Substrate specificities of baking lipases for use in fine bakery goods

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DISSERTATION

von

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Preliminary releases

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Peer-review publications

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Further publications

Stemler CD, Scherf KA. Sensorische Überprüfung der Eignung von Backlipasen zur Nutzung in Feinen Backwaren. Getreide, Mehl und Brot 2022; 3: 18-21

Oral presentations

Stemler CD, Scherf KA. Fehlaromenbildung in Feinen Backwaren durch Backlipasen mit unterschiedlicher Substratspezifität (Off-flavor formation in fine bakery goods caused by baking lipases with different substrate specificities). *Arbeitstagung des Regionalverbandes Südwest der Lebensmittelchemischen Gesellschaft* (Workshop of the Regional Association Southwest within the Society of Food Chemistry); Online, 22.-23.03.2021

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Poster presentations

Stemler CD, Scherf KA. Freie Fettsäuren als Auslöser für Backlipase-induzierte Fehlaromen in Feinen Backwaren. (Free fatty acids as cause of baking lipase-induced off-flavors in fine bakery goods). *49. Deutscher Lebensmittelchemikertag* (49. German Food Chemists' Day); Online, 30.08.-01.09.2021

Stemler CD, Scherf KA. Einfluss von Backlipasen auf die Produkteigenschaften Feiner Backwaren. *50. Deutscher Lebensmittelchemikertag* (50. German Food Chemists' Day); Hamburg, 19.-21.09.2022

Stemler CD, Cutignano A, Scherf KA. Lipidomic insights into the textural impact of baking lipases on fine bakery goods. 36th EFFoST International Conference 2022; Dublin, IE, 07.-09.11.2022

Contents

Abbreviations

3 AFC 3 Alternative Forced Choice

AGC automatic gain control

ANOVA analysis of variance

Cer ceramide

Chol cholesterol

CL cardiolipin

DATEM diacetyl tartaric acid ester of mono and diglycerides (E472e)

DG diacylglycerol

DGDG digalactosyldiacylglycerol

DGMG digalactosylmonoacylglycerol

DOPC 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine (PC (18:1/18:1))

EL egg yolk lecithin

FA fatty acid

FFA free fatty acid

GA gum arabic

GC gas chromatography

HESI heated electrospray ionization

HLB hydrophilic-lipophilic balance

LC liquid chromatography

LVE linear viscoelastic region

MG monoacylglycerol

MGDG monogalactosyldiacylglycerol

MGMG monogalactosylmonoacylglycerol

MS mass spectrometry

MS/MS tandem mass spectrometry

MTBE 2-methoxy-2-methylpropane (methyl *tert*-butyl ether)

NAPE *N*-acyl phosphatidylethanolamine

NALPE *N*-acyl lysophosphatidylethanolamine

PA phosphatidic acid
- **LPA** lysophosphatidic acid

BisMePA bis-methyl glycerophosphate
- **BisMeLPA** bis-methyl lysoglycerophosphate

PC glycerophosphocholine
- **LPC** lysoglycerophosphocholine

PE glycerophosphoethanolamine
- **LPE** lysoglycerophosphoethanolamine
- **dMePE** dimethyl phosphatidylethanolamine
- **LdMePE** lysodimethyl phosphatidylethanolamine

PEt glycerophosphoethanol
- **LPEt** lysoglycerophosphoethanol

PG glycerophosphoglycerol
- **LPG** lysoglycerophosphoglycerol

PI glycerophosphoinositol
- **LPI** lysoglycerophosphoinositol
- **PIP2** glycerophosphoinositol (4,5)-bisphosphate
- **LPIP2** lysoglycerophosphoinositol (4,5)-bisphosphate
- **PIP3** glycerophosphoinositol (3,4,5)-trisphosphate
- **LPIP3** lysoglycerophosphoinositol (3,4,5)-trisphosphate

PS glycerophosphoserine
- **LPS** lysoglycerophosphoserine

PCA Principal Component Analysis

SM sphingomyelin

So sphingosine

SPE solid phase extraction

SPH sphingoid bases

SSL sodium stearoyl lactylate (E481)

TG triacylglycerol
- **LMW-TG** low molecular weight TG
- **MMW-TG** medium molecular weight TG
- **HMW-TG** high molecular weight TG

TNB 2-nitro-5-thiobenzoate

UHPLC ultra high-performance liquid chromatography

Zusammenfassung

Lipasen werden seit Jahrzehnten als *clean-label* Alternativen für herkömmliche Emulgatoren zur Verbesserung der Backeigenschaften von Brot eingesetzt. Für Feine Backwaren gibt es jedoch bisher nur wenige Anwendungen.

Bei der Reaktion von Lipasen mit Butter, einem üblicherweise in Feinen Backwaren eingesetzten Fett, werden geruchsintensive kurzkettige Fettsäuren freigesetzt. Um deren Freisetzung und damit die Bildung von ranzigen Fehlaromen zu begrenzen, werden Lipasen mit geeigneten Fettsäuresubstratspezifitäten benötigt. Dazu wurden 17 Lipasen mithilfe des *p*-Nitrophenyl-Assays auf die Fettsäuren hin untersucht, die sie bevorzugt freisetzen. Das aus dem Assay resultierende, breite Spektrum an Fettsäuresubstratspezifitäten konnte bei der Reaktion mit Backfetten in neu entwickelten Modellemulsionen jedoch nicht bestätigt werden. Stattdessen war die Struktur der Grenzfläche entscheidend für die Freisetzung von Fettsäuren. Sieben Lipasen wurden aufgrund ihrer Reaktionsmuster im *p*-Nitrophenyl-Assay und ergänzenden, sensorischen Experimenten mit den Modellemulsionen für weitere Versuche ausgewählt.

Die Einsatzmöglichkeiten von Lipasen in Feinen Backwaren mit unterschiedlichen Rezepturen wurden am Beispiel eines eifreien Rührkuchens, eines traditionellen Sandkuchens und einer hefebasierten Brioche untersucht. Die Lipasen beeinflussten die Eigenschaften von Kuchenteigen und Backwaren. Das Ausmaß der Veränderung hing dabei sowohl von der Rezeptur, als auch von der Lipase ab. Enthielt die Rezeptur intrinsische, oberflächenaktive Moleküle wie polare Ei-Lipide in Sandkuchen, waren die Effekte der Lipasen schwächer als in der Rezeptur ohne Ei. In Brioche wurden nur geringe bis keine Verbesserungen der Backqualität erreicht. Die Unterschiede der Lipasen wurden auf ihre Reaktionsmuster zurückgeführt.

Diese Hypothese unterschiedlicher Reaktionsmuster wurde durch Lipidomics-Analysen von Lipase-behandelten Teig- und Kuchenproben weiter untersucht. Dazu wurde eine Flüssigkeitschromatographie-Tandem-Massenspektrometrie Methode genutzt. Durch den Vergleich der Substratspezifitätsmuster der Lipasen mit den Ergebnissen zu ihren Auswirkungen auf die Backqualität wurden mögliche Schlüsselverbindungen für die Backqualität ermittelt. Bei Rührkuchen ist die Hydrolyse von Glyceroglycolipiden entscheidend, während in Sandkuchen Lysoglycerophospholipide mit Effekten auf die Textur in Verbindung gebracht wurden. In Brioche wurde die Reaktion der Lipasen nicht, wie ursprünglich vermutet, gehemmt, aber die Art der freigesetzten Lipide hatte keinen Einfluss auf die Backqualität.

Zur Vorhersage der Eignung einer Lipase für den Einsatz in Feinen Backwaren wird ein Assay benötigt. Basierend auf den Ergebnissen der Lipidomics-Analysen ist die Substratspezifität von Lipasen entscheidend für ihre Wirkung. Daher wurde ein fluorimetrischer Assay entwickelt, um die Reaktion von Lipasen mit verschiedenen Substraten zu analysieren. Die mithilfe des Assays ermittelten Reaktionsmuster stimmten jedoch nicht mit den Reaktionsmustern aus Kuchen überein. Ähnlich wie bei den Ergebnissen für die Fettsäurensubstratspezifität war die Substratspezifität ebenfalls von der Verfügbarkeit von Substraten für die Reaktion abhängig.

Diese Arbeit eröffnet neue Einblicke in die Reaktion von Lipasen und Mechanismen der (Fettsäuren)substratspezifität. Außerdem zeigte sie das Potenzial von Lipasen als Backhilfsmittel in Feinen Backwaren und ebnete den Weg für weitere zukünftige Anwendungen von Lipasen.

Summary

Lipases have been used for decades as a clean-label alternative to conventional emulsifiers to improve the baking properties of bread. However, there are only scarce applications in fine bakery goods so far.

When lipases react with butter, a fat commonly used in fine bakery goods, odour-intensive short-chain fatty acids (FA) are released. To limit their release and thus the formation of rancid off-flavours, lipases with suitable FA substrate specificities are needed. Therefore, 17 lipases were screened concerning the FA they preferentially release by the use of the *p*-nitrophenyl assay. However, the broad spectrum of FA substrate specificities resulting from the assay could not be confirmed in the reaction with baking fats in newly developed model emulsions. Instead, the structure of the interface was decisive for the release of FA. Seven lipases were selected for further experiments based on their reaction patterns in the *p*-nitrophenyl assay and supplementary sensory experiments with the model emulsions.

The potential uses of lipases in fine bakery goods with different recipes were investigated using an eggless basic cake, a traditional pound cake and yeast-based brioche as examples for fine bakery goods. Lipases affected the properties of cake doughs or batters and the baked products. The extent of the change depended on both the recipe and the lipase. If the recipe contained intrinsic surface-active molecules like polar lipids from egg in pound cake, the effects caused by the lipases were diminished compared to the recipe without egg. In the yeast-based brioche, little to no improvements of baking quality were achieved. Differences between the lipases were attributed to their reaction patterns.

The hypothesis of different reaction patterns was further investigated by lipidomic analyses of lipase-treated cake samples before and after baking. A liquid chromatography tandem mass spectrometry (LC-MS/MS) method was used for this purpose. By comparing the substrate specificity patterns of lipases with previous results for the corresponding effects on baking quality, possible key compounds for quality improvements were identified. In basic cake, the hydrolysis of glyceroglycolipids is decisive, while in pound cake, lysoglycerophospholipids were linked to textural impacts. In brioche, lipase reactions were not inhibited as originally suspected, but the type of released lipids did not affect the baking quality.

An assay to predict the suitability of a lipase for the application in fine bakery goods is needed. Based on the results from the lipidomic analysis, the substrate specificity of lipases is crucial for their impact. A fluorimetric assay was thus developed to analyse lipase reactions towards different substrates. The patterns determined in the assay did not match the ones previously analysed in cakes. In accordance with the results for the FA substrate specificity, the substrate specificity was also dependent on the availability of substrates for the reaction.

This work provides new insights to lipase reactions and mechanisms defining FA substrate and substrate specificities. Besides, it also demonstrated the potential of lipases as baking improvers in fine bakery goods and paved the way for further future applications of lipases.

1. Introduction

1.1. Fine bakery goods

1.1.1. Definition and legal basis

The term “fine bakery goods”, corresponding to the German term “Feine Backwaren”, was defined by the Deutsche Lebensmittelbuch-Kommission as follows:

> “Fine bakery goods are made from doughs and batters by baking [...] or other processes. The doughs or batters are made using cereals and/or cereal products, starches, fats, sugars, etc. Fine bakery goods are distinguished from bread and small bakery goods by the fact that their content of fat and/or sugars is more than 10 parts per 90 parts of cereals and/or cereal products and/or starches.” (translated from *Leitsätze für Feine Backwaren* [1])

They comprise, e.g., different cakes, cookies, crackers and gingerbread. Within this work, cakes are used as examples of fine bakery goods.

The word “cake” lacks a clear definition. It is derived from the old Norse word “kaka” and was first used to describe sweetened bread-like products [2]. Nowadays, cakes are bakery goods consisting of flour, sugar, eggs, fat or oil and leavening agents. However, different recipes with varying ingredients for cakes are used around the world [3]. A range of categories for the wide range of cakes were therefore suggested. Cakes are commonly divided into batter-type and foam-type cakes. Batter-type cakes contain significant levels of fat and their batters are emulsion-like. Foam-type consist mainly of flour, sugar and eggs but contain only small levels of fat. They have foam-like structures [4]. Due to their low content of fat, foam-type cakes were not considered for this work. Besides batter-type and foam-type cakes, the German “Kuchen” also comprises yeast-based cakes like brioche and *Bienenstich*. Yeast-based cakes are made of doughs, that is, mixtures of mainly flour and liquids which can be kneaded. The more liquid-like mixtures of cake ingredients for, e.g., batter-type cakes, are called “batters”.

1.1.2. Preparation of fine bakery goods

The preparation of fine bakery goods depends on the product. Yeast-based cakes, e.g., are prepared like breads following a sponge-and-dough system: parts of the flour, liquids, yeast and yeast substrates (e.g., sugars) are formed to a loose dough, which is called “sponge”. This sponge is then fermented. During fermentation, the yeast causes the release of CO_2 and leavens the product. Afterwards, the remaining ingredients are added. Further proofing of the dough is done before baking [5].

The classic procedure for the preparation of batter-type cakes is multistage mixing with three steps (Figure 1) [3, 4, 5, 6]:

(i) creaming of fat and sugar

(ii) incorporation of liquids

(iii) incorporation of flour

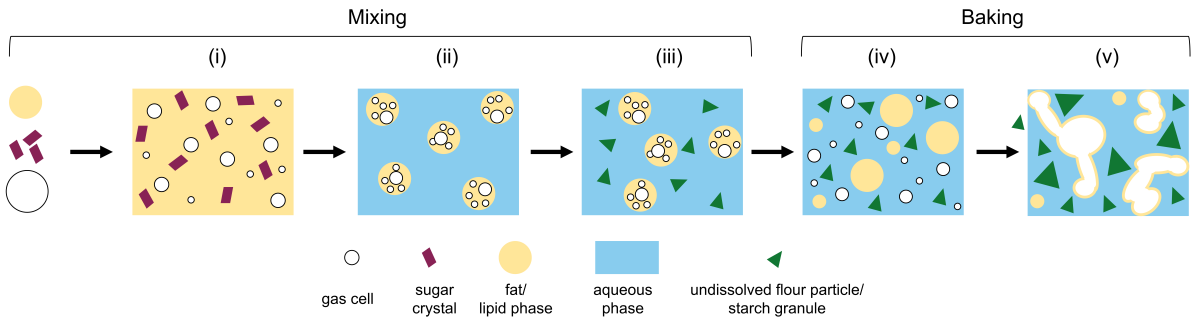


Figure 1: Preparation of batter-type cakes. Figure based on figures by Wilderjans et al. [3] and Lambrecht et al. [6].

During the first step, air is incorporated in the batter while sugar and fat are homogenised. In the second step, a water component is added. Besides water or milk, this component can also be represented by eggs. Sugar dissolves in the water phase, while the fat forms an oil-in-water emulsion. The air is thereby still contained in the fat phase. Finally (step iii), flour is mixed in. The resultant batter is a multiphase system. Fat containing air cells is emulsified in water, in which sugar and proteins are dissolved and flour particles suspended. A high viscosity of the batter helps in maintaining the system and prevents the phases from separating. The retention of incorporated air in the mixture is crucial, as during leavening and baking, no further air cells can be formed. Generated gas can only be incorporated in already existing cells. Contrary to the preparation of bread, the development of a continuous gluten network and concomitant firm texture is detrimental for batter-type cakes. Their texture is expected to be softer than the one of bread. After addition of the flour, the batters are therefore only briefly mixed.

To sum up the main aims of batter preparation, all ingredients in the batter are to be homogenously incorporated and hydrated while gluten network formation is inhibited and a maximum amount of air is incorporated in the batter [2].

During baking, the batter forms into the final product. With rising temperatures during the early baking stage, the fat melts and releases the gas cells into the aqueous phase (Figure 1, step iv). There, the gas cells can be stabilized by egg constituents and expand further. Cells can be lost due to bubble rise, disproportionation or coalescence of bubbles. During the late baking phase, the structure sets by starch swelling and protein denaturation or coagulation. This effect has been compared to bricks and mortar, with swollen starch granules as building bricks and protein as mortar to hold them together [3]. Remaining gas cells coalesce and build a continuous gas system (Figure 1, step v) [6]. During cooling, the expanded gases contract or condense. If the structural strength of cakes is not sufficient, they collapse [3]. After baking, the cake crumb is subject to further changes during storage. Water migration and starch retrogradation alter its texture and thereby also affect its quality [7]. Each ingredient of a batter or a dough contributes to the resulting cake quality [8].

1.1.3. Ingredient functionality

The main functions of typical ingredients of batter-type cakes (flour, sugar, eggs, fat and baking powder) are listed in Table 1.

Table 1: Ingredient functionality in batter-type cakes [2, 3, 5, 6, 9, 10, 11, 12, 13, 14, 15, 16].

Ingredient		Effect on baking quality	Mode of action
Flour	Starch	◦ texture, especially crumb structure and firmness ◦ volume	◦ increase batter viscosity and stabilisation of the batter emulsion resulting in improved entrapment of air in the batter ◦ control of oven rise and structure setting ◦ swelling and amylopectin retrogradation
	Protein	◦ texture ◦ volume	◦ building of a cross-linked protein network with egg proteins crucial for structure setting, resistance to collapse and gas retention during baking ◦ increase of batter viscosity and thereby improved gas retention
Sugar		◦ texture, especially softness ◦ appearance ◦ flavour ◦ shelf-life ◦ volume	◦ influence on structure setting temperature and thereby time for oven rise ◦ increase of batter viscosity leading to increased gas retention ◦ inhibition of gluten network formation by water binding ◦ caramelisation and Maillard reaction ◦ sweet taste ◦ moisture retention post baking ◦ influence on amylose retrogradation
Eggs	White	◦ texture, especially softness ◦ moisture	◦ increase of batter viscosity, enhanced air incorporation and gas retention ◦ protein network formation ◦ high water content
	Yolk	◦ volume ◦ texture, especially crumb structure ◦ appearance	◦ Maillard reaction ◦ surface-active properties of lipoproteins lead to enhanced gas cell stability and more even bubble size distribution ◦ protein network formation with gluten proteins
Fat		◦ texture, especially softness ◦ shelf-life	◦ inhibition of gluten network formation by coating of proteins ◦ air entrapment, hindrance of gas bubble coalescence and increased gas retention during baking by stabilisation of gas bubbles ◦ stabilisation of the gluten-starch matrix ◦ effect on amylopectin retrogradation
Baking powder		◦ volume ◦ texture, especially crumb structure and softness ◦ appearance	◦ release of CO_2 ◦ inhibition of gluten network formation by lowering the pH of the batter

Concerning the flour, both starch and proteins exhibit effects on the final cake quality. They are linked to final product volume and crumb texture. The added sugar affects both the sensory quality of the cake like, e.g., its sweet taste and its texture by water binding leading to increased starch gelatinisation temperatures. Both egg white and egg yolk are crucial for air incorporation and gas retention during baking. Fats typically used in baking are butter, margarine, cocoa butter, shortening and lard. Their primary role are sensory attributes like the mouthfeel of cakes, but they also impact the entrapment of air during mixing. The term “baking powder” describes commercially available mixtures of leavening agents consisting of baking soda ($NaHCO_3$), acid salts and inert ingredients like starch. In the presence of moisture and heat, baking soda and acids react and release CO_2. They thereby add to the cake volume [2, 3, 5, 6, 9, 10, 11, 12, 13, 14, 15, 16].

As for bread, besides the essential ingredients, further substances can be added during cake manufacturing. These further substances either modify the flavour of the product, such as cacao, or act as functional ingredients, that is, baking improvers. Baking improvers can be, e.g., surface-active molecules or different enzymes like amylases or lipases.

1.2. Lipase substrate specificity

1.2.1. Lipases

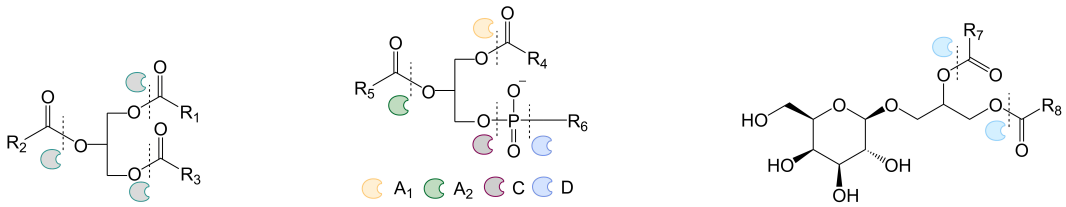
Lipases are naturally occurring enzymes and are part of the metabolism in animals, plants and microorganisms [17]. They are also the third largest group of commercial enzymes following peptidases and carbohydrases and are applied in different industrial processes such as for the production of polymers, detergents and food [18].

Lipases are enzymes, which belong to the class of hydrolases acting on ester bonds. More precisely, they are classified as carboxylic ester hydrolases [19]. In terms of their substrates, the enzyme category “lipases” was stated to be ambiguous [12]. It can refer exclusively to triacylglycerol (TG) lipases (EC 3.1.1.3), which act on the ester bonds of TG and their corresponding hydrolysis products diacylglycerols (DG) and monoacylglycerols (MG) (Figure 2). Besides TG lipases, the term “lipases” can also include the groups of phospholipases (EC 3.1.1.32, EC 3.1.1.4, EC 3.1.4.3 and EC 3.1.4.4) and galactolipases (EC 3.1.1.26) (Figure 2).

Phospholipases catalyse reactions of one or more (phosphodiester) bonds of glycerophospholipids. According to the specific (phosphodiester) bond they can cleave, they are distributed into the four categories A_1, A_2, C and D (Figure 2). In addition to these four categories, lysophospholipases (EC 3.1.1.5) can be included in the group of phospholipases [20, 21].

Galactolipases react with the ester bonds of glyceroglycolipids (Figure 2).

Within this work, a definition based on the suggestions by Gerits et al. [22] and Melis et al. [12] for lipases will be used: Lipases are lipid-degrading enzymes acting on the (phosphodiester) bonds of glycero(phospho/glyco)lipids. This definition comprises all previously mentioned categories.



(a) Triacylglycerol lipase (b) Phospholipases (c) Galactolipase

Figure 2: Types of lipases. R_1 - R_8 stand for any fatty acid residues.

Although lipases differ in terms of their preferred substrates, all lipases have several common features. The first is a conserved alpha/beta-hydrolase fold as their three-dimensional structure [17]. A second feature are disulphide bridges for enzyme stability and support of the catalytic activity [17]. The catalytic activity is exerted by a catalytic triad of amino acids at the active site of all lipases. The three amino acids of the catalytic triad are serine, histidine and aspartic or glutamic acid. Besides the catalytic triad, the active site is also characterised by the occurrence of an “oxyanion hole”, which is a mechanism to stabilize the high-energy oxyanion intermediate formed during the catalysis reaction [23]. In many lipases, the active site is covered by a lid composed of one or more α-helices. This lid is mobile and, in presence of a lipid-water-interface, uncovers the active site and grants access of substrates to the enzyme. This phenomenon is called “interfacial activation” of lipases [17, 24].

In their way of action, lipases resemble esterases. Different suggestions for a clear differentiation between both groups were presented, among which are the types of FA the enzymes interact with, the presence of a lid in the enzyme and the kinetics towards different substrates [17, 22, 23]. However, none of the suggested criteria applied for all enzymes which are typically referred to as either lipases or esterases. Special care therefore needs to be taken when differentiating lipases from esterases and all aforementioned criteria should be considered.

1.2.2. Lipase reactions in aqueous media

The reactions which lipases catalyse depend on the surrounding media. In organic media, they catalyse, e.g., alcoholysis, acidolysis, ester synthesis or transesterification reactions [25, 26]. In aqueous media, lipases lead to the hydrolysis of ester bonds. The hydrolysis reaction needs an aqueous phase, but the presence of water is not sufficient. Lipases exert only low catalytic activity towards molecularly dissolved substrates [27] . They act preferably at lipid-water-interfaces due to the interfacial activation described previously.

