterisation and application of naturally derived polar lipids through lipolysis

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Abstract

Monoglycerides are lipid based emulsifiers extensively used for their broad technical function in the food industry. Commercial monoglycerides are generally manufactured through chemical synthesis; however, lipolysis of triglycerides by lipase enzyme provides a biochemical pathway by which monoglycerides may be produced. This is particularly appealing for consumers for whom all natural and clean labelled food products are a particular driver. Accordingly, rather than replacing monoglycerides from formulations with other types of emulsifiers (and that may lack the requisite functionality), an alternative approach may be to develop a non-chemical and more natural pathway to produce the emulsifier, thereby allowing the particular monoglyceride functionality to be retained within products. Therefore, this study was conducted to investigate the feasibility of using lipase enzyme as a processing tool to synthesise polar lipids, namely monoglycerides, *in situ* of the manufacture of whippable food emulsions, such as cream and ice cream.

This concept idea was initially proven viable through interfacial tension (IFT) measurements obtained using a straight-forward surface characterisation technique. *R. miehei* lipase was found to competitively bind at the interface of vegetable oils-water and that the adsorbed protein (sodium caseinate and whey protein isolate) or surfactant layer (lecithin and Tween 80) did not act as a barrier to lipase adsorption at the oil-water interface. IFT measurements were also able to demonstrate the progressive accumulation of polar lipids at the oil-water interface arising from lipolysis, and were additionally used to indicate how thermal treatment of the enzyme could be used to terminate activity.

In considering how the requisite functionality could be achieved for whippable emulsion formulations, emulsion droplet size, type of emulsifiers used as well as lipase concentration were shown to be key variables by which the extent and rate of lipolysis could be manipulated and controlled. The results showed that formulation (emulsifier types and oil content) and processing conditions (Microfluidizer® pressure and number of passes) had significant effects on the emulsion droplet size.

As part of controlling the extent of lipolysis, the conditions by which the reaction could be terminated were investigated by measuring the viability of *R. miehei* lipase against thermal treatment. Results showed that the *R. miehei* lipase was thermostable up to temperatures of 70 °C. Above this temperature, substantial reduction of the residual activity occurred. However, even elevated temperature of between 90 and 100 ^{o}C did not immediately inactivate the lipase, with heating for ~ 2 min required before activity was no longer detected. In terms of emulsion stability, the palm oil emulsion tested in this study was found to be thermostable up to 100 ^{o}C, thus allowing development of a thermalisation step that was able to inactivate the enzyme without compromising the stability of the emulsion.

The shear stability analyses on lipolysed O/W emulsion showed the lipolysed emulsions were susceptible to shear-induced aggregation, and that the degree of aggregation could be manipulated as a consequence of controlling the extent of lipolysis through either enzyme concentration or holding time. The drastic increase in the viscosity curve between the non-lipolysed and lipolysed emulsion suggested that the shear–induced partial coalescence was primarily due to the lipolysis reaction and was not as a result of the high fat content (30 %). The findings elucidate the ability of the generated polar lipids in the emulsion to displace the

existing sodium caseinate adsorbed layer, thus compromising emulsion stability upon shearing.

The quantification of synthesised polar lipids from the triglyceride component of fat droplets by the lipolysis reaction showed a mixture of fatty acids, di- and mono-glycerides being produced. Palmitic acid was observed to be the main liberated fatty acids. While, monoolein and monopalmitin were the most prominent monoglycerides, with measured concentrations of 3.755 ± 0.895 and 1.660 ± 0.657 mg / g fat respectively after 15 min with lipase concentration of 50 mg /g fat. The relative concentration of polar lipids produced was found to be dependent on the lipase concentration as well as time of lipolysis. Furthermore, up to 30 min of lipolysis (concentration 50 mg /g fat) were seen to have no observable effect on the droplet size distribution of the emulsion suggesting that quiescently stable emulsions could be produced. The results show the importance of controlling reaction conditions (both enzyme concentration and reaction time) in order to provide requisite functionality without excessively destabilising emulsions such that droplet structuring can occur under quiescent conditions.

The generation of monoglycerides at *quantum satis* levels able to impart critical functionality was demonstrated in whipped cream and ice cream. The addition of *R. miehei* lipase at very low concentration of 5 mg /g fat was able to produce a rigid and stable whipped cream with overrun exceeding 100 %. However, good stability of the whipped cream over time was achievable with concentration above 10 mg /g fat. Similarly, ice cream made with the addition of 5 mg /g fat exhibited good melt stability and firmness. The findings proved the feasibility of *in situ* production of polar lipids, namely monoglycerides and fatty acids, in replicating the functionality imparted by commercial monoglycerides in whippable emulsions.

Thus, the findings in this thesis offer an alternative biochemical pathway for the generation of polar lipids to that of commercially available monoglycerides, which are currently produced synthetically. The potential for using this approach as part of the processing step for food emulsion manufacture has also been demonstrated. The concept can be tailored for various emulsion based food products.

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List of Abbreviations

^{o}C	degree celcius
cont.	continue
D(3,2)	particle volume/surface mean (also called the Sauter mean)
D(4,3)	particle mean diameter over volume
FAME	fatty acid methyl ester
FFA_{max}	extent of lipolysis
GC	gas chromatography
h	hour
HLB	Hydrophilic-lipophilic balance
IFT	Interfacial tension
k_{exp}	deactivation rate constant
kg	kilogram
K_m	Michaelis constant
L	Litre
m	metre
mg	milligram
min	minute
ml	millilitre
µm	micrometre
µmol	micromole
MPa	megapascal
N	Newton
NaOH	sodium hydroxide
η_D/η_C	viscosity ratio dispersed over continuous phase
nm	nanometer
O/W	oil-in-water
Pa.s	Pascal second
psi	pounds per square inch
PUFA	polyunsaturated fatty acids
QDs	Quantum dots
rpm	revolution per minute
s	second
[S]	substrate concentration
SMP	skim milk powder
sn	stereospecific numbering
sp.	species
$t_{1/2}$	half-life values
t_c	aggregation time
TLC	thin layer chromatography
U	enzyme activity unit
US FDA	United State of America Food and Drug Administration
UV	Ultra violet
V	velocity
v_o	initial lipolysis velocity
wt %	weight percent
w/w	weight per weight

List of Equations