The mechanism of a lipase-catalysed hydrolysis is shown in Figure 3. It consists of several steps including the catalytic triad of serine, histidine and aspartic or glutamic acid. First, a proton is transferred from serine via histidine to the carboxyl group of the acid. This leads to the activation of serine, which consequently acts as a nucleophile by attacking the carbonyl group of the substrate. The resulting tetrahedral oxyanion reacts to form an acyl enzyme intermediate. During the next step of the hydrolysis, a nucleophile like water attacks the alkoxycarbonyl group of the acyl enzyme intermediate. A second tetrahedral oxyanion including the acylated serine and the nucleophile is formed. The instability of the oxyanion then leads to the release of the hydrolysed product and the regeneration of the catalytic site [17, 24].

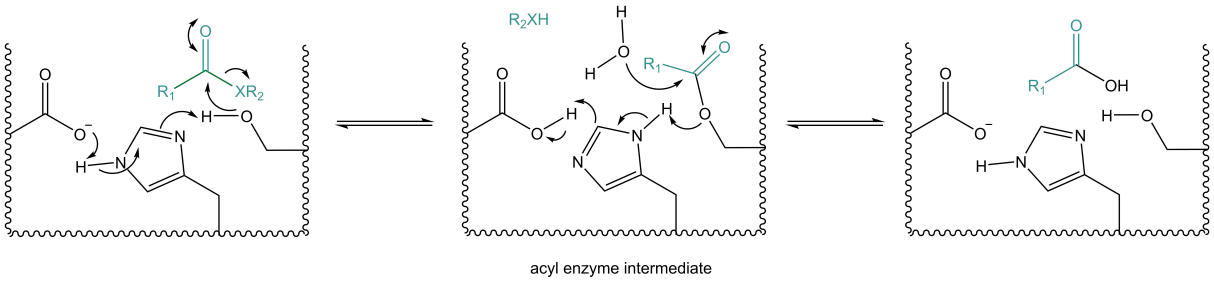


Figure 3: Mechanism of lipase catalysis (figure modified from Reis et al. [24]). R_1 and R_2 stand for any organic residues.

1.2.3. Lipase substrate specificity and selectivity

In the literature, both the terms specificity and selectivity are used to describe the preference of lipases for certain reaction conditions [23]. Within this work, this property of lipases will be referred to solely as “specificity”.

The term “lipase substrate specificity” was first defined by Desnuelle and Savary in 1963 [28]. They described it as “the influence exerted on lipase activity by the physical state and chemical nature of the substrate”. This means that the activity of a lipase towards different substrates can differ depending on the substrate. By “physical state”, they understood the influence of interfaces on the activity. However, this influence can be explained by the previously mentioned interfacial activation and applies to all lipases with a lid in the same way. The causes for different activity rates based on the chemical nature of the substrate were further analysed by Jensen et al. in 1983 [29]. They stated that the specificity of lipases is controlled by molecular properties of the enzyme, the structure of the substrate and factors affecting the binding of enzyme and substrate. According to Jensen et al., different types of specificity can be distinguished: substrate specificity based on the alcohol moiety, FA substrate specificity, regiospecificity, stereospecificity and combinations thereof. Later, chemospecificity was added to the list [23].

The substrate specificity based on the alcohol moiety is similar to the different categories (TG lipases, phospholipases and glycolipases) of lipases as explained in section 1.2.1. However, lipases are variable enzymes and act on a wide range of substrates [22]. A TG lipase can therefore also hydrolyse ester bonds in glycerophospholipids and *vice versa*. The specific activities towards substrates with different alcohol moieties differ according to the preferred substrates of the lipases.

The FA substrate specificity describes the preference of lipases for specific FA depending on chain length and degree of unsaturation. This specificity is controlled by the shape of the lipase binding site and the nature of the amino acid sequences at the binding site [23]. The FA substrate specificities of lipases can be modified by altering the amino acid sequence of the protein causing, e.g., a steric hindrance for long-chain FA. This approach is commonly applied and has been used, e.g., for the design of a lipase for cheese making [30]. However, the design of tailored lipases is expensive and time-consuming.

The regiospecificity of lipases has also been referred to as positional specificity. Some lipases react specifically with 1 or 2 chemical groups on the same substrate molecule [23]. TG lipases, e.g., can be *sn* 1,3-specific and only interact with the outer two FA residues of a TG

molecule.

Stereospecificity and enantiospecificity of lipases lead to the preferred reaction with one stereoisomer or one enantiomer compared to other isomers.

If a lipase is chemospecific, its reaction rates differ towards different esters, e.g., alcohol and thiol esters [23].

The substrate specificities of lipases are decisive factors for their industrial applications. Concerning the production of pharmaceuticals with lipases, their enantioselectivity is crucial. Often only one enantiomer leads to a therapeutic effect while the other enantiomer may be inactive or even detrimental [31]. In the production of foodstuff, lipases can affect the flavour of the resulting product. Therefore, their FA substrate specificity is important: while short-chain FA like butyric acid cause off-flavours at high concentrations, unsaturated long-chain FA are prone to oxidation and can also lead to undesired volatile compounds. Depending on the desired flavour, lipases with matching FA substrate specificities are therefore applied or newly designed, as done for the manufacturing of cheese [30]. For baking purposes, different effects on the baking quality of breads depending on the alcohol moiety substrate specificity of lipases were shown by Melis et al. [32]. Both the FA substrate specificity and the alcohol moiety substrate specificity are therefore of special interest for the application of lipases in fine bakery goods. To ease the readability, the alcohol moiety substrate specificity will be referred to as substrate specificity in the following.

1.3. Use of surfactants in bakery goods

1.3.1. Surface-active molecules

Surface-active molecules or surfactants are substances with emulsifying properties and therefore also referred to as emulsifiers or emulsifying agents. In food applications, surfactants may also include compounds with further functions such as interactions with proteins or starch [33].

Emulsifiers are characterised by their amphiphilic nature. They are composed of polar and non-polar regions and can therefore act as coupling agents between polar and non-polar phases like, e.g., in oil-in-water emulsions. Mostly, long-chain FA residues act as non-polar regions while the polar parts consist of, e.g., hydroxyl groups, amino groups, carboxylic acids like tartaric acid or phosphoric acids [7]. An example of an emulsifier is shown in Figure 4.

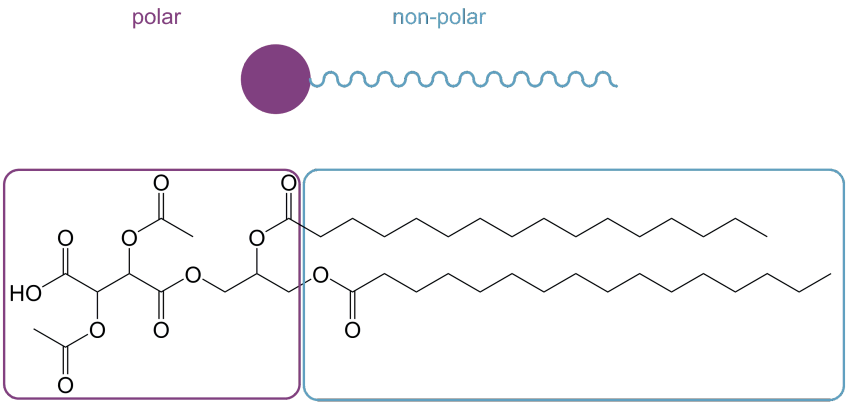


Figure 4: Diacetyl tartaric acid ester of 1,2-dihexadecanoyl-*sn*-glycerol (possible structure of DATEM) as an example of surface-active molecules.

Emulsifiers can be classified according to several categories [7, 34]: Concerning their charge, they can be either anionic, cationic, amphoteric or non-ionic. A common way of describing emulsifiers is their hydrophilic-lipophilic balance (HLB). The HLB values range from 1 to 20 and increase with increasing hydrophilic properties of the molecules. Therefore, emulsifiers with low HLB (2-8) are soluble in oil while emulsifiers with high HLB (14-18) readily dissolve in water [7]. Besides their charge, the HLB and concomitant solubility, emulsifiers are commonly classified based on their functional groups, e.g., the acids in the polar region and their origin (synthetic or natural).

Surfactants enhance the formation and stabilisation of emulsions. Phase separation of emulsions can occur due to various mechanisms such as creaming, coalescence of the dispersed phase or Ostwald ripening. Ostwald ripening describes the process of disproportionation when larger particles grow at the expense of smaller ones [7]. Surfactants suppress these mechanisms via several modes of action: they reduce the surface tension and thereby the work needed to enlarge the contact area between two phases, they can lead to the electric repulsion of droplets due to their charge and they can promote the hydration of droplets or physically prevent the contact of droplets by steric hindrance [7].

Within aqueous media, surfactants arrange in mesophases. Four different types of mesophases depending on the shape of the surfactant were identified and characterised by Krog and coworkers (Figure 5) : cone-shaped surfactants organize themselves in hexagonal I (polar regions inside) or cubic mesophases. Cylindrical surfactants prefer lamellar arrangements similar to biological membranes. Surfactants with an inverted cone shape form hexagonal II mesophases (polar regions outside) [35]. Examples of surfactants preferring the mesophases are sodium salts of FA for hexagonal I, MG and DG for cubic, lecithin and diacetyl tartaric acid ester of MG and DG (DATEM, Figure 4) for lamellar and sodium stearoyl lactylate (SSL) for hexagonal II [36].

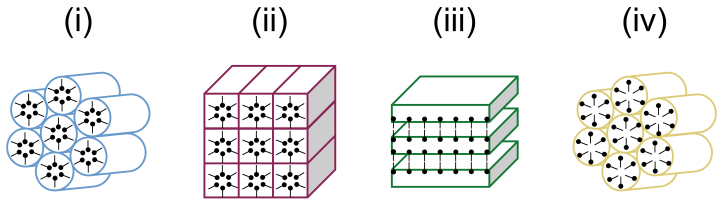


Figure 5: Mesophases built by surfactants in aqueous media. (i) hexagonal II (ii) cubic (iii) lamellar and (iv) hexagonal I. Figure based on a concept by Melis et al. [12].

1.3.2. Commercial surfactants

Commercial surfactants used in the food industry were originally naturally occurring substances like gums, polysaccharides and lecithin [7]. Later, synthetic emulsifiers with improved effects were applied. The first reported use of synthetic emulsifiers was the application of MG and DG in shortening in 1921, more than one hundred years ago [35]. MG and DG as well as their corresponding esters are still widely applied in foodstuff [7].

Within the European Union, the use of surfactants as food additives is regulated by the Regulation (EC) No 1333/2008 [37]. Before usage, surfactants need to be authorised. Authorised surfactants are listed in a Community list and are assigned a standard code (“E number”). Ex-

amples of authorised and commonly applied surfactants in bakery are DATEM (E472e), SSL (E481) and lecithin (E322). All three range among the most used surfactants in bakery goods [15].

Commercial surfactants are available as beads, powders or liquids [7]. In bread, common dosages for surfactants are 0.3% to 1.0% [38].

During the last decades, a trend towards the reduction of commercial surfactants in foodstuff has emerged. Common reasons are their comparably high costs and the consumers' wish for "clean label" products [22]. Additionally, commercial surfactants were recently shown to alter human gut microbiota [39]. However, commercial surfactants can improve the quality of bakery goods in various ways as outlined in the following section.

1.3.3. Functionality of surfactants in baking

The functionality of surfactants in baking relies on four different mechanisms.

First, the surfactants act as emulsifiers. They aid in the homogenous incorporation of all ingredients in the dough or batter [36].

Second, by interacting with lipids, they participate in the formation of joint micelles and ensure a high elasticity of the films which surround gas cells. They thereby enhance the formation and stabilisation of gas cells [40]. The promotion of lamellar mesophases was found to be favourable for gas cell stability [36]. The stabilisation of gas bubbles is especially important in cake formulations with liquid fats which cannot retain gas cells [7].

Third, surfactants act as dough strengtheners by interacting with the side chains of amino acids in gluten. They decrease the electrostatic repulsion of gluten proteins and therefore enable a stronger gluten aggregation [41]. A correlation between gluten aggregation and bread baking quality in terms of loaf volumes was recently confirmed in a study by Schopf & Scherf [42].

Fourth, the hydrophobic parts of surfactants fit into the inside of the hydrophobic helical structures of amylopectin and amylose. The resulting complexes lead to changes in starch swelling, gelatinisation and retrogradation. This mechanism is also referred to as "softener" because starch is the main factor for product firmness [7, 43].

Direct and indirect effects of the four mechanisms on the baking quality are outlined in Table 2. They improve the properties of bakery goods throughout the whole manufacturing chain: doughs and batters have an improved machinability, the resulting products are softer and have even crumb structures as well as increased shelf-lives during storage.

To which degree a surfactant improves the baking quality depends on its dosage and molecular properties. For DATEM, e.g., it was shown that the FA chain length affects product volumes [44].

Table 2: Functionality of surfactants in bread and fine bakery goods [7, 15, 22, 36, 40, 41, 45, 46].

Mechanism	Direct effect	Indirect effect
Emulsifying properties	◦ improved wettability ◦ improved homogenisation of dough/batter ◦ stabilisation of dispersed phases	◦ reduction of kneading/mixing time ◦ improved machinability ◦ improved performance of dried eggs ◦ even crumb structure ◦ possibility of single-stage mixing procedures
Interaction with lipids	◦ enhanced air incorporation ◦ improved gas retention ◦ postponed gas cell opening during baking	◦ reduction of expensive ingredients (eggs, fat) ◦ increased product volume ◦ faster manufacturing due to reduced proofing times ◦ uniformity of the crumb
Interaction with gluten	◦ improved gluten quality by enhancement of gluten aggregation ◦ reduced stickiness	◦ higher shock tolerance of doughs ◦ improved machinability ◦ improved texture ◦ reduced water loss ◦ reduction of expensive ingredients (eggs, fat) ◦ improved crumb gain ◦ uniformity of the crumb
Interaction with starch	◦ starch complexation ◦ inhibition of starch retrogradation ◦ increased starch gelatinisation temperature	◦ improved texture (softness) ◦ increased shelf-life by inhibition of staling ◦ increased volume

1.3.4. Baking lipases

Baking lipases are lipases designed for the application in bakery goods. They have been used as baking improvers since 1990 [21]. Lipases catalyse the hydrolysis of lipids in bakery goods and thereby lead to the *in situ* formation of polar molecules which can act as surfactants. They therefore do not increase the total quantity of lipids participating in the stabilisation of gas cells, but change the lipid composition towards a higher polarity [47].

The development of baking lipases was outlined by Moayedallaie et al. [48]: The first generation of baking lipases were *sn* 1,3-specific TG lipases. They led to the release of free FA (FFA) and MG. The second generation of lipases acted on both polar and non-polar lipids and therefore also cleaved bonds of glycerophospholipids. The third generation was described to be more concentrated and have a better tolerance towards different flour types. In the work of Moayedallaie et al., slight tendencies towards a better performance of the later generation of baking lipases were revealed.

Concerning legal aspects of their use, the application of food enzymes within the European Union is regulated by the Regulation (EC) No 1332/2008 [49]. This regulation prescribes that only approved enzymes may be used. These can be found on a Community list. However, the evaluation of enzymes for the list is currently still ongoing. Until the evaluation is completed, national rules apply. In Germany, this entails that food enzymes in bakery goods are considered as processing aids and are therefore not indicated on the list of ingredients in accordance with

Regulation (EU) No 1169/2011 [50, 51].

Contrary to traditional surfactants, baking lipases can therefore be used as clean-label baking improvers. The formed polar molecules correspond to naturally occurring lipids. Besides, due to lower amounts of lipases needed, their use is cheaper than the application of traditional surfactants [22].

Currently, 16 different lipases are in the authorisation process for the use in bakery goods as listed in the food enzyme database of the national public health institute of Belgium [52]. All of them are produced in fungi like, e.g., *Aspergillus oryzae* or *Candida cylindracea*. The production strain is fermented, the product filtered and then further purified. For eight products, the safety evaluation is already completed and the scientific opinions as provided by the European Food Safety Authority do not list safety concerns for their use. Lipases therefore offer promising possibilities for the improvement of baking quality as replacers for traditional surfactants.

1.3.5. Functionality of lipases in bakery goods

The functionality of lipases in bread has been analysed in depth. The underlying mechanisms of baking quality improvement are similar to the ones of surfactants as outlined in section 1.3.3.

Lipases convert lipids which form hexagonal II or cubic mesophases to lipids which arrange in form of lamellar mesophases [12]. Polar lipids and FFA released by lipases thereby impact gas cell formation and gas retention during baking, resulting in corresponding higher product volumes [46].

The addition of lipases also enhances gluten aggregation [53].

Finally, lipase addition was also shown to fulfil the effects of a “softener” by interacting with starch. Both amylose-lipid complexes and a reduction of amylopectin retrogradation were reported when lipases were used in bread [54, 55].

However, lipase addition does not seem to enhance the distribution of ingredients in doughs. In fact, as the responsible molecules are formed *in situ*, their release takes time. Gerits et al. reported unaltered lipid compositions directly after mixing when lipases were added to bread doughs [22]. They therefore cannot assist in the homogenisation procedure.

Instead of acting as emulsifiers, another mechanism for lipase and their reaction products to improve the baking quality was suggested: As they increase the level of FFA, they provide easily accessible substrates for lipid oxidation processes. These were suspected to influence dough strength and extensibility as well as reduce dough stickiness [22].

In their study on the effect of lipases on wheat flour dough, Colakoglu et al. therefore found an improved machinability of the doughs caused by reduced stickiness and increased stability [56]. Due to identified amylose-lipid complexes, they also predicted a delay in starch retrogradation. Additionally, the detrimental effect of DATEM on the dough extensibility did not occur, hinting at favourable conditions for DATEM replacement. Moayedallaie et al. also applied lipases as bread improvers in comparison to DATEM [48]. They found similar impacts of lipases and DATEM on the baking quality of the final products taking into consideration product volume, brightness, firmness and taste. In contrast, Frauenlob et al. observed a lower impact of lipase addition compared to DATEM [57]. Although lipases acted as softeners and thereby improved the baking quality, they led to decreased bread heights. They also reported differences in the suitability of the six lipases they used which need further clarification.

Different lipases release different reaction products due to differences in their activities or substrate specificities. In a range of studies conducted by Gerits et al. [45, 58, 59] and Schaffarczyk et al. [60, 61], the role of lipid reaction products on the baking quality of bread was therefore analysed. They confirmed that the effect of lipases is dose-dependent and also affected by lipase substrate specificity. The impact of wheat lipid hydrolysis products on the baking quality of bread is further discussed in section 1.4.1.

While lipase application in bread is a well established procedure in the baking industry, there are only scarce reports of lipases in cake manufacturing. In 2006, Guy and Sahi explored the potential of lipase application in cake by analysing the effects of a lipase in a cake with high amounts of sugar [62]. They found a lower surface tension, hinting at a stabilisation effect on gas bubbles in cake batter similar to bread dough. At the same time, the batter had a higher viscosity and the cakes had a higher product volume in combination with a more even crumb. Besides, they also found a small anti-staling effect which was probably due to complexation of starch. Rodríguez-García et al. further analysed the functional effects of two lipases in cakes for fat-reduction purposes. They baked low-fat cakes with inulin and monitored their baking quality and the improvement thereof by lipase addition [63]. The texture of the cakes was slightly improved during storage and they obtained a more even crumb structure. However, the lipases did not lead to an increase of product volume as described by Guy and Sahi. Recently, a new (indirect) approach for the use of lipases in cake manufacturing was presented by Monié et al. [64]. In their work, they used lipase-treated rapeseed oil for the improvement of the baking quality of a foam-type cake. The lipolysis was carried out in emulsion, the oil phase was extracted and added to cake batter. The resulting cakes were softer and maintained a higher degree of softness during storage. Nonetheless, their approach presents a comparably laborious procedure which is not fit for the cake baking industry.

1.4. Lipids in fine bakery goods

No generally accepted definition exists for the term “lipids”. Several approaches based on the solubility of substances or constituents were suggested. Within this work, the definition from Christie and Han will be used:

> “Lipids are fatty acids and their derivatives, and substances related biosynthetically or functionally to these compounds” [65]

According to Fahy et al. [66], lipids can be distributed into eight categories:

1. Fatty acyls. They are characterised by a repeating series of methylene groups and comprise, e.g., FA, fatty esters and fatty alcohols.

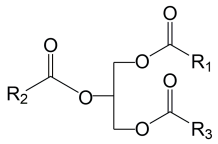
2. Glycerolipids. Glycerolipids contain a glycerol-backbone like the acylglycerols MG, DG and TG. The structure of TG is shown exemplarily in Figure 6a. Besides, also glycero-glycolipids are part of this category. Glyceroglycolipids have one or more glycosidically linked sugar residues attached to glycerol. An example for glyceroglycolipids are monogalactosyldiacylglycerols (MGDG, Figure 6c). They consist of a diacylglycerol linked to a galactose moiety. Because of the wide abundance and importance of glycerophospholipids, they were given a separate category.

3. Glycerophospholipids. Glycerophospholipids have a phosphatidyl ester bond derived from phosphatidic acid (PA). They are further differentiated according to the phosphatidyl ester groups. Common glycerophospholipids are, e.g., glycerophosphocholines (PC, Figure 6b), glycerophosphoethanolamines (PE) and glycerophosphoserines (PS).

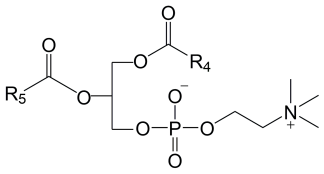
4. Sphingolipids. Sphingolipids have a sphingoid based backbone and comprise, e.g., sphingomyelin (SM), sphingoid bases (SPH) and ceramides (Cer).

5. Sterol lipids. This group is based on its biological function. An example for sterol lipids is cholesterol (Chol).

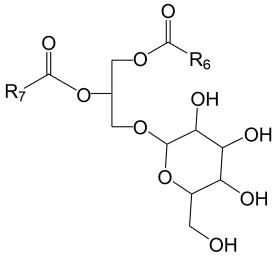
Besides, prenol lipids consisting of isoprenoid units, saccharolipids with a FA linked directly to the sugar backbone and polyketides with a polyketide backbone are named as categories.



(a) Structure of triacylglycerols.



(b) Structure of glycerophosphocholines.



(c) Structure of monogalactosyldiacylglycerols.

Figure 6: Examples of lipid structures of glycerolipids (a), glycerophospholipids (b) and glyceroglycolipids (c). R_1 - R_7 stand for any fatty acid residues.

In fine bakery goods, lipids are introduced by several ingredients. The main contributors for the lipid content of fine bakery good is the fat component. In this work, butter was used as fat component to comply with traditional recipes and deal with the matter of possible off-flavours by short-chain FA. Besides butter, mainly wheat flour and eggs contribute to the total lipid composition.

1.4.1. Wheat flour lipids

Wheat flour made from common wheat (*Triticum aestivum* L.) typically contains 2.0 - 3.0% lipids [12]. The amount and the composition are influenced by the genetics of the plant, the environmental conditions during the cultivation and the techniques applied for milling and final extraction of the lipids [38].

Several possibilities for the classification of wheat lipids are commonly used. They can be distinguished according to their polarity (neutral lipids like TG and DG and polar lipids like glycerophospholipids), their extractability (free and bound lipids) or their occurrence (starch and non-starch lipids) [12, 58, 67, 68] .

About 60% of wheat flour lipids are non-starch lipids. They consist mainly of TG and PC. Besides, also *N*-acyl glycerophosphoethanolamine (NAPE) and the glyceroglycolipids digalactosyldiacylglycerol (DGDG) and MGDG were identified (Figure 7) [12]. Starch-lipids are mainly composed of lysoglycerophospholipids like lysoglycerophosphocholine (LPC), lysoglycerophosphoethanolamine (LPE), lysoglycerophosphoglycerol (LPG) and lysoglycerophosphoinositol (LPI)

[69]. Lipidomic analysis of wheat flour revealed a total of 62-85 lipid species [68, 70, 71]. In addition to the already mentioned lipid classes, FA, DG, PE, glycerophosphoinositol (PI), glycerophosphoglycerol (PG), PS and PA species were identified. Depending on the wheat flour sample, further lysolipids, e.g., *N*-acyl lysoglycerophosphoethanolamine (NALPE), lysoglycerophosphoserine (LPS) and MG can occur [38, 72, 73]. The most abundant FA residues in wheat flour are linoleic acid (C18:2) and palmitic acid (C16:0) [68].

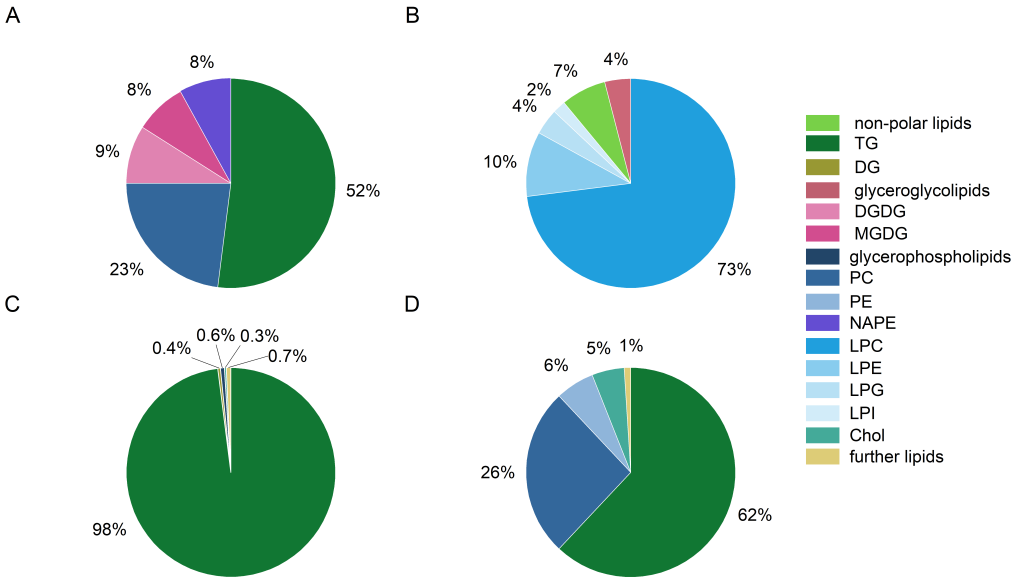


Figure 7: Lipid distributions in wheat flour non-starch lipids (A), wheat flour starch lipids (B), milk (C) and egg yolk (D) [12, 69, 74, 75, 76]. All abbreviations correspond to the abbreviations used in the text.

Despite the low share of lipids in wheat flour, their impact on the baking quality of bread is crucial as shown in several lipase-based approaches [58, 60, 61]. Melis et al. concluded from the pool of studies upon the topic that both the flour lipid level and the flour lipid composition affect the baking quality [12]. Concerning the composition, non-polar lipids have no impact, but FFA could be detrimental. Polar lipids act dose-dependently and can increase the bread loaf volume. Within the class of polar lipids, the effect of the level of glyceroglycolipids was found to be greater than the one of glycerophospholipids [77, 78]. Schaffarczyk et al. additionally showed synergistic effects between different lipid classes and an optimum effect on bread loaf volumes for a mixture of DGDG, monogalactosylmonoacylglycerol (MGMG) and NALPE [61]. This mixture of lipids leads to the formation of lamellar mesophases complemented by further hexagonal I and hexagonal II mesophases for optimal gas cell stability [61].

1.4.2. Milk lipids

Butter contains 81-85% of fat originating from cow milk [67]. The lipid distribution of milk fat is clearly dominated by the class of TG with a total share of 98% (Figure 7) [76]. Besides, further glycerolipids (DG, MG), fatty acyls (FA), glycerophospholipids (PA, PC, PE, PI, PS) and lysoglycerolipids (LPC, LPE), sphingolipids (SM, Cer) and the sterol lipid Chol were identified [76, 79]. Milk lipids contain up to 45 different FA residues which are mostly saturated and long-chain FA [80]. The FA residues range from acetic acid (C2:0) to behenic acid (C22:0) [81].

1.4.3. Egg lipids

Although egg yolk makes up for only 36% of the weight of fresh eggs, they are the main source of egg lipids [67, 75]. The lipid content of egg yolk is 32.6% while egg white contains only 0.03% of lipids [67].

The lipids of egg yolk are exclusively organized in supramolecular assemblies of different lipid classes and proteins, called lipoproteins. The lipids within these lipoproteins consist of 62% TG, 33% glycerophospholipids and less than 5% Chol (Figure 7) [75]. The comparably high content of glycerophospholipids in egg yolk has been studied extensively. Main glycerophospholipid classes are PC (78.5%), PE (17.5%) and PI (0.6%) [74]. Besides, PS and LPC were found in a mass spectrometric approach by Ali et al. [82]. Ali et al. identified the predominant molecules of the different glycerophospholipid classes as PC (16:0/18:1), PE (18:0/20:4), PI (18:0/18:2), PS (18:0/18:2) and LPC (16:0) [82]. This is in line with the main FA residues in egg yolk lipids, namely oleic acid (C18:1, 40-45%), palmitic acid (C16:0, 20-25%) and α-linolenic acid (C18:2, 15-20%) [75]. Still, it has to be stated that the exact FA composition strongly depends on the used feed [75].

The data on egg white lipids is scarce. A study by Sato et al. from 1972 reported a glycerophospholipid content of 13-15% and further the occurrence of TG, DG, FA, Chol and Chol derivatives as well as SM [83].

1.4.4. Further lipids

Further lipids can be introduced by further ingredients. Water, sugar, salt and baking powder are free of lipids. Yeast, however, contains lipids and can thereby contribute to the total lipid distribution of fine bakery goods. The lipidome of baker's yeast, *Saccharomyces cerevisiae*, was analysed in depth by Ejsing et al. [84]. They identified 162 different lipid species belonging mainly to glycerolipids (TG, DG) and glycerophospholipids (PA, lysoglycerophosphatidic acid (LPA), PC, LPC, PE, LPE, PI, LPI, PS and cardiolipin (CL)). Remarkably, PI was found to be the major class of glycerophospholipids with a share of 17-30% of the yeast's lipidome. However, the lipid content of yeast is only about 1% of its dry matter [73]. In combination with the comparably small amounts of yeast which are used in bakery goods, its influence on the population and extractability of other lipids is probably more important than its addition of lipids [73].

1.5. Methods for the determination of the baking quality of fine bakery goods

The baking quality of fine bakery goods comprises both the quality of batters/doughs and the quality of the final product.

1.5.1. Characterisation of batter and dough quality

The characterisation of batter and dough quality is of interest to determine the machinability of batters and doughs. The properties of batters and doughs can be optimized to achieve optimal handling properties, which include, e.g., a low density and a low stickiness. Besides the importance during manufacturing, the properties of the batters and doughs also affect the properties of the resulting products. Indeed, a predictive power of cake batter quality as defined by cake ingredients and processing methods was presented by Christaki et al. [85].

Common parameters for the quality are: density, stickiness, the rheological behaviour and the temperature-dependent behaviour of batters and doughs.

The density of cake batters and doughs is assessed by weighing a known volume of batter or dough [85, 86, 87].

For the stickiness, an objective method to assess dough stickiness was first developed by Chen and Hoseney in 1995 [88]. It relies on the force needed to separate a probe from the dough surface. This procedure is still commonly applied as done by Colakoglu et al. [56]. More recently, the Warburtons Dough Stickiness System was introduced. The batter or dough sample rests in a box and a blade is driven through a slot of the retaining plate on top. The force needed to withdraw the blade is then used to calculate the stickiness.

The rheological characterisation studies the viscoelastic properties, that is the flow and the deformation of cake doughs and batters. It gives information about viscous portions of a sample, causing it to flow, and elastic portions of samples, responsible for its deformation when external forces are applied to the sample.

For bakery products, parallel plate geometries are commonly used. The dough or batter is put on a fixed plate and a second plate is lowered onto the sample. A defined deformation or force is applied to the sample by movement of the upper plate and the response of the sample is measured. The movement of the upper plate can be oscillatory or rotational and both tests are applied for cake batters [63, 85, 86, 87]. For doughs, which are more solid-like, mostly oscillatory tests are recommended [89].

In oscillatory rheology, the movable plate oscillates at a sinusoidal strain of a defined amplitude γ_0.

$$\gamma = \gamma_0 sin\omega t \tag{1}$$

The sample responds with a sinusoidal stress. This stress is shifted by a factor of δ, the phase lag. The phase lag depends on the sample itself and is δ=0 for totally elastic samples and δ=1 for totally viscous samples.

$$\sigma = \sigma_0 sin(\omega t + \delta) \tag{2}$$

Taking the formulas (1) and (2), the elastic portion of samples can be calculated as their storage modulus G' and the viscous portion as their loss modulus G".

$$G' = \frac{\sigma_0}{\gamma_0} cos\delta \tag{3}$$

$$G'' = \frac{\sigma_0}{\gamma_0} sin\delta \tag{4}$$

The ratio of G" and G' is referred to as loss factor (tanδ). The higher tanδ, the greater the viscous portion and therefore "liquid-like" the sample is.

$$tan\delta = \frac{G''}{G'} \tag{5}$$

Typical tests carried out in oscillatory rheometry are amplitude sweep, frequency sweep and temperature sweep tests. All of them are common procedures for the characterisation of cake batter and dough samples and were used by different groups [63, 85, 90, 91].

In an amplitude sweep (test), the movable plate oscillates with an increasing amplitude γ_0 while the frequency ω is kept constant. A typical diagram of an amplitude sweep of a cake batter sample is given in Figure 8. An amplitude sweep allows the identification of the linear viscoelastic region (LVE). The LVE corresponds to the range of deformation in which the inner structure of the sample is not destroyed as G' and G" and thereby also tanδ remain constant. Within the LVE, G' represents the structural strength of the sample. Besides the LVE and G' in the LVE, the crossover point is assessed. This is the deformation at which G' corresponds to G" and the sample changes from viscoelastic solid to viscoelastic fluid.

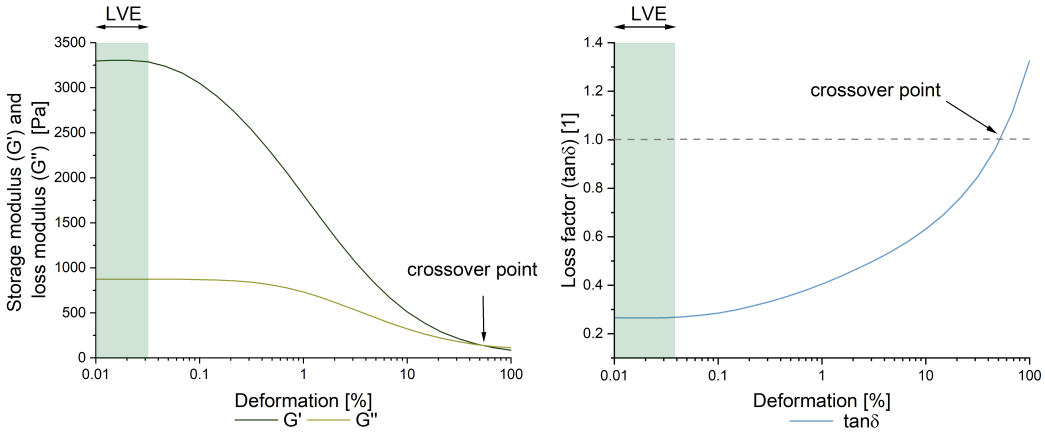
Frequency sweep tests measure at varying frequencies ω while the amplitude γ_0 is kept constant. They are thereby used to monitor the time-dependent behaviour of samples. Low frequencies correspond to long-term behaviour and high frequencies to short-time behaviour.

In temperature sweep tests, both amplitude γ_0 and frequency ω are kept constant while the temperature during the measurement increases. They mimic baking of cakes and monitor the structural changes during heating. An example of the course of tanδ during heating from 25 °C to 100 °C is shown in Figure 8. For cake batter temperature sweeps, three characteristic phases can be distinguished [85, 92, 93, 94]:

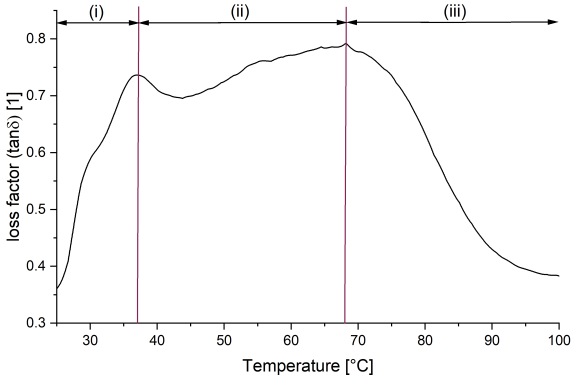
1. An increase of tanδ at the beginning of the sweep which represents a liquefaction mainly due to the liquefaction of fat.

2. A second increase of tanδ between 40 °C and 70 °C as interactions between the ingredients are reduced and CO_2 is released.

3. The final decrease of tanδ at temperatures above 75 °C due to protein denaturation and starch gelatinisation.



(a) Typical diagram of an amplitude sweep in cake batter samples. LVE - linear viscoelastic region.



(b) Example of a temperature sweep of a cake batter sample.

Figure 8: Rheological characterisation of cake batters.

1.5.2. Characterisation of product quality

The product quality of fine bakery goods can be expressed by a range of parameters. Commonly measured variables comprise the colour of products, the weight loss during baking referred to as baking loss or moisture/water loss, product density and the crumb structure which influences the product texture. In combination with effects caused by lipases, especially the parameters baking loss, density and texture are of interest, as they were shown to be affected by lipase addition to bakery goods [48, 57, 59, 60, 63, 64].

The baking loss of bakery goods is determined by comparing the weight of batter or dough to the weight of the resulting product after baking and cooling to room temperature.

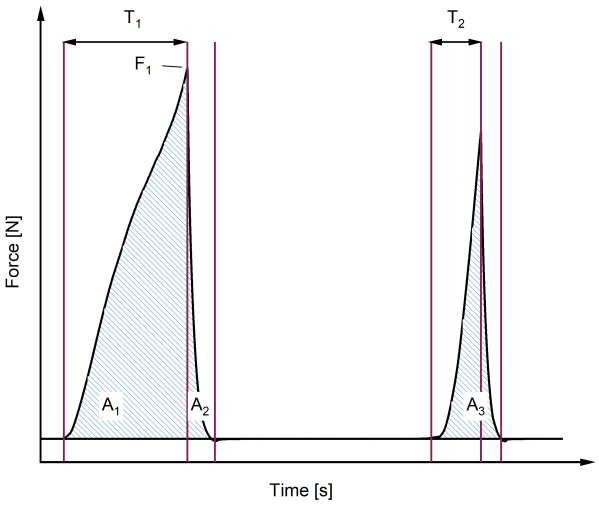
Product density is calculated from the weight of the product and the corresponding product volume as measured by, e.g., a laser-based scanner.

For texture analysis, a classification system for the texture of food products was developed by A. Szczesniak in 1963 and has been used with small modifications ever since [95]. Modern texture analysis comprises the parameters firmness, springiness, cohesiveness and resilience.

Firmness describes the force which is necessary to achieve a fixed deformation of the product. The ability to regain the original shape is referred to as springiness. By cohesiveness, the inner strength of the product is described as ability to withstand repeated applied force. Resilience is a parameter to assess the ability to recover after a compression [87, 95, 96].

Depending on the already described parameters, two more dependent parameters can be calculated. The gumminess of products is a measurement for the energy which is required to disintegrate food before swallowing and chewiness for the length of time to masticate before a food can be swallowed [87, 95, 96].

All six parameters can be measured using the Texture Profile Analysis. The Texture Profile Analysis consists of a double compression test. It was described to either simulate the process of biting and chewing in the mouth [85] or to mimic the approach consumers use to assess the freshness of baked products by squeezing, e.g., a packaged bread loaf [96]. Similar to the rheological characterisation, the Texture Profile Analysis relies on the relationship between applied force and resulting effect on a sample in a parallel plate geometry. In case of the Texture Profile Analysis, the force applied is uniaxial in form of a compression while in rheology, it is applied as a shear force parallel to the surface of the sample. The force and the time needed to achieve a fixed deformation of a product are recorded during a repeated compression of the sample. From the resulting diagram, all textural parameters can be deduced (Figure 9).



Textural property	Calculation
Firmness	= F_1
Springiness	= T_1/T_2
Cohesiveness	=(A_1+A_2)/ A_3
Resilience	=A_1/A_2
Gumminess	=Firmness x Cohesiveness
Chewiness	=Gumminess x Springiness

Figure 9: Typical diagram of a Texture Profile Analysis. Figure modified from Stemler & Scherf [97].

A further parameter linked to the texture of bakery products is staling. Staling was described as the deterioration of bread quality during storage, which includes a loss of crispiness and an increase of crumb firmness [34]. To determine the effect of additives on the staling of bakery products, the development of their texture during storage is monitored. For lipases, antistaling effects in bread were described by Frauenlob et al. after measuring the product firmness of lipase-treated and untreated breads daily during 10 days of storage [57].

1.6. Methods to analyse lipase reactions

There are numerous ways to analyse lipase reactions. An overview about different approaches will be given in section 1.6.1. Two kinds of methods will be discussed in detail, first methods which are suitable for the analysis of lipase substrate specificity (section 1.6.2) and second methods comprising natural substrates (section 1.6.3).

1.6.1. Lipase activity assays

Lipase activity assays can be categorised according to four different criteria:

1. Lipase activity can be determined both qualitatively and quantitatively. Qualitative approaches include, e.g., gel diffusion assays. In the present work, commercial lipases were used. Qualitative assays were therefore not needed and will not be further dealt with. Instead, quantitative assays were applied for the characterisation of lipases.

2. The activity assays can monitor either the disappearance of the substrate, the release of a reaction product, coupled reactions or physical changes of the reaction media based on the reaction, e.g., the clarification of an emulsion after TG hydrolysis [98].

3. The assays can rely on end-point determinations or measure lipase activity continuously. Continuous measurements allow the monitoring of reaction progress curves. Disturbances of the reaction can therefore be excluded from the determinations. For lipase reactions, the determination of enzyme activity should rely on a linear relationship between reaction time and progress of the reaction. As the substrate is depleted, the reaction velocity slows down and finally ceases. In end-point determinations, it is unclear whether only the linear part of the reaction rate was measured or if the reaction was already completed before the measurement was undertaken. Continuous methods should therefore be preferred over end-point determinations [99].

4. The final criteria for lipase activity assays is the analytical method which is used for the determination. The analytical methods reported for the analysis of lipase activity comprise, e.g., titrimetry, radioactivity, immunochemistry, microscopy, chromatography, interfacial tensiometry, conductimetry and spectroscopy [25, 98, 100, 101]. Among these methods, only three are suitable for continuous measurements: interfacial tensiometry, conductimetry and spectroscopy. Compared to the other two, spectroscopy is most widespread.

The spectroscopy methods can be further divided into visible spectrophotometry and fluorometry. For both approaches, a variety of methods has been developed, including a wide range of substrates. In terms of visible spectrophotometry, the *p*-nitrophenyl assay is a commonly used approach [102]. It relies on the hydrolysis of artificial *p*-nitrophenyl esters and the detection of the released *p*-nitrophenol, which is a yellow chromophore (Figure 10). It was, e.g., applied by Frauenlob et al. [57] and Gerits et al. [58] for the characterisation of lipase activities of baking lipases for the use in bread.

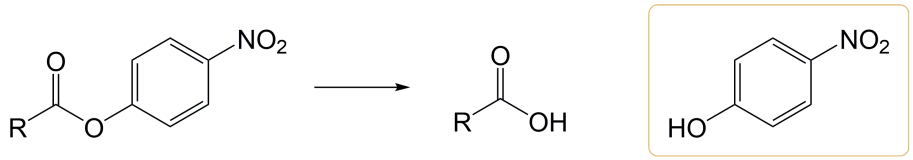


Figure 10: Hydrolysis of a *p*-nitrophenyl ester to the corresponding free fatty acid and the chromophore *p*-nitrophenol (marked). R stands for any fatty acid residue.

Concerning fluorometric methods, several artificial substrates are commercially available. Among them are, e.g., 4-methylumbelliferyl esters as applied by Menden et al. [103] and methylresorufin derivatives as used by Sokolova et al. [104]. However, these artificial and bulky substrates are different from natural lipase substrates and their use has therefore been criticized.

1.6.2. Lipase substrate specificity

For the analysis of lipase substrate specificity, only methods for the determination of lipase activity depending on FA chain length (FA substrate specificity) have been established so far. For this means, the use of the aforementioned *p*-nitrophenyl assay is common and has been applied by a range of groups [30, 31, 105] . The *p*-nitrophenyl esters are available with FA residues of varying chain lengths. For each of the substrates, the lipase activity is determined and then compared to the activity towards other substrates. The lack of commercially available esters with unsaturated FA has been dealt with by the group of Nalder et al., who successfully synthesized a range of *p*-nitrophenyl esters with unsaturated FA [106]. Besides the *p*-nitrophenyl assay, further artificial substrates with varying chain-lengths have been synthesized and used by single groups, as tailor-made fluorogenic triglycerides [107] or 4-hydroxy-n-propyl-1,8-naphthalimide esters [108].

In terms of end-point determination-based methods, the use of chromatographic techniques for the analysis of lipase substrate specificity is possible. FFA of the reaction of lipases with an oil with randomized FA chain lengths [109] or of the reaction with several ethyl monoesters [110] can be identified and quantified by gas chromatography (GC). However, it remains unclear in how far the results obtained with single approaches and artificial substrates as, e.g., *p*-nitrophenyl esters, can be reproduced in competitive reactions with natural substrates and thus serve as a prediction for the reaction in foodstuff.

1.6.3. Lipase reactions with natural substrates

To overcome the shortcomings of artificial substrates, systems to measure lipase reactions with naturally occurring lipids are needed. Lipases are water-soluble enzymes, but their substrates are typically hydrophobic. Emulsions are therefore required for the reaction. For their preparation in terms of lipase reaction analysis, there is no common procedure. Although the droplet size of lipid micelles and therefore the interfacial area is crucial for lipase activity [111], a range of methods has been proposed and used without further analysis of the resulting emulsions. Among the methods for emulsion preparation are ultrasonic treatment [112], simple stirring [113], shaking [114] and homogenisation procedures [115, 116]. Incubation times range from

2 min up to 48 h [115, 116, 117] and different emulsifiers such as gum arabic (GA) and Triton X-100 are used [117, 118]. It is not clear how far these approaches are comparable.

There are several possibilities for the determination of lipase activities after the reaction with natural substrates. Most of them rely on chromatographic techniques, e.g., the quantification of FFA via GC as done by Wu et al. [119]. By using a suitable chromatographic procedure, the FA substrate specificity of the reaction could also be included in the analysis. However, this approach is challenging for the quantification of short-chain FA such as butyric acid from butter [120]. Recently, the group of Mannion et al. presented a method for the accurate determination of FFA of butter based on their derivatisation to butylesters [121]. The method could be transferred for the analysis of lipase FA substrate specificities in reactions with emulsified butter. Besides GC methods, the use of an LC-MS/MS system is possible. Thereby, both FFA and lipid residues can be analysed after extraction and clean-up. This approach was already successfully used for the analysis of lipase reactions with tuna oil by Xuan et al. [122].

Chromatographic techniques are end-point analyses and need specific laboratory equipment. For a simpler spectroscopy method, a way to detect either hydrolysed lipid residues or released FFA is needed. First experiments with the use of the fluorescent dye Rhodamine B (Figure 11) for the quantitative detection of FFA have been carried out by Zottig et al. [123] and van Gaelen et al. [124]. They used emulsions of triolein, olive oil and soy bean oil in water. However, their works did not include lipid classes besides TG or information on the influence of different FA chain lengths on the sensitivity of the dye. The use of Rhodamine B could enable the development of a new lipase activity assay with continuous measurement including different natural substrates. Therefore, the determination of lipase substrate specificities towards different lipid classes could be achieved.

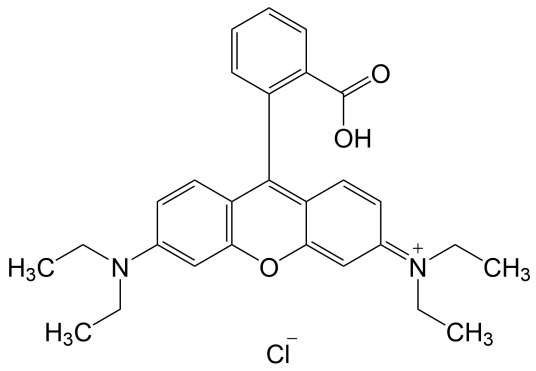


Figure 11: The molecular structure of Rhodamine B.

2. Aim of the work

Lipases offer promising potential for the use in fine bakery goods towards the replacement of traditional surfactants. However, a deeper understanding of their functionality in fine bakery goods is missing. In contrast to bread, fat is an essential ingredient during the preparation of, e.g., cakes with a variety of functional effects and concomitant high levels. While in bread, the substrates for lipase reactions originate mainly from wheat flour, in fine bakery goods, further ingredients add to the range of available substrates. They thereby enlarge the pool of possible reaction products with unknown effects on the baking quality. In addition to the effects on the baking quality, the flavour of fine bakery goods can be affected by the use of lipases. Butter is used in traditional cake recipes. The lipids of butter are known for their share of short-chain FA, especially butyric acid. When short-chain FA are released, they entail a rancid off-flavour which is undesirable for cakes and thus also prevents lipase application so far.

These challenges result in four main aims of this study:

First, lipases with suitable FA substrate specificities for use in fine bakery goods were needed. They should not release short-chain FA but preferentially interact with long-chain FA. To achieve this aim, a range of lipases currently applied in bread was first characterised in terms of FA substrate specificity by using the *p*-nitrophenyl assay. Due to the unknown transferability of the results to “natural” substrates like the fats currently used in baking, model systems for the reaction were needed. For the quantification of released FFA, a GC method with special regard to short-chain FA was developed. The formation of possible further off-flavours from the model systems was monitored by a trained sensory panel. Based upon the results, lipases for further experiments were selected.

The second aim was to explore potential uses of baking lipases in cakes. Three recipes, an eggless basic cake (German *Rührkuchen*), a traditional pound cake (German *Sandkuchen*) and a yeast-based brioche were chosen for the analysis. The effects of seven lipases on the baking quality were compared to the effects of the traditional surfactant DATEM. The analysis comprised the effects of the quality of cake batters and doughs including the parameters density, stickiness and a rheological characterisation as well as the quality of the baked products including density, baking loss and texture during storage.

Third, the underlying reactions responsible for effects on the baking quality were assessed. Therefore, a LC-MS/MS method was optimised and applied to analyse the lipidome of lipase-treated cake samples before and after baking. The resulting patterns for substrate specificities were compared to the results for the baking quality and key reaction products were identified.

Fourth and last, a method to predict the suitability of a lipase for the use in fine bakery goods was needed. The lipases chosen for the baking trials were first further characterised by the use of three commercially available lipase activity assay kits. Then, a new assay for the analysis of substrate specificity was developed.

3. Results and discussion

3.1. Model systems to characterise lipase substrate specificities

The following parts were partly already published in *LWT - Food Science and Technology* [125] and *Getreide, Mehl und Brot* [126].

The FA substrate specificities of 17 lipases were first analysed using the *p*-nitrophenyl assay. The results were compared with the FA substrate specificities of the same lipases towards fats frequently used in baking. Emulsions were needed for the water-soluble enzymes to come into contact with fats. Therefore, a procedure for the preparation of stable emulsions of rapeseed oil, margarine, butter and wheat germ oil was established. The FA substrate specificities of the lipases in the emulsions were determined by quantifying the released FFA by GC. Additionally, a sensory panel was trained to detect rancid off-flavours in the emulsions. The panel evaluated the flavours of the emulsions after treatment with lipases. Based on the results, a range of seven lipases with suitable FA substrate specificities was chosen for further analysis.

3.1.1. *p*-Nitrophenyl assay

The *p*-nitrophenyl assay was performed with 17 baking lipases to determine their FA substrate specificity towards eight *p*-nitrophenyl esters with FA residues from C4:0 (butyric acid) to C18:0 (stearic acid). 16 of 17 lipases led to activities within the quantifiable range (Table 19 and Figure 12). For lipase D, no measurable activity was detected towards any of the substrates.

The total activities of the lipases against all substrates were from 0.23 ± 0.1 $s^{-1}g^{-1}$ to 99.4 ± 4.1 $s^{-1}mg^{-1}$, indicating a 497,000-fold range between the single activities. While some lipases had average activities ranging between 20.2 $s^{-1}mg^{-1}$ (lipase A) up to 60.1 $s^{-1}mg^{-1}$(lipase I), others reacted only with maximum activities of 13.3 $s^{-1}g^{-1}$(lipase F) or 9.4 $s^{-1}g^{-1}$(lipase L). To ease the comparability between the lipases, the FA substrate specificities were therefore indicated as relative activities per lipase. Therefore, the percentage of the activity of the lipase towards a specific substrate was compared to the sum of activities of the lipase towards all analysed substrates.

The resulting patterns showed no clear trend (Figure 12). However, the release of C8:0, C10:0, C12:0 and C14:0 seemed to be preferred by most lipases (average percentage of total activities: 19.7%, 15.1%, 16.3% and 13.4% for C8:0, C10:0, C12:0 and C14:0, respectively). Twelve of the 16 lipases had similar specificities towards the substrates and showed low activity towards C4:0 and C6:0, high activities towards C8:0 - C14:0 and again low activities towards C16:0 and C18:0. Only four lipases differed from this trend:

First, lipase A selectively released only C8:0, C10:0 and C12:0. The percentages of activities towards the remaining substrates were all below 5%. Second and third, the lipases L and N reacted mostly with C4:0, C6:0 and, in the case of lipase L, with C8:0. The percentage of activity towards longer chain substrates was 8.6% at most (lipase L towards C10:0). They thus resembled more to esterases than lipases. Fourth and last, lipase Q cleaved all substrates with similar activities and showed no preference for certain chain lengths. Its percentage activities all ranged between 7.0% (C18:0) and 17.1% (C14:0).

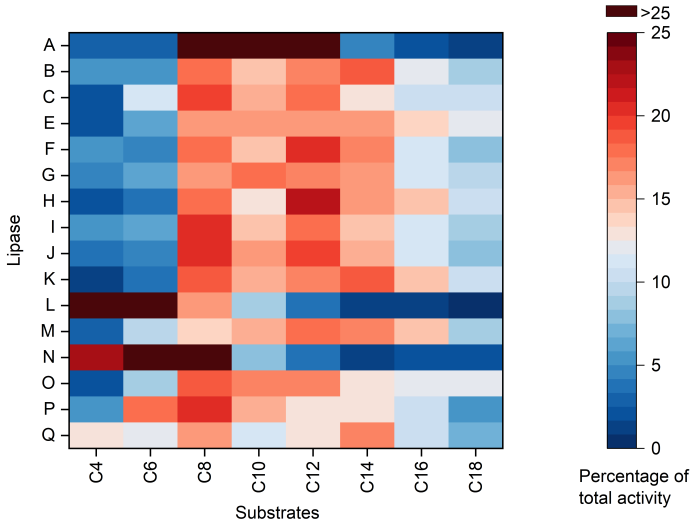


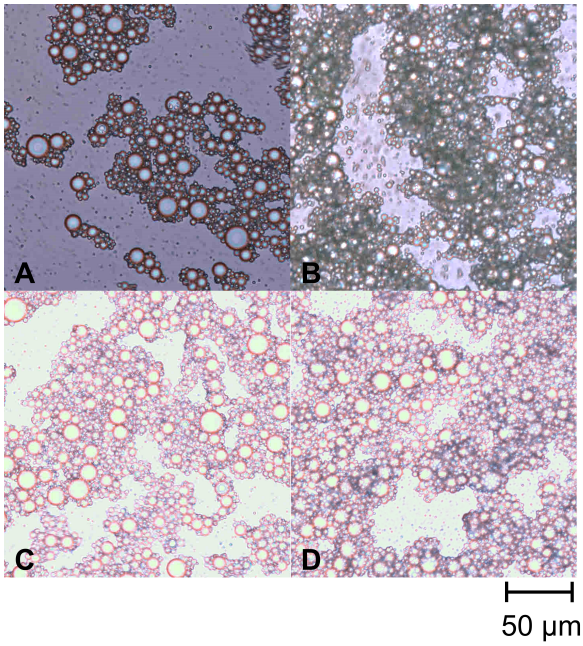
Figure 12: Activity of different lipases A to Q towards the *p*-nitrophenyl-derivatives of different fatty acids from C4:0 to C18:0. All results (means, n = 3) are shown as percentage of the total activity (sum of all activities) of the respective lipase for better comparability (Table 19). Lipase D showed no reaction under the conditions of the assay. Figure modified from Stemler & Scherf [125].

3.1.2. Establishment of stable emulsions as model systems

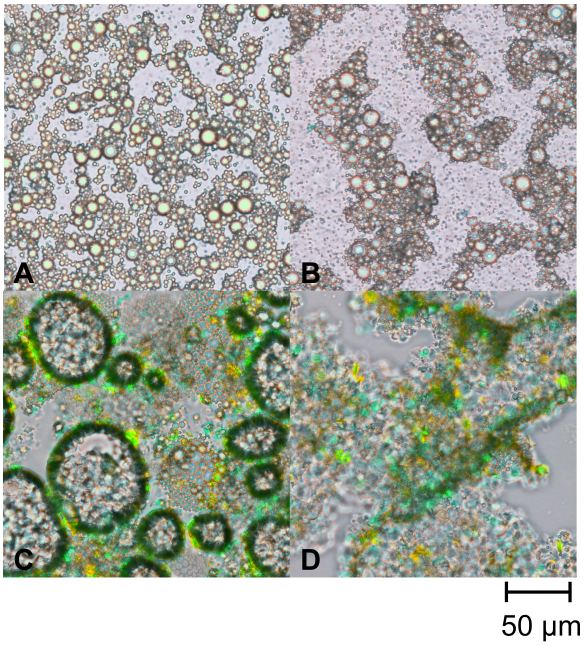
To analyse the reaction of lipases with baking fats, suitable model systems were needed. For the water-soluble enzymes to come into contact with their lipid-soluble substrates, the substrates were transferred into an emulsion. The reaction of baking lipases with the baking fats rapeseed oil, margarine, butter and wheat germ oil was monitored. The latter was taken as a control sample for the reaction of lipases with lipids from wheat flour.

The stable emulsions which were to serve as model systems for the reaction of lipases with baking fats had to fulfil three requirements:

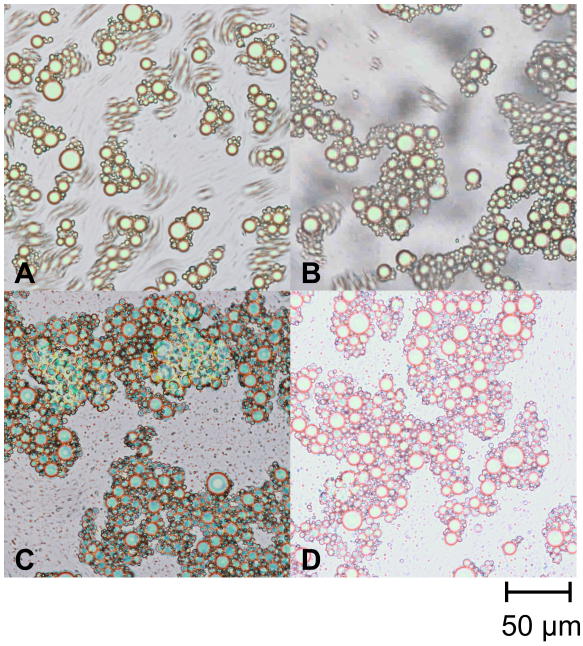
1. First, the procedure had to be suitable for the complete range of fats. While rapeseed oil and wheat germ oil could be emulsified without further treatment, butter and margarine are spreadable fats and thus solid at room temperature. Therefore, melt emulsions were prepared according to the following procedure: The emulsifying solutions were heated to 50 °C before adding them to the fats. The fats were melted gently in order to maintain their FA composition. However, even after the emulsions were prepared the fats could recrystallize which would then influence the interaction of the fats with lipases. Petrographic microscopy was used to determine whether crystals were formed in the emulsions with or without lipase treatment 1 h and 24 h after preparation (Figure 13). For rapeseed oil and wheat germ oil, no crystals were found in the emulsions. For margarine, recrystallisation occurred both for lipase-treated and untreated emulsions after 24 h. For butter, no crystals occurred 3 h after preparation. Interestingly, for longer incubation times of butter, crystals were found in the samples without lipase only. The addition of lipase therefore inhibited the recrystallisation of emulsified butter.



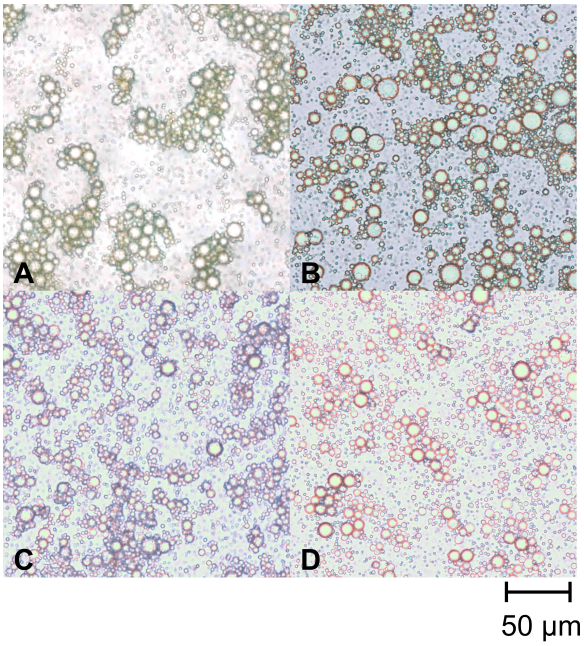
(a) Rapeseed oil



(b) Margarine



(c) Butter



(d) Wheat germ oil

Figure 13: Petrographic microscopy of rapeseed oil/margarine/butter/wheat germ oil emulsions 1 h after preparation without lipase (A) or with lipase (B) and 24 h after preparation without lipase (C) or with lipase (D). Figures modified from Stemler & Scherf [125].

2. The second requirement was that the comparability of the results from the model systems and the *p*-nitrophenyl assay had to be assured. Therefore, similar reaction conditions were needed. For the procedure as described in section 5.2.1, the same emulsifier (Triton X-100), the same pH value (7.5), the same concentration of $CaCl_2$ and the same temperature for the reaction (room temperature, approx. 22 °C) were chosen.

3. As a third requirement, the properties of the model systems had to be reproducible and stable throughout the reaction. Therefore, the particle size distributions of the emulsions were monitored 3 h and 24 h after preparation. The results were compared using the Sauter diameter (Table 3). The Sauter diameter indicates a specific surface area based on the mean spherical size of the disperse phase. It can therefore be used to compare the particle size distributions of different emulsions. The Sauter diameter depended strongly on the fat used for the emulsion. The diameters ranged from 1.45 ± 0.09 µm to 4.22 ± 0.16 µm. They were lowest in the margarine emulsion, higher for wheat germ oil and highest in butter and rapeseed oil. For rapeseed oil, margarine and wheat germ oil, no significant change in Sauter diameter occurred during incubation ($p>0.05$). In the butter emulsion, the Sauter diameter of the butter droplets decreased from 3 h after preparation to 24 h after preparation about 20%.

Table 3: Sauter diameter of the different fats in emulsion measured 3 h and 24 h after preparation. Data partly already published in Stemler & Scherf [125]. Results are indicated as mean values ± standard deviation (n = 3). Numbers with different superscript letters are significantly different (ANOVA with Tukey's test, $p<0.05$).

Fat	Sauter diameter [µm]	
	After 3 h	After 24 h
Rapeseed oil	4.16 ± 0.15^{a}	4.12 ± 0.15^{a}
Margarine	1.45 ± 0.09^{d}	1.47 ± 0.23^{d}
Butter	4.22 ± 0.16^{a}	3.34 ± 0.12^{b}
Wheat germ oil	2.50 ± 0.16^{c}	2.10 ± 0.17^{c}

3.1.3. Analysis of fatty acids released from the model emulsions

During the reaction of lipases with emulsions, FFA were released. For their determination, a GC method was established including C4:0, caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), C18:0, oleic acid (C18:1), linoleic acid (C18:2) and α-linolenic acid (C18:3) (Figure 14). The FFA were derivatised to butyl esters and quantified using the internal standards valeric acid (C5:0), undecanoic acid (C11:0) and nonadecanoic acid (C19:0) (section 5.2.3.1). The total runtime was 32 min.

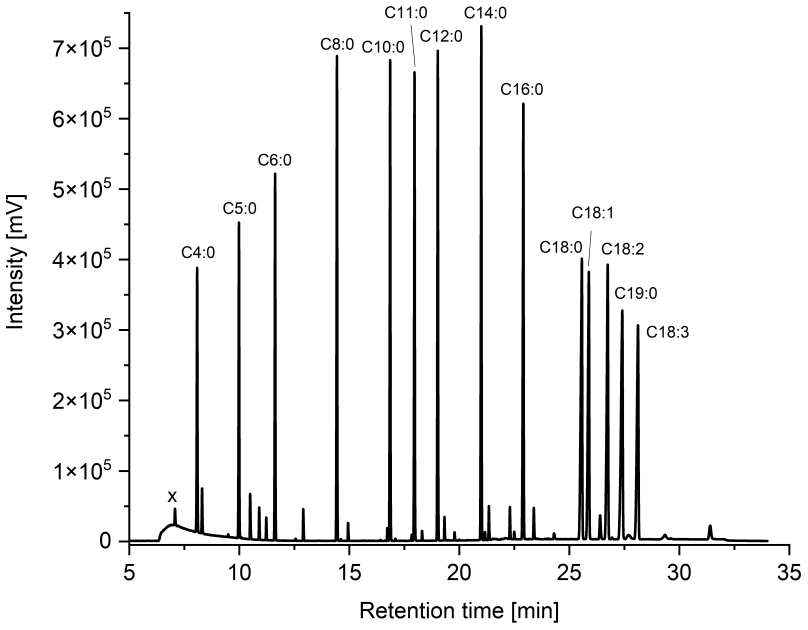


Figure 14: Gas chromatographic separation of the free fatty acid butyl esters. x butanol, C4:0 butyric acid, C5:0 valeric acid, C6:0 caproic acid, C8:0 caprylic acid, C10:0 capric acid, C11:0 undecanoic acid, C12:0 lauric acid, C14:0 myristic acid, C16:0 palmitic acid, C18:0 stearic acid, C18:1 oleic acid, C18:2 linoleic acid, C19:0 nonadecanoic acid, C18:3 α-linolenic acid. Figure modified from Stemler & Scherf [125].

The intermediate precision of the method was checked by comparing the average relative standard deviation for all analytes within one day (6.82%, one emulsion analysed in triplicate, n = 3) and on three consecutive days (7.80%, three emulsions analysed in triplicate, n = 9). They did not differ significantly (Tukey's test $p>0.05$). The method was thus reproducible.

The GC experiments were used for the determination of substrate specificities for FA with certain chain lengths. The specificities were calculated as substrate specificity factors. The factors were based on the quotient of the percentage of a FFA within the FFA distribution of a lipase-treated sample and the percentage of the respective FFA within the FFA distribution of the corresponding control sample (emulsion of the same fat without lipase treatment). If the share of a FFA was increased by a lipase, the lipase showed a higher specificity for this FA and had thus a higher specificity factor for this FA. The FFA distribution of the control sample was used for normalisation to take into account all factors influencing the share of FFA in contrast to bound FA, e.g., volatility and oxidative degradation rates.

3.1.3.1. Rapeseed oil

The rapeseed oil control sample contained 0.70 mg g^{-1} FFA after emulsification and incubation based on its rapeseed oil content (Table 20). The FFA distribution of the control sample was 45.9% C18:1, 21.0% C16:0, 13.7% C18:2, 14.0% C18:0 and 5.4% C18:3.

The lipase-treated samples had total FFA contents ranging from 0.57 mg g^{-1} to 129.29 mg g^{-1} (mean: 43.10 mg g^{-1}). The lipases O, C and P released the highest amounts of FFA (129.29 mg g^{-1}, 113.02 mg g^{-1} and 99.31 mg g^{-1}, respectively) and the lipases D, N and L the lowest amounts

of FFA (0.57 mg g^{-1}, 0.67 mg g^{-1} and 0.74 mg g^{-1}, respectively).

The specificity factors of the lipases showed a clear trend towards the preferential release of unsaturated FFA for all lipases besides D, N and L (Figure 15). The lipases D, N and L all had very low activities and led to FFA distributions corresponding to the one of the control sample. Their average substrate specificities towards all substrates were 0.98 ± 0.09 (lipase D), 0.98 ± 0.09 (lipase N) and 1.01 ± 0.09 (lipase L). The remaining 14 lipases all had low specificities towards C16:0 (mean: 0.28 ± 0.05) and C18:0 (mean: 0.13 ± 0.05), a higher specificity towards C18:1 (mean: 1.28 ± 0.06) and high specificities towards C18:2 (mean: 1.75 ± 0.23) and C18:3 (1.78 ± 0.30).

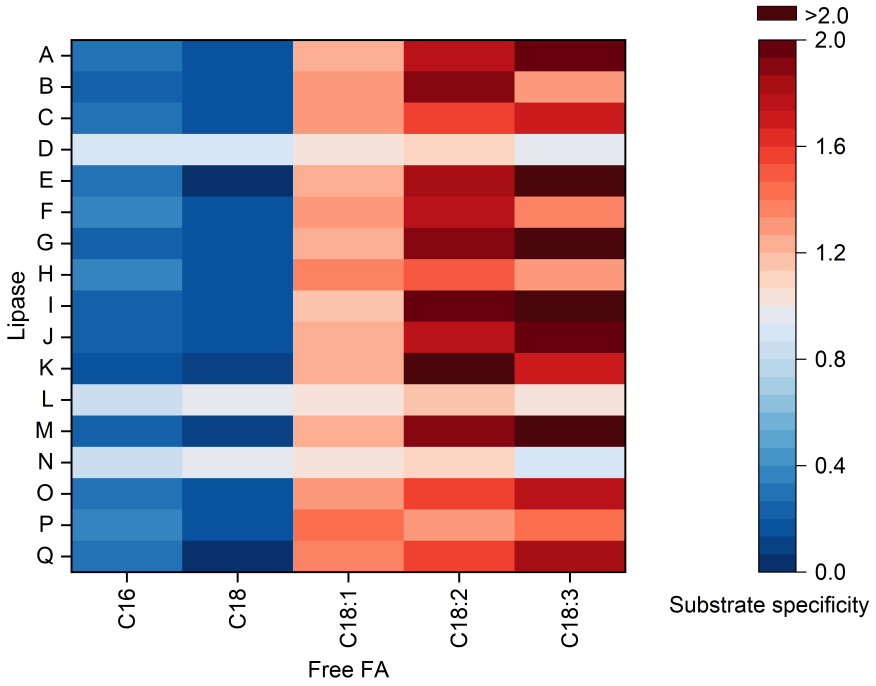


Figure 15: Distribution of free fatty acids (FFA) released by 17 different lipases A to Q from rapeseed oil emulsified with Triton X-100 in water. All means (n = 3) were normalised to the results of a control sample without lipase to account for the natural occurrence of the different FFA in the rapeseed oil emulsion. Figure modified from Stemler & Scherf [125].

3.1.3.2. Margarine

The control sample for margarine emulsions had a FFA content of 1.15 mg g^{-1} margarine (Table 21). It consisted mainly of long-chain FA (35.1% C16:0, 28.6% C18:0). Besides C16:0 and C18:0, the FFA C18:1, C18:2, C12:0, C18:3 and C14:0 were present with a share of 17.4%, 10.7%, 5.9%, 1.2% and 1.1%, respectively.

The lipases released 1.05 mg g^{-1} - 397.49 mg g^{-1} FFA (mean: 216.92 mg g^{-1}) from margarine emulsions. The lowest activities were again attributable to the lipases D (1.05 mg g^{-1}), L (1.09 mg g^{-1}) and N (1.12 mg g^{-1}). The lipases C (397.49 mg g^{-1}), E (392.27 mg g^{-1}) and M (379.79 mg g^{-1}) released the highest amounts of FFA from margarine emulsions. The lipases O and P, which had high activities towards rapeseed oil, also led to comparably high FFA contents of 308.70 mg g^{-1} and 269.30 mg g^{-1}, respectively.

Concerning the substrate specificities in margarine, the lipases showed again a trend towards the preferential release of unsaturated FFA (Figure 16). The lipases D, L and N did not match the trend and all had FFA distributions similar to the control sample. Lipase F cleaved preferentially the bonds of C18:1, but not of C18:2 or C18:3. All other lipases released specifically C18:3 (specificity factor 5.84 ± 1.38), C18:1 (3.19 ± 0.20) and C18:2 (1.76 ± 0.20). The substrate specificity factors towards the saturated FA C12:0 (0.22 ± 0.05), C14:0 (0.56 ± 0.22), C16:0 (0.41 ± 0.11) and C18:0 (0.09 ± 0.05) were all below 1, indicating a lack of specificity for saturated FA.

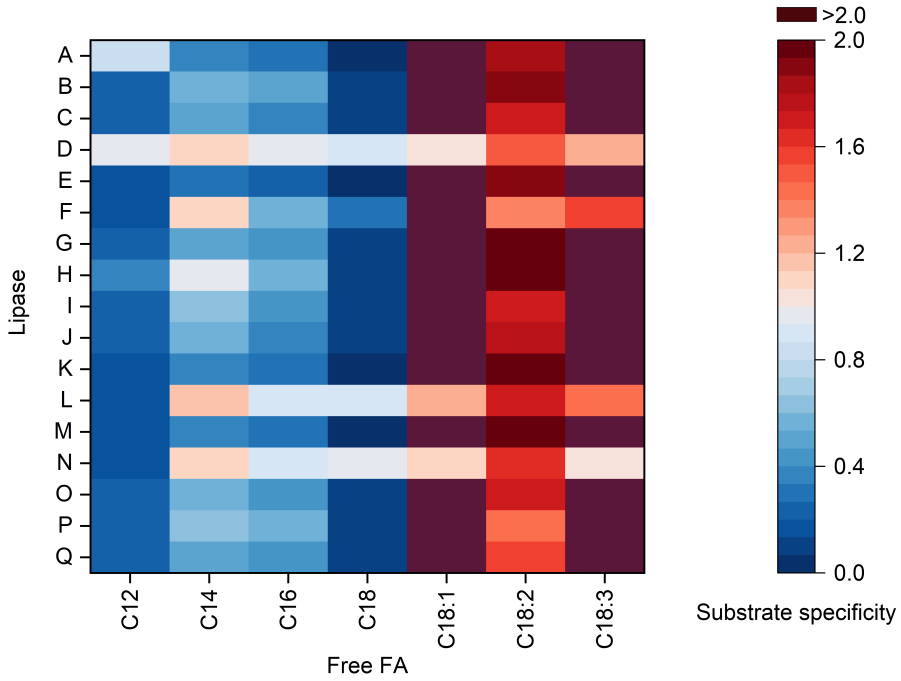


Figure 16: Distribution of free fatty acids (FFA) released by 17 different lipases A to Q from margarine emulsified with Triton X-100 in water. All means (n = 3) were normalised to the results of a control sample without lipase to account for the natural occurrence of the different FFA in the margarine emulsion. Figure modified from Stemler & Scherf [125].

3.1.3.3. Butter

The butter emulsion without lipase addition (control sample) contained 1.04 mg g^{-1} FFA based on its butter content (Table 22). They consisted of 39.8% C16:0, 22.0% C18:1, 17.1% C18:0, 7.8% C14:0, 4.5% C4:0, 3.4% C12:0, 2.3% C18:2, 1.6% C10:0, 0.9% C6:0 and 0.6% C8:0.

After incubation with lipases, the FFA content ranged between 1.35 mg g^{-1} and 258.99 mg g^{-1} (mean: 136.18 mg g^{-1}). As described for rapeseed oil and margarine, the lipases D, N and L released the lowest amounts of FFA (1.35 mg g^{-1}, 1.63 mg g^{-1} and 1.96 mg g^{-1}, respectively). In butter emulsions, the lipases C (258.99 mg g^{-1}), I (254.47 mg g^{-1}) and O (251.14 mg g^{-1}) were most active. The lipases P, E and M, which had released high amounts of FFA in the rapeseed oil and margarine emulsions, led to FFA contents of 186.07 mg g^{-1}, 110.18 mg g^{-1} and 158.87 mg g^{-1}, respectively.

In butter emulsions, the substrate specificity patterns for all but the lipases D, F and N had a trend towards the preferential release of short-chain FA and unsaturated FA (Figure 17). The majority of lipases had high specificities towards the unsaturated FA C18:1 (1.47 ± 0.17) and C18:2 (1.27 ± 0.23) and rather low specificities towards C16:0 (0.79 ± 0.07) and C18:0 (0.54 ± 0.10). Additionally, the majority of lipases also released specifically C4:0 (1.30 ± 0.33), C6:0 (2.96 ± 0.70), C8:0 (2.95 ± 0.64) and C10:0 (1.82 ± 0.23) and less specifically C12:0 (0.91 ± 0.13) and C14:0 (1.01 ± 0.14). Only the lipases D, F and N did not match this trend and had a low activity towards C4:0 (0.28 ± 0.02), C6:0 (0.53 ± 0.07) and C8:0 (0.94 ± 0.04) as well as towards C16:0 (0.76 ± 0.04) and C18:0 (0.76 ± 0.04) while releasing C10:0 (1.53 ± 0.09), C12:0 (1.13 ± 0.07), C14:0 (1.23 ± 0.05) and the unsaturated FA C18:1 (1.42 ± 0.08) and C18:2 (2.00 ± 0.55) with higher specificities.

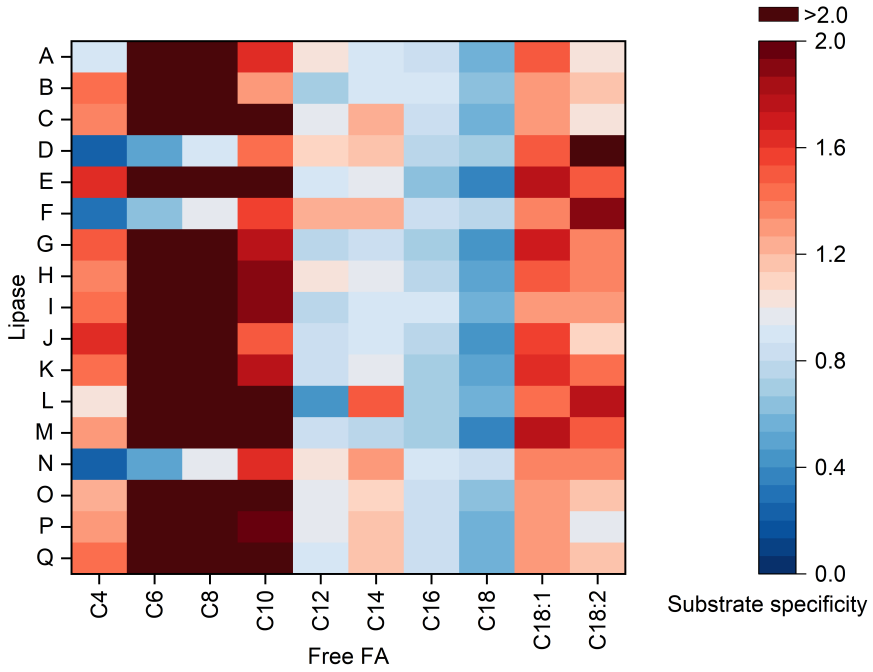


Figure 17: Distribution of free fatty acids (FFA) released by 17 different lipases A to Q from butter emulsified with Triton X-100 in water. All means (n = 3) were normalised to the results of a control sample without lipase to account for the natural occurrence of the different FFA in the butter emulsion. Figure modified from Stemler & Scherf [125].

3.1.3.4. Wheat germ oil

The wheat germ oil control emulsion contained 86.93 mg g^{-1} FFA based on its wheat germ oil content (Table 23). The FFA distribution was 55.5% C18:2, 19.9% C16:0, 15.8% C18:1, 7.9% C18:3 and 0.9% C18:0.

The lipase-treated samples had 58.47 mg g^{-1} to 687.54 mg g^{-1} FFA (mean: 388.48 mg g^{-1}). Again, the lipases D, L and N had the lowest activities leading to FFA contents of 58.47 mg g^{-1}, 75.67 mg g^{-1} and 86.90 mg g^{-1}, respectively. The highest release of FFA was determined for the lipases O (687.54 mg g^{-1}), P (633.34 mg g^{-1}) and M (588.31 mg g^{-1}).

The substrate specificities in wheat germ oil emulsions were less distinct than in the other

emulsions (Figure 18). All substrate specificity factors were between 0.70 and 1.49. The highest specificities of all lipases were the ones of lipase Q towards C16:0 (1.49) and C18:0 (1.23) as well as lipase D towards C18:1 (1.33). The average substrate specificities of all lipases towards the different substrates were close to 1 (C16:0 0.97 ± 0.14; C18:0 0.86 ± 0.12; C18:1 0.99 ± 0.12; C18:2 1.02 ± 0.05; C18:3 0.97 ± 0.07) with a slight tendency towards the preferred release of C18:2.

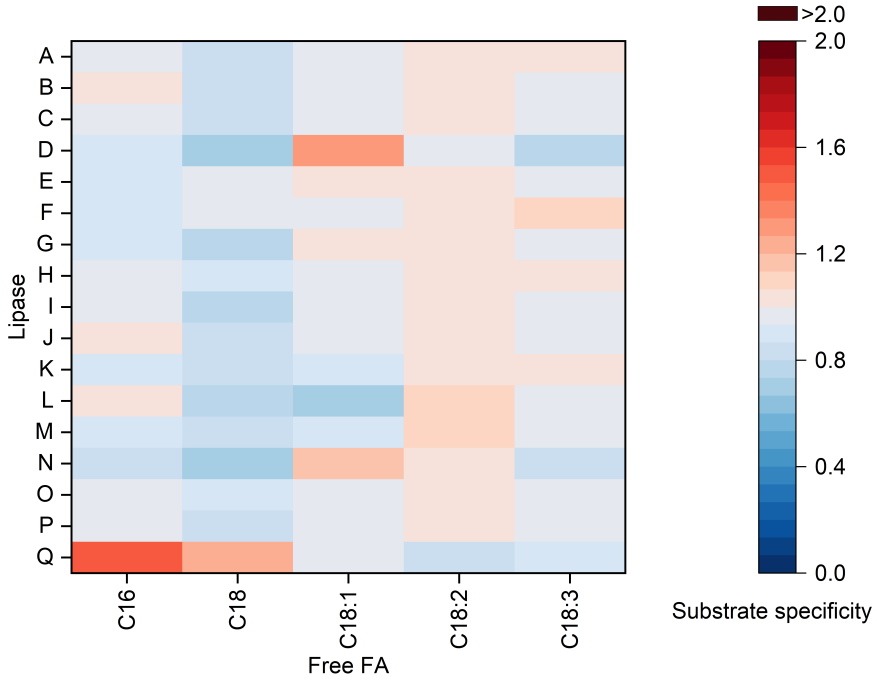


Figure 18: Distribution of free fatty acids (FFA) released by 17 different lipases A to Q from wheat germ oil emulsified with Triton X-100 in water. All means (n = 3) were normalised to the results of a control sample without lipase to account for the natural occurrence of the different FFA in the wheat germ oil emulsion.

3.1.3.5. Time course of the reaction

The influence of the incubation time on the release of FFA and the concomitant substrate specificity patterns was analysed for the reaction of lipase A with butter. Shorter incubation times (60 min - 480 min) instead of 960 min (16 h) were applied (section 5.2.3.4).

The total amount of released FFA varied over time (Table 24). Starting with a total amount of 60.27 mg g^{-1} after 60 min of incubation time, more FFA were released within the following hour, leading to a total amount of 79.53 mg g^{-1} after 120 min. During the next two hours, the FFA amount decreased by 24.7% to 59.91 mg g^{-1} and increased again to 79.04 mg g^{-1} at 360 min of incubation time. At 480 min of incubation time, only 46.49 mg g^{-1} FFA could be detected. The corresponding sample with an incubation time of 960 min had a total FFA content of 235.87 mg g^{-1}.

Although the contents varied, the substrate specificity patterns remained mostly constant during the incubation (Figure 19). The specificity factors towards the long-chain FA C16:0, C18:0, C18:1 and C18:2 varied to a maximum of 22.9%. The greatest variances were measured for the substrate specificity towards C8:0 (33.7%), C10:0 (43.5%), C12:0 (48.3%) and

C14:0 (38.7%). For the short-chain FA C4:0 and C6:0, the maximum variance was only 15.5%. The reaction time therefore had a minor influence on the FFA distribution.

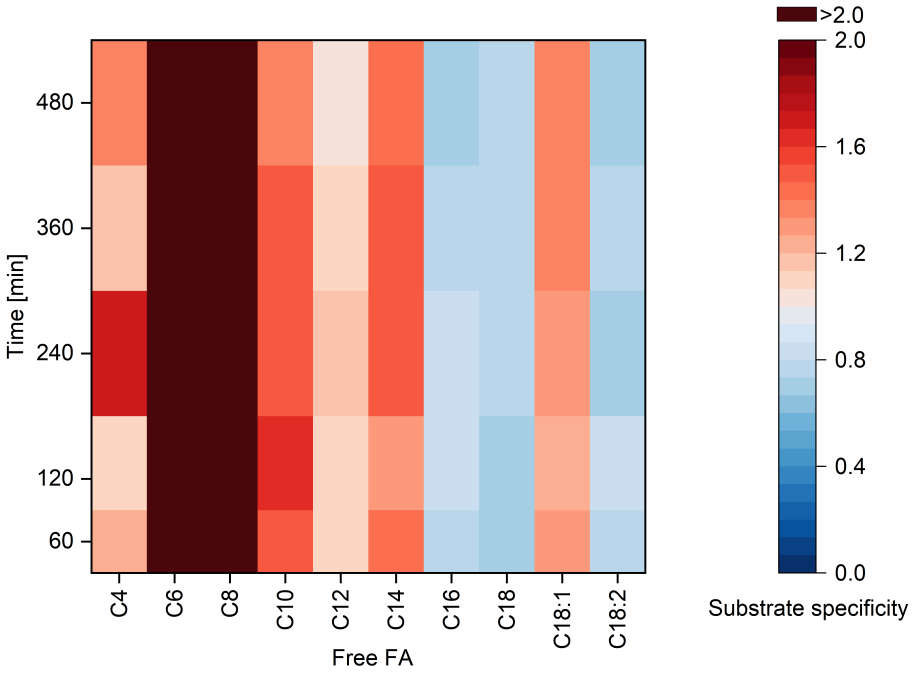


Figure 19: Distribution of free fatty acids (FFA) released by lipase A within several time periods (60 min–480 min) from butter emulsified with Triton X-100 in water, calculated as mean (n = 2). All amounts were normalised with the results of a control sample without lipase to account for the natural occurrence of the different FFA in butter. Figure modified from Stemler & Scherf [125].

3.1.3.6. Impact of the emulsifier

By changing the emulsifier used for the stabilisation of the fat micelles in water, the surface of the micelles is modified. The impact of this modification on the substrate specificity was analysed by testing the three lipases A, J and Q in emulsions stabilised by GA and egg yolk lecithin (EL) instead of Triton X-100. Triton X-100 is a nonionic surfactant used for laboratory applications and cleaning products. It is not commonly used in foodstuff. GA and EL, on the other hand, are both applied in food. GA is a mixture of arabinose- and galactose-based polysaccharides and glycoproteins. EL consists mainly of glycerophospholipids.

Both emulsifiers had an impact on the FFA content of the untreated samples (Table 25). GA led to a decrease of 60% (rapeseed oil emulsion), 43% (margarine emulsion) and 71% (wheat germ oil emulsion) while increasing the FFA content of the untreated butter emulsion by 24%. When EL was used, the FFA content of the control samples was increased by 200% (rapeseed oil), 79% (margarine) and 38% (butter) but decreased by 24% for wheat germ oil.

The total lipase activity was also affected. Whether the release of FFA was enhanced or inhibited was sample-specific and depended on the lipase, the fat and the used emulsifier. In rapeseed oil, e.g., the activity of lipase A was decreased by the use of GA and increased by EL. Lipase J led to higher contents of FFA in both modifications compared to the Triton X-100 emulsion. The activity of lipase Q was lower in the GA-emulsion and comparable to the

Triton X-100 emulsions for the EL-emulsion. In the margarine emulsion and the wheat germ oil emulsion, lipase activities were also both inhibited (lipases A and Q both in the GA- and the EL-emulsion, lipase J in the GA-emulsion) and enhanced (lipase J in the EL-emulsion) in comparison to the Triton X-100 emulsion. In butter, the effect seemed to be lipase-specific, as the activities of the lipases J and Q were lower and the ones of lipase A higher in both the GA- and the EL-emulsion.

The substrate specificities of the lipases again had similar patterns, but depended on the emulsifier (Figure 20).

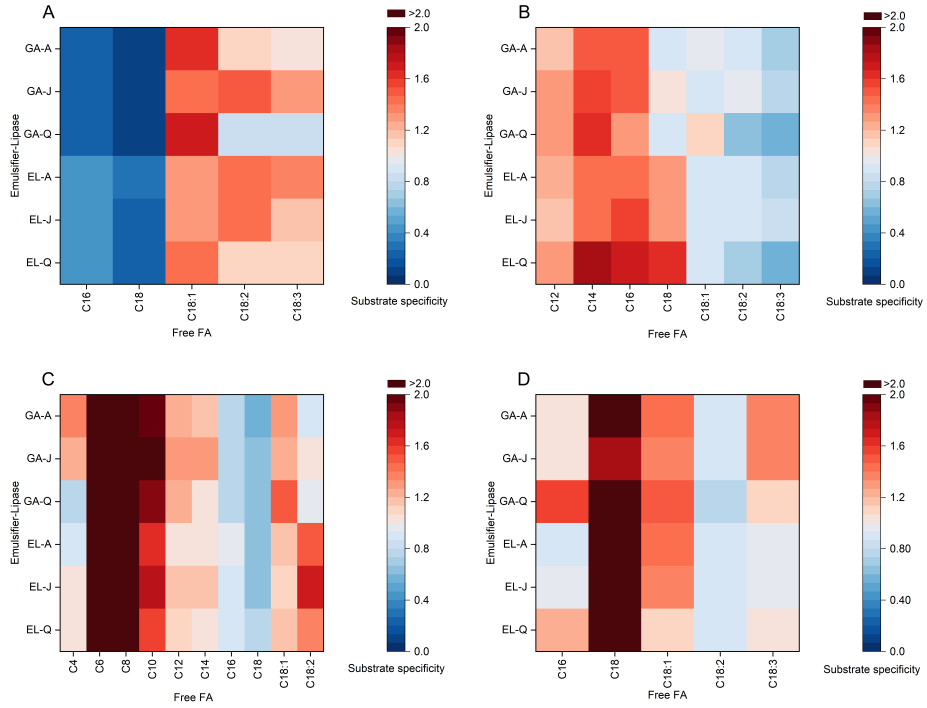


Figure 20: Distribution of free fatty acids (FFA) released by the lipases A, J and Q from rapeseed oil (A), margarine (B), butter (C) or wheat germ oil (D) emulsified with gum arabic (GA) or egg yolk lecithin (EL) in water. All means (n = 3) were normalised to the results of a control sample without lipase to account for the natural occurrence of the different FFA in the emulsions, respectively. Figure modified from Stemler & Scherf [125].

In rapeseed oil, mostly unsaturated FFA were released by all 3 lipases. GA enhanced the release of C18:1 compared to the EL-emulsion (mean substrate specificity factors 1.58 ± 0.11 instead of 1.36 ± 0.07). The trend towards the preferential release of the unsaturated FA C18:2 and C18:3 was smaller than in the Triton X-100 emulsion when the emulsifier was changed (mean substrate specificity factors for C18:2: 1.14 ± 0.27 for GA, 1.32 ± 0.14 for EL and 1.72 ± 0.10 for Triton X-100 and for C18:3 1.04 ± 0.20 for GA, 1.20 ± 0.11 for EL and 1.93 ± 0.07 for Triton X-100).

For the margarine emulsions, the substrate specificity patterns for the lipases were similar for the GA- and the EL-emulsions. The specificities towards C12:0, C14:0, C16:0, C18:1, C18:2 and C18:3 were related, e.g., for C14:0 with a mean specificity factor of 1.58 ± 0.07 in GA-emulsions and 1.56 ± 0.18 in EL-emulsions. The emulsifiers differed in their impact on the

release of C18:0. The share of C18:0 in the FFA distribution of GA-emulsions was diminished after incubation with lipases (mean substrate specificity factor 0.94 ± 0.06) and increased in EL-emulsions (1.42 ± 0.16). The results from both the GA- and the EL-emulsions are in contrast to the findings for the Triton X-100 emulsions, were mostly unsaturated FFA were released from margarine.

In the butter emulsions, the patterns resembled the ones for the Triton X-100 emulsions. GA and EL had a similar impact on the release of C4:0, C12:0, C14:0, C16:0, C18:0 and C18:1. The activity of the lipases towards C6:0, C8:0 and C10:0 was less in the EL-emulsions than in the GA-emulsions, e.g., for C10:0 with mean substrate specificities of 2.01 ± 0.12 in GA-emulsions and 1.66 ± 0.10 in EL-emulsions. By contrast, EL enhanced the release of C18:2 (mean substrate specificity factor 1.50 ± 0.14 instead of 0.97 ± 0.06 for GA-emulsions).

For wheat germ oil, the trends for the substrate specificity were similar with both GA and EL as emulsifier. Compared to the Triton X-100 emulsion, more C18:0 was released (mean substrate specificity factor 2.07 ± 0.21 for GA and 2.41 ± 0.28 for EL compared to 0.96 ± 0.20 for Triton X-100).

To sum up, a change of the emulsifier had an impact on the substrate specificity patterns of the three lipases A, G and J. Within one system (same emulsifier and same fat), they again behaved similarly.

3.1.4. Sensory analysis of lipase-treated model emulsions

The effect of released FA on the flavour of the model emulsions was analysed with the help of a sensory panel. This approach was chosen to include all possible effects on the flavour and not to limit the analysis to certain classes of substances like FFA, as would have been necessary in an instrumental setup.

3.1.4.1. Determination of odour thresholds

Before the sensory analysis of lipase-treated model emulsions was carried out, the panel was trained to detect rancid off-flavours. C4:0 was used as a model substance and the sensitivity of the panel was checked by evaluating its odour thresholds for C4:0 in different matrices (Table 4). Besides water, the odour thresholds were also determined for the previously established emulsions with fats frequently used in baking (rapeseed oil, margarine, butter and wheat germ oil emulsified with Triton X-100). In water, the panel recognised an off-flavour starting from a C4:0 concentration of 0.97 mg L^{-1}. The odour threshold was lower in rapeseed oil emulsions (0.87 mg L^{-1}) and margarine emulsions (0.92 mg L^{-1}). The panel stated that the inherent odour of the emulsions facilitated the detection of a different flavour. The odour threshold in butter corresponded to the one in water, probably due to the inherent content of C4:0 in butter. The strong inherent odour of wheat germ oil inhibited the detection of C4:0 and led to an odour threshold of 8.00 mg L^{-1}.

Table 4: Odour thresholds for butyric acid in different matrices. Table modified from Stemler & Scherf [126].

Matrix	Odour threshold for butyric acid [mg L^{-1}]
Water	0.97
Rapeseed oil emulsion	0.87
Margarine emulsion	0.92
Butter emulsion	0.97
Wheat germ oil emulsion	8.00

3.1.4.2. Description of the flavours of short-chain fatty acids

Besides C4:0, the panel was also trained to recognize the flavours of the FFA C6:0, C8:0 and C10:0. All were described as off-flavours (Table 5). The concentrations used influenced the description of the flavour. While low concentrations (1 mg L^{-1}) of butyric acid were described as “buttery”, higher concentrations (10-50 mg L^{-1}) led to a vomit-like flavour.

Table 5: Flavours of the short-chain fatty acids butyric acid (C4:0), caproic acid (C6:0), caprylic acid (C8:0) and capric acid (C10:0). Table modified from Stemler & Scherf [126].

Fatty acid	Flavour description by the panel
C4:0	buttery, cheesy, vomit
C6:0	pungent, musty
C8:0	like plastic, rancid
C10:0	pungent, acidic, fruity

3.1.4.3. Sensory analysis

All lipases were characterised by sensory analysis concerning the flavours caused by their reaction with emulsified rapeseed oil, margarine, butter and wheat germ oil. The results were evaluated using the Thurstone model (section 5.2.4.3) and samples which were correctly identified by at least 67% of the panellists were considered as significantly different.

If significant off-flavours were developed depended both on the lipase and the fat (Table 6). Originally, only rancid off-flavours were expected. They were caused by, e.g., lipase A in the butter emulsion (“pungent, vomit”) or lipase C in the wheat germ oil emulsion (“rancid, buttery”). However, some lipases also led to off-flavours which were not rancid or considered as negative off-flavours, such as the combination of lipase L and wheat germ oil (“less intense”) or lipase P and margarine (“grassy, fruity”). This phenomenon only occurred for specific combinations of lipases and fats.

In the butter emulsion, 70% of all lipases led to an off-flavour. This was more than in the plant-based fat emulsions: in the rapeseed oil emulsion, only 35% of all lipases caused an off-flavour, only 29% in emulsified wheat germ oil and only 18% in emulsified margarine.

For the rapeseed oil emulsion, rancid off-flavours were caused by the lipases B, E, G, J, N, P and Q. The lipases H and I also had a significant impact on their flavour.

Table 6: Sensory analysis of the reaction of lipases in emulsions of rapeseed oil/margarine/butter/wheat germ oil. Results are given as the percentage of panellists who were able to correctly identify the sample and the corresponding descriptions of the deviating odour. Values greater than 67% are marked in colour: rancid off-flavours are marked in yellow and other flavours in green. Table modified from Stemler & Scherf [126].

Lipase	rapeseed oil emulsion	margarine emulsion	butter emulsion	wheat germ oil emulsion
A	66.7% sweaty, buttery	60.0% buttery, rancid	100.0% pungent, vomit	60.0% not buttery
B	100.0% cereal-like, rancid	75.0% cereal-like, pungent	100.0% pungent, vomit	100.0% buttery, acrid
C	66.7% nutty	50.0% milky, rancid	100.0% pungent, vomit	100.0% rancid, buttery
D	50.0% fresh	40.0% buttery, cereals	42.9% slightly rancid	60.0% rancid
E	71.4% nutty, rancid	33.3% buttermilk	100.0% acidic, buttermilk	42.9% sweetish
F	40.0% cheesy	20.0% rancid	33.3% pungent, rancid	50.0% chemical
G	100.0% like plastic, musty	66.7% old, rancid	100.0% cheesy, vomit	100.0% rancid
H	80.0% milky, flowery	66.7% pungent, grassy	80.0% cheesy	50.0% like gas
I	100.0% sweetish, milky	60.0% milky, like yoghurt	100.0% pungent, vomit	80.0% buttery, acidic
J	100.0% rancid	100.0% pungent, nutty	100.0% pungent, acidic	88.9% fishy, green
K	66.7% fatty, rancid	66.7% oily, pungent	100.0% cheesy, pungent	50.0% less intensive
L	42.9% rancid, flowery	83.3% hay, cheesy	62.5% rancid, cheese	100.0% less intensive
M	66.7% cheesy	75.0% cheesy, sweaty	100.0% cheesy, pungent	75.0% milder
N	80.0% rancid	75.0% green, chemical	50.0% rancid	33.3% milder
O	60.0% pungent, rancid	100.0% cereal, sweaty	100.0% cheesy, acidic	100.0% fresh, pungent
P	100.0% rotten, foul	100.0% grassy, fruity	100.0% cheesy, salty	100.0% fishy, grassy
Q	100.0% mushroomy, like manure	62.5% buttery, acidic	100.0% cheesy, vomit	83.3% rancid, musty

In margarine emulsions, the lipases B, J, L, M, N and O led to either rancid or “chemical-like” off-flavours. Lipase P caused a “grassy and fruity” flavour.

In the butter emulsion, only four lipases (D, F, L and N) did not cause an off-flavour. Lipase E was the only lipase leading to a non-rancid flavour, namely “acidic and buttermilk-like”.

For the wheat germ oil emulsion, the lipases B, C, G, I, O and Q caused rancid off-flavours. The lipases J and P led to a “fishy” flavour. The flavours caused by the lipases L and M were neither rancid nor fishy but significantly “less intense” or “milder”.

3.1.5. Discussion

The FA substrate specificity of 17 lipases was screened by using both artificial substrates (*p*-nitrophenyl assay) and a competitive assay with natural substrates in emulsions as model systems. In the emulsions, in addition to the released FFA, the concomitant off-flavours were monitored by the use of a trained sensory panel.

The *p*-nitrophenyl assay was suitable for the analysis of FA substrate specificities of 16 out of the 17 lipases. Lipase D did not react with any of the *p*-nitrophenyl esters under the conditions of the assay. According to the manufacturer’s instructions for lipase D, its maximum activity occurs at a pH range from 4.0 - 6.5. The *p*-nitrophenyl assay was performed at a pH of 7.5 due to the fact that *p*-nitrophenol, which is used for detection, is colourless under acidic conditions. Additionally, lipase D was described to work best at a temperature of 45 °C while the assay was done at room temperature (approx. 22 °C). Therefore, the chosen reaction conditions were not suitable for the reaction of lipase D. This is a drawback of the assay and pH-independent methods to monitor lipase FA substrate specificities are needed for the characterisation of further lipases.

The *p*-nitrophenyl assay revealed several different patterns for FA substrate specificities of the lipases. Interestingly, most lipases reacted faster with middle-chain FA such as C8:0, C10:0 and C12:0. This phenomenon has not been described previously in comparable studies on the FA substrate specificities of lipases [127, 128] and might present a property specific for baking lipases.

The transferability of the results from the *p*-nitrophenyl assay to competitive assays using natural substrates was checked by evaluating the FA substrate specificity of the lipases when reacting with fats frequently used in baking. The model systems developed for this reaction fulfilled all three previously stated requirements (inclusion of spreadable fats, comparability to the *p*-nitrophenyl assay and stability, section 3.1.2). First, the melting process for spreadable fats led to reliable results, although partly recrystallisation occurred over time. Second, the reaction conditions were similar to the conditions used for the *p*-nitrophenyl assay. Third, the emulsions were stable with unaltered particle size distributions for rapeseed oil, margarine and wheat germ oil as fat phase. Only small changes between storage times of 3 h and 24 h in the Sauter diameter were measured for butter emulsions. The established emulsions were thus suitable for the analysis of lipase reactions.

The GC method which was developed for the quantification of FFA included all main FA known for the FA distributions of the used fats [129, 130]. Compared to the method established by Mannion et al. [121], the internal standard margarinic acid (C17:0) was changed to nonadecanoic acid (C19:0) to account for the natural occurrence of C17:0 in margarine. Additionally,

the GC runtime was slightly shortened (32 min instead of 33.67 min) and the detection of C4:0 was improved by removing excess *n*-butanol from the mixture before injection (section 5.2.3.1). If *n*-butanol was left in the mixture, its signal overlapped with the signal of C4:0. This problem was dealt with by the introduction of a washing step prior to injection. The combination of lipase reaction in emulsion and analysis of the released FFA was reproducible.

When applied to rapeseed oil, the method revealed a FFA content of 0.70 mg g^{-1} for untreated rapeseed oil emulsions 16 h after preparation. This is less than the contents reported for rapeseed oil in literature (5 mg g^{-1} - 29 mg g^{-1} [131, 132]). The difference is probably due to the emulsification and the long incubation time. During both processes, losses due to evaporation of FFA or oxidative degradation could occur.

The total lipase activity as measured by contents of FFA after treatment with lipases were similar to the activities determined in the *p*-nitrophenyl assay. The lipases D, F, L and N showed the lowest activities in both approaches. The lipases with highest activities, however, were not consistent in both experiments. This is probably due to the differences in substrates and reaction times.

The FFA distribution of the rapeseed oil control sample corresponded to the FFA distributions reported in literature [132]. Compared to the FA in the TG fraction [129], it contained less unsaturated FA. The preferential release of unsaturated FA therefore led to FFA distributions similar to the FA distribution of bound FA. However, this shift of the FFA distribution back to the FA distribution of bound lipids and therefore towards more unsaturated FA did not occur for lipases with low activities. When they were applied, the FFA distribution after lipase treatment was similar to the one of the control sample.

The loss of unsaturated FFA at low FFA concentrations could be due to the fact that they are more prone to oxidation than saturated FFA and accordingly, they are degraded faster. FFA are more polar than TG and thus migrate to the surface of lipid micelles. In oil-in-water emulsions, oxidative processes occur mostly at the surface of lipid micelles. At the lipid-water-interface, lipids come into contact with pro-oxidative substances from the water phase which accelerate their oxidative degradation [133]. This mechanism applies to low levels of FFA as found in the control sample and the samples of lipases with low activities. With increasing levels of FFA, the micelle surfaces become saturated with FFA. Therefore, high amounts of FFA including unsaturated FFA do not come into contact with the pro-oxidative substances from the water phase. They are therefore not degraded and stay intact. This hypothesis is supported by the fact that with increasing lipase activities, the share of unsaturated FFA within the FFA fraction increased. Alternatively, the FA substrate specificity of the lipases could cause the phenomenon. This seems, however, rather unlikely as 14 of 17 lipases reacted similarly.

Besides FA substrate specificity, also further lipase specificities as positional preferences could influence the release of FFA. FA are not distributed randomly over the TG backbone but show trends between the outer *sn*1/*sn*3 positions versus the inner *sn*2 position. Again, this explanation seems not applicable, as it would need to affect the majority of lipases.

For margarine, no data on the contents or the distribution of FFA was available. The total lipase activities were again similar albeit not corresponding to the ones from the *p*-nitrophenyl

assay. Compared to rapeseed oil, overall higher contents of FFA were released. This could be due both to varying lipase activities towards the substrates and the lower particle sizes of the margarine emulsion. With decreasing particle sizes, more substrates become accessible for lipases and thus increase the reaction rates [111]. Unlike rapeseed oil, margarine is an emulsion. During its production, different emulsifiers such as proteins or lecithins are added for stabilisation. These emulsifiers are amphiphilic and reattach to the surface of the new oil-in-water emulsion. They influence the structure of the surface and could facilitate the release of FFA. Additionally, the aggregate state of fats has an impact on the processes at the surface of micelles [133] and could also affect the lipase activity. The FFA distribution of the margarine control sample corresponded to the FA distribution of the TG fraction reported in literature [130, 134, 135].

Again, most lipases released preferentially unsaturated FFA. Although differences between the lipases concerning their specificities towards short- and middle-chain FA were determined in the *p*-nitrophenyl assay, the lipases had similar specificity patterns when reacting with emulsified margarine. The results from the *p*-nitrophenyl assay could not be confirmed. It has already been stated before that the results from the *p*-nitrophenyl assay are not easily reproduced when working with natural substrates, even if non-competitive approaches are used [136]. Instead, other factors seemed to be more important. As these factors were the same for most lipases, they were most probably linked to the emulsion and not to the lipases. In accordance with the effects on the lipase activity, the specificity could also be determined by the accessibility of different FA at the micelle surface. The different structure of unsaturated and saturated FA could cause differences and thus influence their possibilities for hydrolysis.

According to literature, untreated butter samples contain 1.49 - 1.96 mg g^{-1} FFA [120, 121]. Similar as described for rapeseed oil, the FFA contents of the emulsions were lower than described for non-emulsified samples. Again, this was probably due to emulsification and incubation times. The FFA distribution of the butter control sample corresponded to the one reported for untreated butter [120, 121]. Compared to the TG fraction [129], the share of C6:0, C8:0, C10:0 and C12:0 was lower. This could be due to their higher volatility compared to longer-chain FA. However, this phenomenon did not occur for the share of C4:0 in the FFA distribution of the control sample, which was not reduced compared to the values reported for the TG fraction. As described for rapeseed oil and margarine emulsions, the total lipase activities did not directly correspond to the ones detected by the *p*-nitrophenyl assay.

Also as described for rapeseed oil and margarine emulsions, similar FA substrate specificity patterns were found for most lipases when reacting with butter. They had high specificities towards C4:0, C6:0, C8:0 and C10:0 and also preferentially released unsaturated FFA. The apparent specificity towards C6:0, C8:0 and C10:0 can be explained by the adjustment of the FFA distribution to the distribution of FA in the TG fraction. The alleged specificity was therefore due to normalisation by the FFA fraction of the control sample, which contained only low amounts of free C6:0, C8:0 and C10:0. The high specificity factors towards C4:0, however, were not caused by normalisation. Its share of the FFA fraction of the control sample corresponds to the one reported for the TG fraction. Most lipases therefore had a true preference for the release of C4:0. The determined FA substrate specificity patterns for the reaction with emulsified butter

did not correspond to the results from the *p*-nitrophenyl assay. Similar as described for margarine, the FA substrate specificity was not crucial for the reaction. Instead, the accessibility of FA seemed to determine their hydrolysis by lipases. Probably, high amounts of C4:0 were located at the micelle surface due to its higher polarity compared to longer chain FA.

The wheat germ oil control sample was found to be comparably rich in FFA. This is in accordance with the FFA reported in literature, which were described to range from 50 mg g^{-1} to 250 mg g^{-1} [137]. The FFA distribution of the wheat germ oil control sample also corresponds to FFA distributions including high levels of unsaturated FFA [129, 138, 139]. Compared to rapeseed oil, both the higher contents of FFA and antioxidant substances including tocopherols and carotenoids, which were described for wheat germ oil [139], could prevent the loss of unsaturated FFA. The total lipase activities were higher than in the emulsions of rapeseed oil, margarine or butter. For rapeseed oil and butter, the smaller particle sizes of the wheat germ oil micelles could be the cause for this phenomenon, however, the margarine emulsion had an overall smaller particle size than the wheat germ oil emulsion. The lipase activities were therefore also influenced by the fat and not only the particle size.

Concerning the FA substrate specificity, all lipases had similar specificity factors towards the complete range of FFA in wheat germ oil. Although similar FFA as in rapeseed oil were released, there was no trend towards the release of unsaturated FFA. This supports the hypothesis that with increasing contents of FFA, less unsaturated FFA are lost by oxidative degradation. Additionally, these findings underline the importance of the emulsion for the resulting FA substrate specificity patterns.

The reaction time only had a minor influence on the FFA distribution of a butter emulsion incubated with lipase A. This is in accordance with previous findings from Schmitt et al. [109], who also reported a time-independent FA substrate specificity when analysing the reaction of lipases in a randomised oil. The reaction time is therefore not decisive for the FFA distribution after treatment with lipases.

The assumption that the emulsion and the properties of the micelle surface determine the release of FA was further analysed by using the emulsifiers GA and EL instead of Triton X-100. The properties of the micelle surface are mainly controlled by the chosen emulsifier [133]. Differences in the changes of total lipase activity were probably due to the different properties of the emulsions such as viscosity and droplet size. Additionally, the use of EL introduced further substrates to the reaction. This result matches similar findings as described by Reis et al. [24], who also reported a dependency of enzyme activity on the chosen emulsifier.

The change of the emulsifier influenced the resulting FA substrate specificity patterns. The specificities were consistent within samples of the same fat and the same emulsifier. While the lipase activity mostly depended on the lipase, the specificity was determined by the emulsifier. The assumption was thus confirmed.

The impact of the determined lipase activities and FA substrate specificities on the flavour of the emulsions was analysed by the use of a trained sensory panel.

It detected C4:0 in water with a similar sensitivity than other panels reported in literature [140, 141]. Similar limits of detection as in water were found in rapeseed oil, margarine and butter emulsions while the panel worked with a lower sensitivity in wheat germ oil emulsions. The sensitivity of the panel was most likely affected by the comparatively strong inherent odour of wheat germ oil compared to the other fats. The panel recognised the flavours of C4:0, C6:0, C8:0 and C10:0 as off-flavours and used common descriptions for the off-flavours [142, 143, 144]. Low amounts the FFA were described with deviating flavours which were not necessarily rancid. An example of a non-rancid deviating flavour was, e.g., “buttery” for low amounts of C4:0.

Indeed, the lipases did not only lead to rancid off-flavours in the emulsions, but also caused other changes in flavour. Besides low amounts of FFA, these could also be due to degradation products of FFA. *Cis*-3-hexenal was reported to smell “green” and *cis*-4-heptenal “fishy” [145]. Both are oxidative degradation products of C18:3.

Both the odour threshold for rancid off-flavours and the fat influenced the number of lipases leading to off-flavours. Less lipases than for the rapeseed oil emulsions caused off-flavours in wheat germ oil emulsions. However, this was not applicable for the margarine emulsion, where even less lipases caused off flavours than in the wheat germ oil emulsions, although the sensitivity in margarine was reported to be higher than in wheat germ oil. This might be due to the fact that the total lipase activities as determined by GC in wheat germ oil were higher than in margarine. Additionally, more unsaturated FFA were released and thus probably more oxidative degradation reactions with concomitant flavour-active reaction products occurred.

In the rapeseed oil emulsions, the total amount of released FFA was not in accordance with the detection of off-flavours. The lipases P and E had high activities and caused an off-flavour, however, so did lipase N which had the second lowest activity of all lipases in the rapeseed oil emulsion. Further substances therefore seemed to influence the resulting flavour.

This trend was similar for the margarine emulsion, where also no accordance between total content of released FFA and resulting off-flavour could be established. Although the lipases N and L had low activities, they led to off-flavours, while the lipases A, C, I and K all released high amounts of FFA and did not lead to an off-flavour.

For the butter emulsion, the detection of rancid off-flavours by the panel correlated with the total amount of released FFA. Only lipases which were found to release only low amounts of FA via GC did not cause off-flavours. In butter, the release of short-chain FA was most likely decisive for the occurrence of rancid off-flavours, while in plant-based oils, the degradation of unsaturated FFA was more important.

This assumption was highlighted by the results for wheat germ oil, which also did not match the total FFA contents after incubation with lipases.

Possible candidates for further experiments according to the results from the sensory analysis were the lipases A and K which both caused a rancid off-flavour exclusively in combination with butter and the lipase E which only led to a rancid off-flavour in the rapeseed oil emulsion.

To sum up, when reacting with fats frequently used in baking, 17 lipases showed similar FA substrate specificity patterns. Still, their FA substrate specificities had been determined as different from each other with the use of the *p*-nitrophenyl assay. The lipase activity seemed to

influence the FA substrate specificity, as shown for the lipases D, F, L and N. Similarities between the lipase reactions in emulsions were most probably due to different accessibilities of the FA at the micelle surfaces. Both the fat structure and the surface properties of micelles were therefore suggested as decisive factors. This hypothesis was further proven by varying the reaction time and the emulsifiers used for the reaction. The time did not impact the FA substrate specificity patterns, but a change of emulsifiers led to shifts in the patterns. The FA substrate specificity as determined in the *p*-nitrophenyl assay therefore describes inherent properties of the lipases which are only applicable if the availability of all FA is similar. The assay can thus not be used for the prediction of lipase reactions with complex fats. Neither can it be used for the prediction of off-flavours caused by lipases. The occurrence of off-flavours was most likely linked to lipase activity if short-chain FA were present and linked to further reactions of the FFA if unsaturated FFA were released. For following baking trials, lipases which do not lead to off-flavours by releasing short-chain FA were needed. However, whether short-chain FA were released did not depend on the lipases themselves, but on their surroundings. Further sensory experiments with lipase-treated fine bakery goods were therefore needed. In case all FA were similarly accessible, the specificities as characterised by the *p*-nitrophenyl assay would come into play. They therefore serve as a reference point for the selection.

For further experiments, a range of seven lipases was chosen (Table 7). The lipases all showed low FA substrate specificities in the *p*-nitrophenyl assay. Additionally, several manufacturers were included in the following experiments.

Table 7: Lipases chosen for further experiments.

Lipase	Type of lipase	Manufacturer
A	Phospholipase	1
E	Phospholipase	2
G	Unknown	3
J	Phospholipase	4
K	Galactolipase	2
M	Phospholipase	2
O	TG lipase	2

3.2. Lipases as improvers for the baking quality of fine bakery goods

The following parts were already published in *LWT - Food Science and Technology* [146] and *Food Chemistry: X* [97].

The effects of the seven lipases A, E, G, J, K, M and O on the baking quality in terms of both improvement of batter and dough quality and the improvement of product quality were assessed. Three recipes, an eggless basic cake, a traditional pound cake and a yeast-based brioche, were chosen for the experiments. For comparison, both an untreated control sample and the traditional emulsifier DATEM were included in the analysis.

3.2.1. Improvement of batter and dough quality

The analysis of batter and dough quality comprised the parameters density and stickiness. Additionally, a rheological characterisation of the samples including an amplitude sweep, a frequency sweep and a temperature sweep was carried out.

Before analysing the effect of lipase addition, the untreated batters and doughs were characterised in terms of their pH value (Table 8). Basic cake batter was slightly basic while pound cake batter had a neutral pH. For brioche, both pre-dough and dough were rather acidic with a maximum pH of 5.7.

Table 8: pH values of batters and doughs.

Recipe	pH value
Basic cake	7.7-8.0
Pound cake	7.0-7.1
Brioche (pre-dough)	5.5-5.7
Brioche	5.4-5.5

3.2.1.1. Density and stickiness

The density of lipase-treated basic cake batters ranged from 1.04 - 1.09 g mL^{-1} (Table 9). The two lipases G and J led to significantly lighter batters (1.04 g mL^{-1} and 1.05 g mL^{-1}, respectively) compared to the control batter without lipase treatment (1.08 g mL^{-1}). DATEM had no significant effect on the density of basic cake batter. Pound cake batters had a lighter density compared to basic cake batter with values ranging from 1.00 - 1.06 g mL^{-1} (control batter: 1.01 g mL^{-1}). Neither lipase nor DATEM affected the density to a significant extent. The density of brioche doughs was higher than the densities of basic cake and pound cake batters. It was between 1.22 g mL^{-1} and 1.23 g mL^{-1} for all samples and not significantly affected by the addition of lipases or DATEM.

Table 9: Density, linear viscoelastic region (LVE), storage modulus at the end of LVE (G') and crossover point of basic cake batter, pound cake batter and brioche batter. Asterisks indicate a significant difference to the respective control (ANOVA with Dunnett's t-test, $p \leq 0.05$, n = 3-9). Data already published in Stemler & Scherf [146].

Modification	Density [g mL^{-1}]	LVE [% strain]	G' [Pa]	Cross-over point [% strain]
Basic cake				
Control	1.081 ± 0.003	0.079 ± 0.000	3126.7 ± 166.8	51.55 ± 1.96
DATEM	1.090 ± 0.007	0.084 ± 0.004	2216.7* ± 363.5	50.28 ± 2.37
A	1.060 ± 0.003	0.129* ± 0.002	1483.3* ± 203.7	30.99* ± 1.01
E	1.092 ± 0.009	0.086 ± 0.004	1930.0* ± 196.1	47.72 ± 2.91
G	1.041* ± 0.004	0.133* ± 0.005	1040.0* ± 134.9	27.85* ± 2.31
J	1.050* ± 0.004	0.135* ± 0.005	811.0* ± 127.1	20.12* ± 3.56
K	1.092 ± 0.007	0.087 ± 0.003	1563.3* ± 204.2	35.79* ± 1.56
M	1.091 ± 0.012	0.086 ± 0.001	1673.3* ± 159.2	45.09 ± 3.80
O	1.094 ± 0.009	0.081 ± 0.005	1740.0* ± 153.0	38.90* ± 3.37
Pound cake				
Control	1.009 ± 0.003	0.123 ± 0.007	2136.7 ± 191.5	20.98 ± 2.36
DATEM	1.064* ± 0.004	0.128 ± 0.009	1793.3 ± 293.2	20.12 ± 1.29
A	1.028 ± 0.015	0.125 ± 0.006	971.3* ± 191.0	11.46* ± 2.59
E	1.033 ± 0.010	0.130 ± 0.004	1440.0* ± 129.6	21.47 ± 1.70
G	1.003 ± 0.005	0.127 ± 0.004	1203.3* ± 107.8	14.54* ± 2.00
J	1.042 ± 0.009	0.126 ± 0.003	1106.7* ± 66.0	14.58* ± 0.30
K	1.058* ± 0.033	0.117 ± 0.002	1443.3* ± 26.2	15.38* ± 0.79
M	1.048 ± 0.011	0.121 ± 0.002	1553.3* ± 216.4	19.49 ± 2.23
O	1.051* ± 0.015	0.124 ± 0.003	1570.0* ± 77.9	19.21 ± 1.69
Brioche				
Control	1.217 ± 0.004	0.083 ± 0.005	53300.0 ± 12221.3	70.24 ± 4.28
DATEM	1.228* ± 0.003	0.080 ± 0.004	71433.3 ± 4160.4	71.60 ± 7.30
A	1.219 ± 0.003	0.078 ± 0.001	59333.3 ± 1755.6	71.03 ± 8.16
E	1.224 ± 0.002	0.077 ± 0.002	67233.3 ± 3680.9	75.30 ± 2.68
G	1.227* ± 0.001	0.081 ± 0.002	57766.7 ± 3880.1	69.55 ± 6.75
J	1.226 ± 0.004	0.081 ± 0.002	45466.7 ± 4129.8	74.37 ± 2.10
K	1.221 ± 0.001	0.080 ± 0.005	57433.3 ± 6715.3	70.25 ± 3.61
M	1.224 ± 0.003	0.082 ± 0.004	56566.7 ± 8622.6	64.09 ± 3.93
O	1.226* ± 0.003	0.079 ± 0.002	64666.7 ± 3578.0	70.84 ± 4.23

The stickiness of basic cake batter ranged from 150.17 g s - 269.22 g s (Figure 21). The stickiness of the control sample (262.13 g s) was reduced significantly by the lipases A (by 32.7%), G (by 42.7%) and J (by 36.5%). Pound cake batter had a similar stickiness as basic cake batter (203.58 g s - 241.24 g s). None of the added lipases had a significant effect on its stickiness. For brioche, the stickiness of both the control and the treated doughs was higher compared to basic cake and pound cake (812.32 g s - 1172.93 g s). Similar as for pound cake, the stickiness was not affected by the addition of lipases. DATEM did not lead to significant improvements of the stickiness in any of the three recipes.

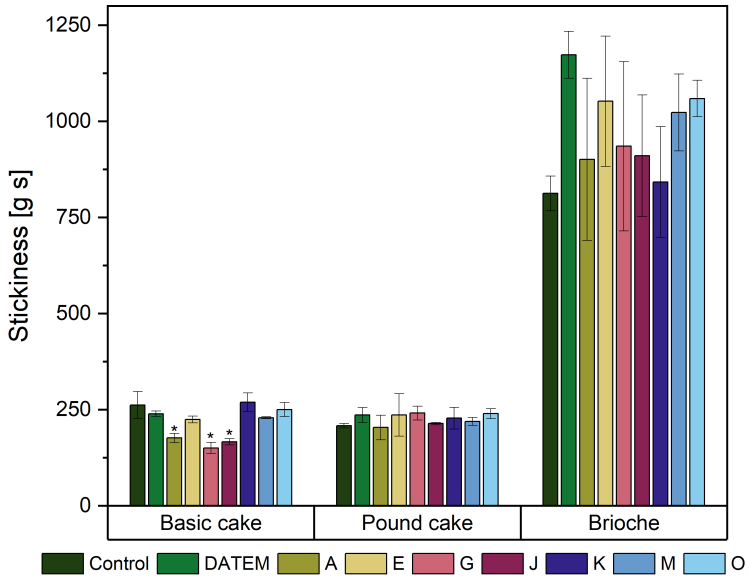


Figure 21: Stickiness of differently treated batters of basic cake, pound cake and brioche batters. Control: sample without addition of improver; DATEM: sample with addition of DATEM; A-O: sample with addition of the respective lipase. Asterisks show a significant difference to the respective control (ANOVA with Dunnett's t-test, $p \leq 0.05$, n = 3). Figure modified from Stemler & Scherf [146].

3.2.1.2. Basic cake

The amplitude sweeps of basic cake batters showed increasing tanδ for increasing strains (Figure 22). The lipases A, G and J led to higher tanδ and therefore enhanced the liquefaction of the batters at higher strains. This was in accordance with their enlargement of the LVE by 63.3% (lipase A), 68.4% (lipase G) and 70.9% (lipase J) compared to the control (Table 9). All lipases and DATEM reduced G' at the end of the LVE significantly with reductions ranging from 29.1% (DATEM) to 74.1% (lipase J). The onset of flow (the cross-over point) occurred at significantly lower strains for the lipases A (reduction by 39.9%), G (46.0%), J (61.0%), K (30.6%) and O (24.5%).

During the frequency sweep (Figure 22), all batters led to curves with similar shapes and a low-point around 3 rad s^{-1}. The batters had solid-like behaviour during fast and slow motions with tanδ<1 throughout the whole measurement. The batters containing either DATEM or lipases had higher tanδ than the control: At 1 rad s^{-1}, tanδ of the control was 0.30 while the batters treated with DATEM or the lipases E and M ranged between 0.34 - 0.35 and the batters treated with the remaining lipases A, G, J, K and O between 0.37 - 0.40. After the low point, tanδ increased for all lipases. Two lipases led to a deviation during this increase: Lipase A inhibited the increase of tanδ after the low point, resulting in a final tanδ of 0.38 at 100 rad s^{-1}. Lipase K led to an increase of tanδ at the highest frequencies and caused a final tanδ of 0.53 at 100 rad s^{-1}.

For the temperature sweep (Figure 22), the batters could be divided into three groups according to their behaviour during increasing temperatures. The first group comprised the control and the batters treated with DATEM and the lipases K and O. For this group, tanδ increased up to its first peak at 35 °C (tanδ 0.61-0.64). After the peak, tanδ decreased until 70 °C and had a second peak around 76 °C (tanδ 0.44-0.47). Final tanδ were between 0.24 - 0.29. The second group included the batters treated with the lipases E and M. Their behaviour was similar to group 1. Tanδ first increased until its first peak (34.4 °C, tanδ 0.66 - 0.67) and further decreased up to 70 °C. During this decrease, tanδ was higher for group 2 than for group 1, e.g., at 60.1 °C it was 0.52 - 0.53 for group 1 and 0.57 for group 2. The second peak was again at around 76 °C with a tanδ of 0.48 for lipase E and 0.47 for lipase M. The final tanδ were 0.28 (lipase E) and 0.29 (lipase M). The third group consisted of the batters treated with the lipases A, G and J. For the third group, tanδ also increased to the first peak, which was earlier than for the other two groups (around 30 °C instead of 35 °C) . The tanδ of the first peak was at 0.5 for all three batters. After the first peak, tanδ slightly varied from 45 °C to 70 °C between 0.56 and 0.60 and then had a second peak around 76 °C and a tanδ of 0.54. The final tanδ for group 3 was between 0.35 to 0.38. The maxima of tanδ occurred between 34.4 °C and 36.3 °C for groups 1 and 2 and from 63.2 °C and 65.7 °C for group 3.

Basic cake batters were thus liquefied and their firming during baking was retarded by the addition of lipases. The three lipases A, G and J had the greatest impact.

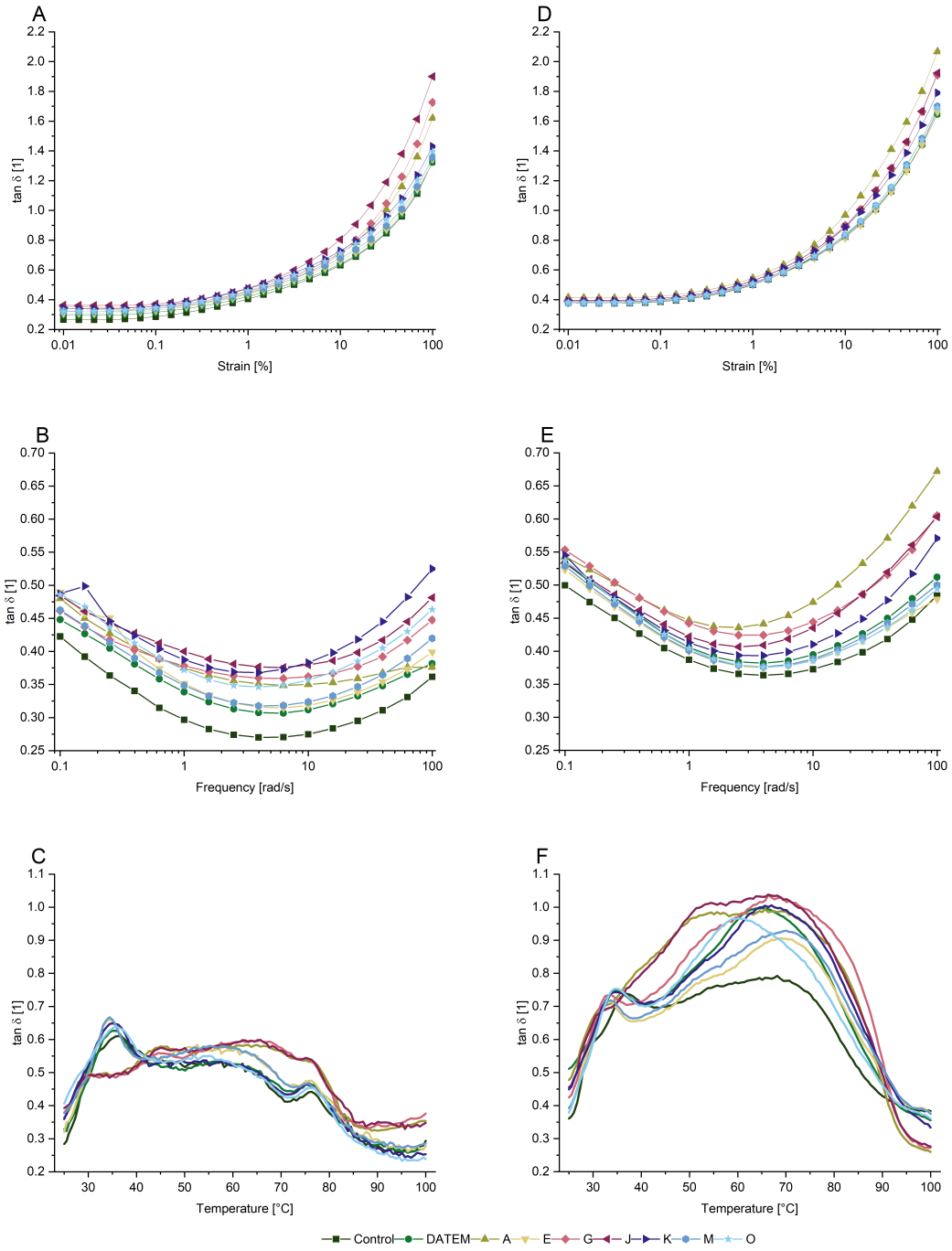


Figure 22: Rheological characterisation of basic cake (A-C) and pound cake (D-F) batters. Amplitude sweeps (A, D), frequency sweeps (B, E), and temperature sweeps (C, F). All curves represent the average of three (amplitude sweep and frequency sweep) and two measurements (temperature sweep), respectively. Control: sample without addition of improver; DATEM: sample with addition of DATEM; A-O: sample with addition of the respective lipase. Figures modified from Stemler & Scherf [146].

3.2.1.3. Pound cake

The curves of pound cake batters with or without treatment by lipases or DATEM during the amplitude sweep were similar to the ones from basic cake (Figure 22). The tanδ varied between 0.4 - 1.9 and increased with increasing strain. The lipases A, G, J and K led to higher tanδ compared to the control (2.07, 1.91, 1.92 and 1.79 compared to 1.68 at a strain of 100%). However, there were no significant effects on the LVE by either lipases or DATEM (Table 9). The LVE of the control sample (0.123%) was 55.7% greater than the one for the control sample of basic cake (0.079%). G' at the end of the LVE was reduced significantly by all lipases, but not by DATEM. The reductions ranged from 26.5% (lipase O) to 54.5% (lipase A). The greatest effects were again achieved by the lipases A, G (43.7% each) and J (48.2%). The cross-over point occurred at significantly lower strains for batters treated with the lipases A, G, J and K. They reduced the strain of the cross-over point of the control by 45.4%, 30.7%, 30.5% and 26.7%, respectively.

For the frequency sweep (Figure 22), all curves of pound cake had a low point around 3 rad s^{-1}. Again, tanδ was below 1 for all measurements, indicating a solid-like behaviour during fast and slow movements. At 1 rad s^{-1}, tanδ was lowest for the control (0.39), higher for the batters treated with the lipases M, O and E (0.40 each) and even higher for the batters treated with DATEM or lipase K (0.41 each). The highest tanδ at 1 rad s^{-1} were caused by the lipases J (0.42), G (0.44) and A (0.45). The three lipases A, G and J also caused the highest tanδ at high frequencies, e.g., at 100 rad s^{-1} it was 0.60 - 0.67 compared to 0.48 for the control.

During the temperature sweep (Figure 22), all pound cake batters behaved in their own way. No clear distinction into groups was possible as done for basic cake. The tanδ of the batters increased to a first peak, followed by a small decrease before it increased again to its maximum between 60.1 °C and 70.1 °C and decreased afterwards. The first peak was between 31.8 °C and 37.6 °C for all batters. The control had its first peak at the highest temperature of all batters (37.6 °C). The lipases A and J liquefied the batter in a way that the slope of tanδ was too steep for a proper first peak and the area resembled more to a shoulder than a peak. The maximum tanδ was lowest for the control (0.79) and higher for all treated batters (0.91 - 1.04). The highest maxima were caused by the lipases K (1.01), G and J (1.04 each). At 100 °C, the final tanδ were between 0.33 - 0.38 for all batters besides the ones treated with the lipases A, G and J which caused a reduction to tanδ of 0.26 - 0.28.

The rheological behaviour of pound cake batter was therefore affected by lipase addition, although to a smaller extent than for basic cake.

3.2.1.4. Brioche

The rheological characterisation of brioche also included an amplitude sweep (Figure 23). Tanδ increased from 0.4 to 1.1 with increasing strain. This indicates that brioche doughs are stiffer than basic cake and pound cake batters. The LVE of the control was 0.083% and not affected significantly by either lipases or DATEM (Table 9). The LVE of all brioche samples ranged from 0.077% - 0.083%. Similar as for the LVE, the lipases or DATEM changed neither G' at the end of the LVE nor the cross-over point in brioche doughs significantly.

During the frequency sweep, all doughs behaved similarly. Tanδ at a frequency of 0.1 rad s^{-1} was 0.54 - 0.55 for all doughs, tanδ at 100 rad s^{-1} was 0.45 - 0.46. The curves all had a low

point around 4 rad s^{-1}. Similar to the results for basic cake and pound cake, all doughs had solid-like behaviour at all tested frequencies.

As described for the amplitude sweep and the frequency sweep, neither the lipases nor DATEM affected t he temperature-dependent behaviour of doughs. All curves of tanδ started at a tanδ of 0.38 - 0.42 and a slightly higher tanδ (0.46) for the batter treated with lipase K. The, the curves increased to 0.45 - 0.48 for a first peak around 35 °C and decreased afterwards. A second peak occurred at approximately 80 °C with tanδ between 0.34 and 0.35. Then, tanδ decreased to a final tanδ of 0.25 for all doughs. The maxima of tanδ were between 33.1 °C and 47.6 °C at tanδ of 0.45 - 0.48 without a clear trend of being affected by lipases.
To sum up, the characteristics of brioche dough were not influenced to a significant extent by lipase addition.

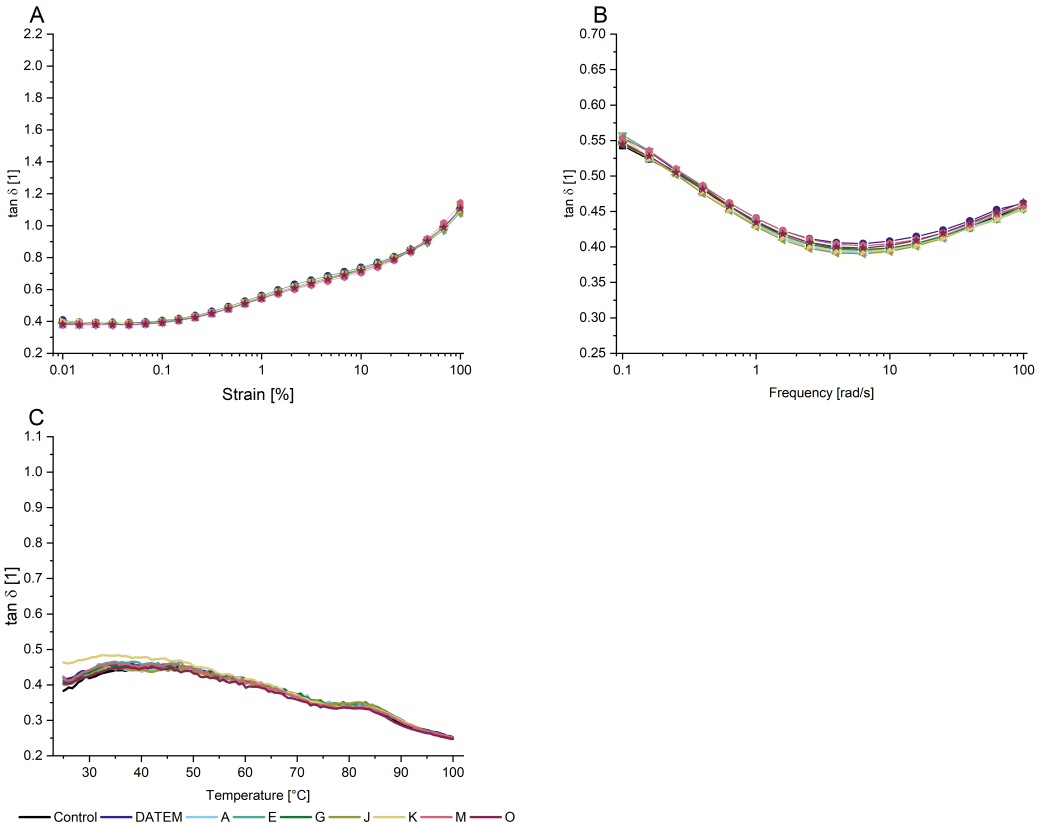


Figure 23: Rheological characterisation of brioche doughs. Amplitude sweep (A), frequency sweep (B), and temperature sweep (C). All curves represent the average of three (amplitude sweep and frequency sweep) and two measurements (temperature sweep), respectively. Control: sample without addition of improver; DATEM: sample with addition of DATEM; A-O: sample with addition of the respective lipase. Figure modified from Stemler & Scherf [146].

3.2.2. Improvement of product quality

For the characterisation of the product quality of basic cake, pound cake and brioche with and without treatment by lipases or DATEM, the parameters product density, baking loss and their texture including firmness, resilience, springiness and cohesiveness were assessed.

3.2.2.1. Density and baking loss

The product density of basic cake was between 0.48 g mL^{-1} and 0.62 g mL^{-1} (Figure 24, Table 26). Lipase A caused a significant reduction of the density (0.48 g mL^{-1} compared to 0.58 g mL^{-1} of the control). For pound cake, the density ranged from 0.61 g mL^{-1} to 0.66 g mL^{-1} and was thus higher than in basic cake. Lipase O was the only lipase leading to a significant adulteration in pound cake batter and increased the density to 0.66 g mL^{-1} compared to 0.63 g mL^{-1} for the control sample. In brioche, none of the lipases had a significant impact on the density. Brioche samples were lighter than basic cake and pound cake (density 0.46 g mL^{-1} - 0.53 g mL^{-1}). In brioche, DATEM led to a significant increase of product density compared to the control (0.65 g mL^{-1} instead of 0.63 g mL^{-1}).

The baking loss of basic cake was 12.2% - 14.0% (Figure 24, Table 26). Compared to the control (baking loss 12.7%), the lipases A and K led to significantly higher losses (14.0% and 13.5%, respectively). For lipase A, this was in accordance with the reduction of density as described above. Lipase A also caused a significantly higher baking loss in pound cake (9.6% compared to 8.8% for the pound cake control). In pound cake, the baking loss was lower overall than for basic cake, ranging from 8.4% to 9.6%. In brioche, three lipases led to a significant increase of the baking loss, namely the lipases E (11.8% compared to 10.6% for the brioche control), G (11.5%) and J (11.4%). The overall range for the baking loss in brioche was from 10.4% to 11.8%.

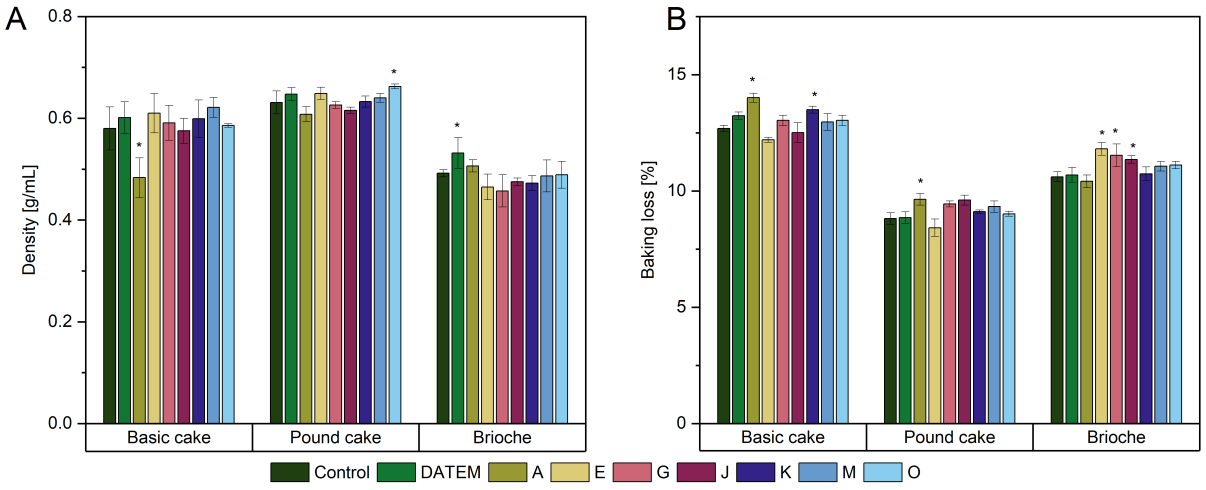


Figure 24: Density (A) and baking loss (B) of differently modified basic cake, pound cake and brioche samples (Control: sample without lipase addition, DATEM: sample with addition of DATEM, A-O: samples with addition of the respective lipase). Asterisks show a significant difference to the control (ANOVA with Dunnett's test, $p \leq 0.05$, n = 6). Figures modified from Stemler & Scherf [97].

3.2.2.2. Basic cake

The texture of basic cake was affected in various ways by the addition of lipases (Figure 25, Tables 26, 27 and 29).

The overall firmness of basic cake increased during storage. The average firmness of the samples measured directly after baking (3.7 N) was significantly lower than the average firmness after 96 h of storage (8.6 N). The firmness of the control increased from 4.9 N to 11.0 N during 96 h.

Concerning the initial firmness, the lipases exerted only small effects. Lipase K was the only lipase leading to a significant decrease by 42% compared to the control. During storage, the impact of lipases on the firmness increased, as the overall firmness increased. After 24 h of storage, all lipases led to significantly softer products compared to the control. The firmness of lipases-treated products ranged from 3.1 N to 7.4 N compared to 9.5 N for the control. The lipases A, G and J were most effective and reduced the firmness by 57%, 65% and 67%, respectively. Also after 48 h and 96 h of storage, the three lipases A, G and J led to the greatest reductions by 59%, 66% and 65% after 48 h and 58%, 65% and 66% after 96 h, respectively. For a storage time of 48 h, also the lipases E, M and K caused significantly softer products, but to a maximum extent of 33%. DATEM did not exert a softening effect at any of the included storage times. The total increase of firmness was 120% for the control and 38% for lipase A, 13% for lipase G and 24% for lipase J.

The resilience of basic cake decreased after the first day of storage and remained stable afterwards. The impact of lipases decreased during storage. Again, the lipases A, G and J exerted the greatest effects compared to all lipases by reducing the resilience by 63%, 67% and 67% directly after baking, respectively. After 96 h of storage, the effects were smaller and caused a reduction by 41%, 33% and 34%, respectively. Besides A, G and J, only lipase E led to a significant reduction of resilience directly after baking (29%) and 24 h after baking (19%). DATEM did not exert any significant effects on the resilience of basic cake.

The overall springiness of basic cake decreased between 24 h and 96 h of storage. After 24 h, it ranged from 0.66 - 0.80, after 96 h from 0.59 - 0.79. Neither DATEM nor the lipases affected the springiness significantly.

Basic cake cohesiveness behaved similarly to basic cake resilience. It decreased significantly during the first and the second day of storage, while the effects caused by lipases also decreased during storage. Directly after baking, the lipases A (39%), E (16%), G (41%) and J (48%) significantly reduced the cohesiveness compared to the control. After 24 h, only the effects exerted by the lipases A, G and J were still significant (39%, 30% and 27%, respectively) and after 48 h, only the effect by lipase A (21%) remained significant. DATEM did not reduce the cohesiveness, but led to an increase after 48 h of storage by 22%.

In accordance with the results for firmness and cohesiveness, the dependent textural parameters gumminess and chewiness were also improved by lipase addition (Table 28).
To sum up, lipases improved the texture of basic cake, while the extent of improvement was greatest for the lipases A, G and J.

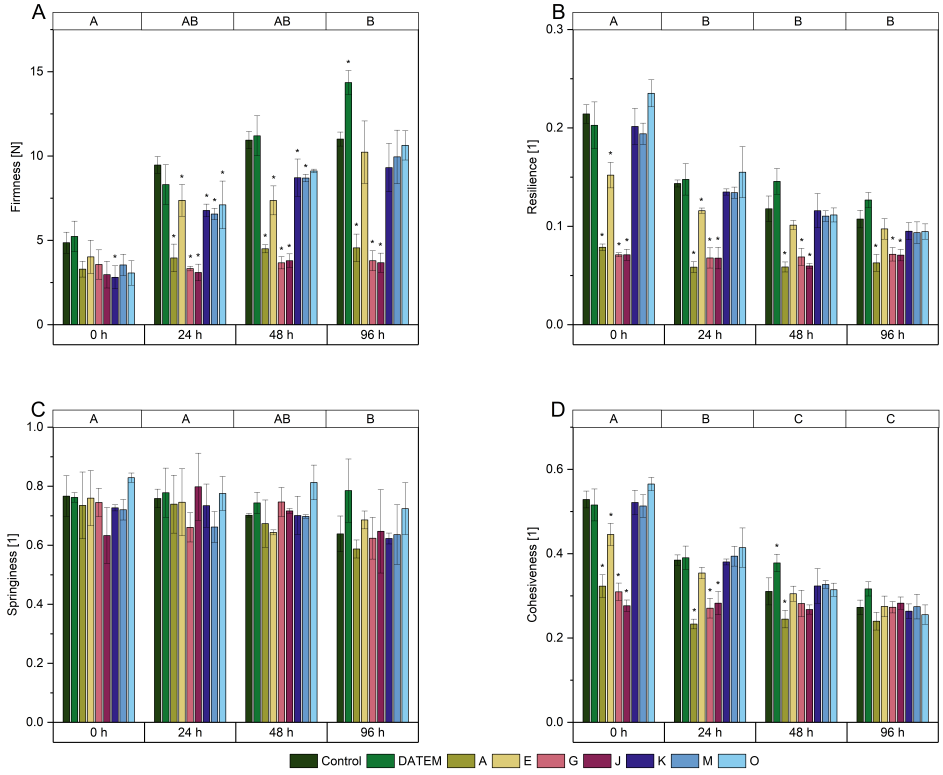


Figure 25: Firmness, resilience, springiness and cohesiveness after 0 h, 24 h, 48 h and 96 h of differently modified basic cake samples (Control: sample without lipase addition, DATEM: sample with addition of DATEM, A-O: samples with addition of the respective lipase). Asterisks show a significant difference to the control sample of the respective time (ANOVA with Dunnett's test, $p \leq 0.05$, n = 6). Capital letters on the top indicate significant differences between means of all values of a certain time (ANOVA with Tukey's test, $p \leq 0.05$, n = 6). Figures modified from Stemler & Scherf [97].

3.2.2.3. Pound cake

Lipases also altered the texture of pound cake (Figure 26,Tables 26, 27 and 29). The overall firmness of control, lipase-treated and DATEM-treated pound cakes increased during the first 24 h and the following 72 h significantly. The control sample gained 10.9 N of firmness during the total storage time of 96 h.

The lipases softened the products. The extent of softening increased with increasing storage times. Directly after baking, lipase O was the only lipase leading a significant reduction of firmness (by 26%), while lipase A caused an increase by 33%. After the first 24 h of storage, no significant changes occurred. 48 h after baking, the use of the lipases G (25%), J (21%) and M (22%) softened the products significantly. 96 h after baking, these effects were partly increased to 25% (lipase G), 25% (lipase J) and 24% (lipase M). Additionally, also lipase A caused significant effects 96 h after baking by reducing the firmness by 23%. DATEM did not exert any effects on the firmness of pound cake. Compared to a total firmness increase of 117% for the control sample after 96 h of storage, the lipase-treated samples only hardened by 27% (lipase A), 37% (lipase G), 54% (lipase J) and 108% (lipase M).

For the resilience of pound cake, an overall decrease could be observed. In accordance with

the results for basic cake, the extent of resilience reduction caused by the lipases decreased with decreasing resilience. The three lipases A, G and J were again found to exert the greatest effects. Directly after baking, they decreased the resilience by 37%, 46% and 46%, respectively. The lipases E and M also caused a significant reduction of the resilience, albeit to only 33% and 19%. 24 h after baking, all lipases but lipase K had a significant impact on the resilience with a maximum reduction by 33% exerted by lipase J. All lipases significantly reduced the resilience 48 h after baking (maximum reduction by lipase E, 29%). 96 h after baking, the lipases E, G, J and O caused a significant reduction of pound cake resilience with a maximum of 21% by lipase E. DATEM did not exert any significant effects on pound cake resilience.

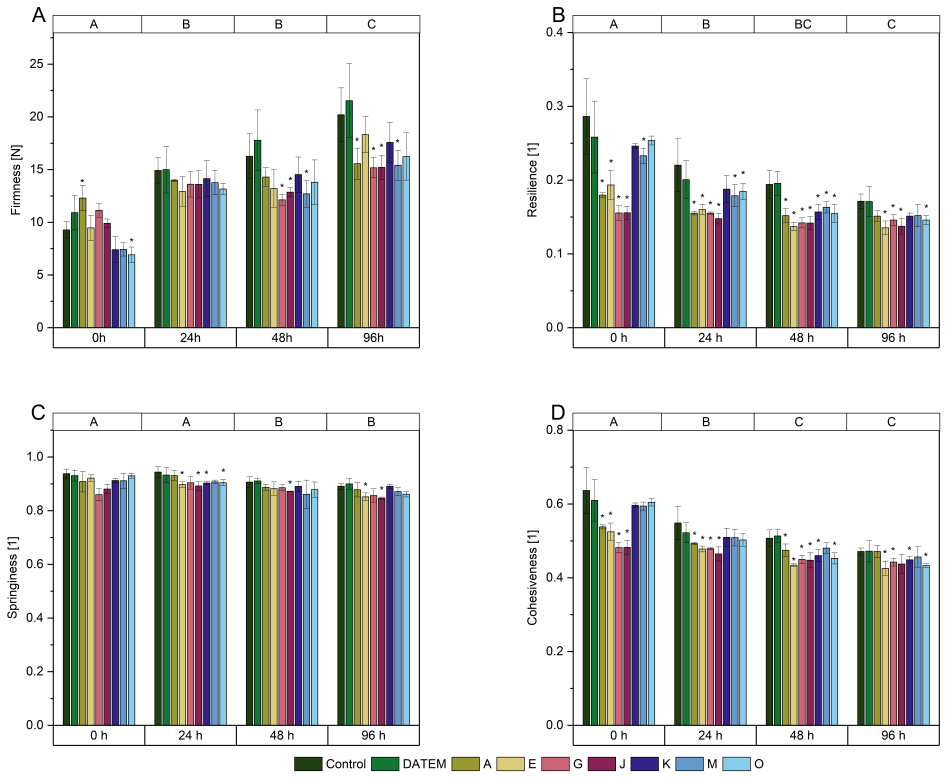


Figure 26: Firmness, resilience, springiness and cohesiveness after 0 h, 24 h, 48 h and 96 h of differently modified pound cake samples (Control: sample without lipase addition, DATEM: sample with addition of DATEM, A-O: samples with addition of the respective lipase). Asterisks show a significant difference to the control sample of the respective time (ANOVA with Dunnett's test, $p \leq 0.05$, n = 6). Capital letters on the top indicate significant differences between means of all values of a certain time (ANOVA with Tukey's test, $p \leq 0.05$, n = 6). Figures modified from Stemler & Scherf [97].

The overall springiness of pound cake decreased from an average of 0.91 to 0.89 between 24 h and 48 h of storage. The lipases only had a slight impact on this parameter. Out of all combinations of lipases and storage times, only lipase G reduced the initial springiness by 8% and lipase E the springiness 24 h after baking by 5%. Besides, no significant effects were exerted by either the lipases or DATEM.

Pound cake cohesiveness behaved similarly to pound cake resilience. An overall decrease occurred within the first (24 h) and the second day (48 h) of storage while the effect of lipases

was diminished. Directly after baking, the lipases A, E, G and J all decreased the cohesiveness with a maximum reduction of 24% (lipase G). 24 h after baking, the maximum effect was 15% by lipase J, 48 h after baking 14% by lipase E and 96 h after baking 10%, also by lipase E. DATEM did not affect the cohesiveness of pound cake.

As expected from the results for pound cake firmness and cohesiveness, both chewiness and gumminess were also improved by the addition of lipases, whereas the effects in terms of chewiness were greater than the ones in gumminess.

Lipases could therefore also be applied for the improvement of textural characteristics of pound cake. The extent of improvement was smaller than the one achieved in basic cake.

3.2.2.4. Brioche

In brioche, the impact of lipase addition on the texture was smaller compared to basic cake and pound cake (Figure 27, Tables 26, 27 and 29).

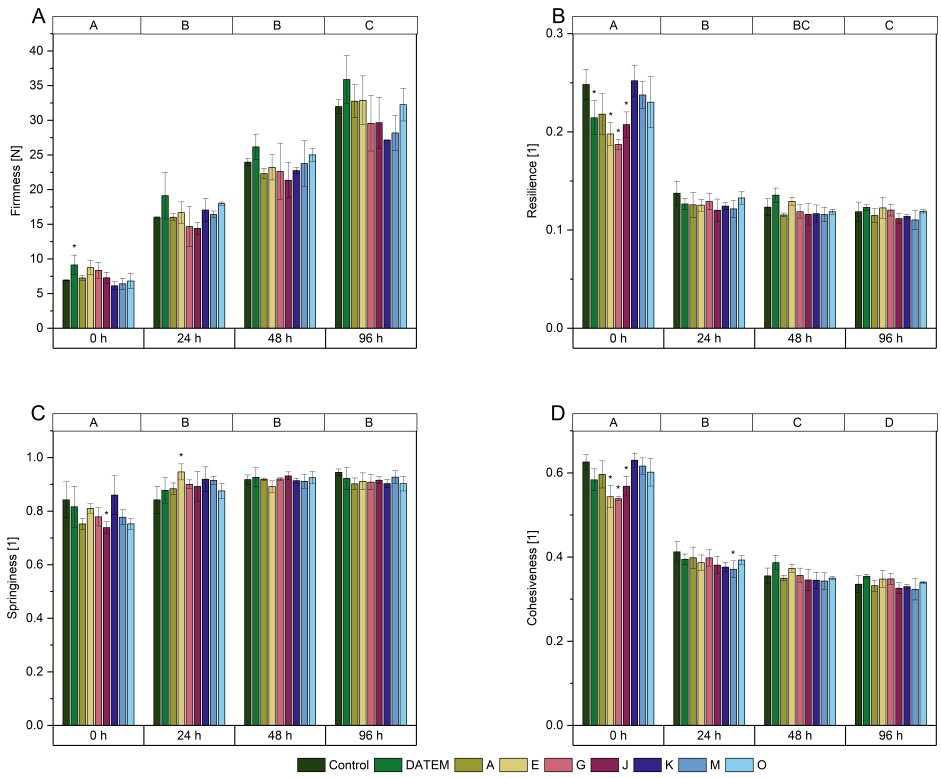


Figure 27: Firmness, resilience, springiness and cohesiveness after 0 h, 24 h, 48 h and 96 h of differently modified brioche samples (Control: sample without lipase addition, DATEM: sample with addition of DATEM, A-O: samples with addition of the respective lipase). Asterisks show a significant difference to the control sample of the respective time (ANOVA with Dunnett's test, $p \leq 0.05$, n = 6). Capital letters on the top indicate significant differences between means of all values of a certain time (ANOVA with Tukey's test, $p \leq 0.05$, n = 6). Figures modified from Stemler & Scherf [97].

The overall firmness of brioche increased during the first 24 h of storage from an average value of 7.5 N to 16.5 N. During the second day of storage (48 h), no significant increase of the average firmness was measured, whereas the values of 96 h after baking were again

significantly firmer (31.1 N). None of the lipases had an impact on the firmness of brioche at any of the chosen measurement times. DATEM led to an increase in firmness directly after baking (32%), but also had no effect during storage.

Brioche resilience decreased during storage similar to the one of basic cake and pound cake. Significant effects by DATEM and the lipases E, G and J were measured directly after baking as a reduction by 14%, 20%, 25% and 17%, respectively. No further significant effects occurred.

Contrary to the results for basic cake and pound cake, the overall springiness of brioche increased during storage. For the control sample, the original springiness rose from 0.84 to 0.94 after 96 h of storage. In two cases, a significant impact of lipase addition occurred: directly after baking, lipase J reduced the springiness by 12% compared to the control, 24 h after baking, lipase E led to an increase of 12%. The effects followed no clear trend.

The cohesiveness decreased steadily during storage. Comparable to the resilience, the lipases exerted effects at short storage times: directly after baking, the lipases E (13%), G (14%) and J (9%) caused a significant decrease of the cohesiveness compared to the control, 24 h after baking, lipase M led to a significant decrease of 10%. Further effects were not significant.

For brioche gumminess and chewiness, the impact of lipases and DATEM was not significant.

Concerning the textural characteristics of brioche, the lipases exerted little to no effects in the chosen setup.

3.2.2.5. Sensory analysis

An untrained panel evaluated the flavours of lipase-treated cakes compared to a corresponding control sample without lipase addition. The lipases E, J and K were chosen for the analysis. Their effects on the sensory profiles of the cakes were evaluated by 3 Alternative Forced Choice tests and the Thurstone model (section 5.3.3.4). Only the combination of lipase J in basic cake led to a significantly different flavour than the control (Table 10). It was recognised by 82.4% of the panellists as different. The deviation was described as buttery and egg-like. 35.3% of the panellists also indicated “rancid” as a description of the deviation.

Table 10: Sensory analysis of basic cake, pound cake and brioche treated with the lipases E, J and K.

Recipe	Lipase	Correctly identified by	Description of the flavour
Basic cake	E	23.5%	more intense
	J	82.4%	buttery, egg-like, rancid (35.3%)
	K	29.4%	more intense
Pound cake	E	25.0%	different
	J	43.8%	buttery
	K	18.8%	sweetish
Brioche	E	14.3%	old
	J	50.0%	acidic
	K	21.4%	buttery

3.2.3. Discussion

The results for the analysis of lipase effects on the baking quality of fine bakery goods will be discussed in the following. First, the effects on the quality of batters and doughs will be evaluated with special regard to the differences between the three recipes. Second, the alternations of product quality will be assessed and compared to the results from corresponding batter or dough quality. Therefore, the underlying mechanisms responsible for textural properties will be presented and putative modes of action for lipases in fine bakery goods will be formulated.

Concerning the pH of doughs and batters, the values were in accordance with findings from literature for the pH values of cake batters, wheat doughs and the influence of specific ingredients [9, 147, 148, 149]. Doughs consisting of wheat flour and water were reported to have an acidic pH [148], while baking powder [9] and to a lower amount also eggs [147] both increase the pH value. Eggless cake batters without baking powder were reported to have pH values ranging from 6.52 - 6.66 [149], which is accordance with the slightly basic pH of basic cake batter including baking powder and the neutral pH of pound cake batter with eggs and baking powder from this study.

The density of cake doughs and batters depends on the volume of the entrapped gas within the dough or batter. The polar lipids released by lipases in dough and batter can enhance the stabilisation of gas bubbles and thus increase the share of air [40, 47, 150]. In basic cake batter, this phenomenon occurred when the lipases G and J were used. The effects of the other lipases were not significant, probably due to insufficient amounts of lipids favouring the formation of lamellar mesophases. As discussed in section 1.3.3, lamellar mesophases are more effective than other mesophases for the stabilization of micelles. The insufficient amounts could be caused both by low overall activities and/or unsuitable substrate specificities, e.g., because the released lipids led to the formation of hexagonal I or II mesophases.

In pound cake, the batter density was not improved by lipase addition. In contrast to basic cake, pound cake contains eggs (approx. 25%), including 12% of egg fat, of which 25% are PC [67, 75]. PC are known to have excellent emulsifying properties [15]. Possibly, the included PC in pound cake are sufficient for the stabilisation of gas bubbles and the lipids released by lipases cannot further improve the process of air incorporation. This hypothesis is supported by the fact that the overall density of pound cake was lower than the one of basic cake, hinting at a higher share of air in pound cake compared to the eggless basic cake. Additionally, a similar effect has also been described by Rodríguez-García et al., who found no improvements of the density of low-fat cake batter including eggs after the addition of lipases [63].

Brioche also contains eggs and therefore PC. Similar mechanisms as discussed for pound cake could lead to the lack of effects by lipases concerning its density. The overall density of brioche was higher than for basic cake and pound cake, probably because the dough was prepared without leavening agent for the determination of dough characteristics, while baking powder was included as a leavening agent in both basic cake and pound cake batter. Although also active during baking, baking powder already releases CO_2 during mixing and enlarges the volume of entrapped air in batters. Brioche dough, on the other hand, was prepared without yeast for the determination of dough properties in order to enable its rheological characterisa-

tion.

Both the cake recipe with special emphasis on the presence of inherent emulsifiers like PC from eggs and the chosen lipase therefore influenced the improvement of cake batter and dough density.

The stickiness of cake batters and doughs was affected similarly to the density. The lipases A, G and J caused a significant reduction of stickiness in basic cake, whereas no effects occurred in pound cake and brioche. Concerning the stickiness of cake batter, no studies on the influence of specific ingredients have been undertaken so far. Stickiness has been linked to co-oxidation products of lipid oxidation processes [56] which are enhanced by lipases. Additionally, stickiness could be affected by the strength of the gluten network and thereby by lipases, as the released polar lipids increase its strength [41]. Although pound cake contains PC and therefore high levels of polar lipids even without lipase addition, its overall stickiness was similar to the one of the basic cake control samples. Still, pound cake stickiness was not improved by lipase addition. Neither was the stickiness of brioche affected.

The impact of lipases on the rheological properties of cake batters and doughs was greatest in basic cake, smaller in pound cake and nearly no effects occurred in brioche.

For pound cake, this could be due to the polar lipids originating from eggs, which already altered its properties compared to basic cake. Brioche contains only 24% of the egg lipid content of pound cake and thus less polar lipids. Therefore, it was expected to be affected by lipase addition to a greater extent than pound cake. Still, there were only little to no effects on its dough quality. There are several possible causes for this phenomenon:

1. According to the manufacturers' instructions for the lipase dosage, smaller lipase dosages (17%) were used in brioche compared to basic cake and pound cake (section 5.3.1). Smaller amounts of lipases could lead to less release of polar lipids and concomitant less effects caused by polar lipids.

2. In a study of lipase effects in bread, the authors suggested that a total lack of effects could be due to the structure of lipids within the dough [151]. As lipids are structured in micelles, they hypothesised that lipases only interact with them during mixing and not during fermentation. Contrary to basic cake and pound cake, brioche dough was kneaded and not whipped which could lead to differences, e.g., a diminished interaction of lipases with lipids.

3. Other authors put forward the assumption of lipase shear sensitivity and their inactivation by mixing [60], leading to similar effects as described under 1.

4. The lower pH compared to basic cake and pound cake of brioche dough could also affect lipase activity. However, the lipases were designed for the use in bread, which also has an acidic pH value. They should therefore tolerate acidic pH values.

5. For lipid hydrolysis, lipases need available water. The amount of water in brioche is lower than in basic cake: Water was either added directly to the batters and doughs or introduced as part of the eggs (water content of eggs approx. 76% w:w [67]). Brioche

dough contains only 14% of directly added water and 4% of water added by egg (total water content 18%) compared to 32% water in basic cake. Based on the water content, the effects in brioche were expected to be less than in basic cake. Pound cake, however, also contains only 19% of water and thus a similar content than brioche. While brioche has a higher content of flour to bind the water than pound cake (56% compared to 25%), pound cake contains more sugar which dissolves in water than brioche (25% compared to 6%). Both flour and sugar reduce the amount of available water, resulting again in similar amounts of available water in both pound cake and brioche. The water content is therefore another possible explanation for the high impact of lipases in basic cake, but cannot explain the differences between pound cake and brioche.

6. Besides a lack of lipase activity, also their substrate specificity could influence their impact on the baking quality. The substrate specificity of lipases was shown to affect their performance in bread as different lipid classes differently affect bread loaf volumes [61]. However, the same lipases were used in all recipes. If the differences between the recipes were due to lipase substrate specificities, this would mean that the recipe had an influence on the substrate specificity.

To sum up, both lipase activities and lipase substrate specificities could be the cause of the differences of lipase effects in the three recipes. This question will be dealt with in detail in section 3.3.

Besides the analysis of batter/dough density and stickiness, the rheological properties of the batters and doughs were analysed in detail by oscillatory measurements comprising an amplitude sweep, a frequency sweep and a temperature sweep.

During the amplitude sweeps of basic cake and pound cake, the lipases A, G, J and K had the greatest impacts on the rheological behaviour of the batters. All led to a liquefaction as expressed by higher tanδ at higher strains, lower G' at the end of the LVE and lower cross-over points. Additionally, they also increased the LVE of the batters.

The results are in accordance with a lower degree of system structuring and a concomitant liquefaction caused by lipase addition to low-fat cakes as reported by Rodríguez-García et al. [63]. Lipases therefore act contrary to emulsifiers, which increase G' and stiffen batters [63, 86]. For emulsifiers, these effects were attributed to their water-binding capacity or their interaction with other ingredients. This would mean that lipase reaction products reduce the water-binding capacity of the batters and doughs or disrupt network formation. For lipases, the liquefaction could also be caused by the interaction of the released polar lipids with other ingredients. Gluten stiffens bread dough [152] and therefore causes high G'. Lipase reaction products cause a decrease of gluten extensibility [32], which could alter the gluten network and therefore diminish its stiffening effect. Another factor influencing the rheological properties could also be the lipid residues after the release of polar lipids. The properties of the lipid phase after the interaction with lipases are affected by its hydrolysis. Similar to decreasing fat contents or changing the fat type in cake batters [93], this could affect the flow behaviour of batters. Differences between the lipases could again be due to their activities or their substrate specificities. Either insufficient amounts of lipids were released, or the released lipids did not lead to functional effects due to their unsuitable structure or polarity.

The liquefaction of batters was further demonstrated by the frequency sweeps. Again, the lipases A, G, J and K had the greatest effects on basic cake and pound cake, while the properties of brioche were not affected. The rheological properties of the batters were only mildly dependent on the movement speed. All batters remained solid-like at all tested frequencies. There is only scarce data on the time-dependent behaviour of cake batters. Two studies reported steadily increasing tanδ with increasing frequencies [92, 153]. This is in contrast to the findings of this study, where all batters of basic cake and pound cake as well as brioche dough first showed a decrease of tanδ to a low point of frequencies from 3-4 rad s^{-1}. As this was the same for all tested samples, the effect does not seem to be due to the ingredients or lipases. It is not clear what caused the difference to the other studies.

During the temperature sweeps, baking of batters and doughs was imitated. The three characteristic phases of temperature sweeps for cake batters as described in section 1.5.1, namely fat melting with concomitant increase of tanδ as the first phase, a second increase of tanδ with the release of CO_2 as the second phase and protein denaturation and starch gelatinisation leading to decreasing tanδ as the third phase [85, 92, 93, 94], occurred in pound cake. In basic cake batters, however, the phases could not be identified. Instead, tanδ decreased between 45 °C and 70 °C, hinting at an early stiffening of the dough. This was unexpected, as basic cake contained baking powder and should thus also release CO_2. A similar curve as for basic cake batters was reported before by Migliori et al. [91]. They analysed the properties of Yorkshire pudding batter which did not contain sugar and only low amounts of fat. Sugar is known to increase the gelatinisation temperature of starch [154]. Compared to 25% of sugar in pound cake, basic cake only contains 8% of sugar. The lower sugar content of basic cake could therefore explain the changed profile of tanδ during heating. This assumption is supported by the findings for brioche (sugar content 6%). In brioche dough, tanδ started decreasing at 45 °C, also showing an early starch gelatinisation.

Lipases affected the temperature dependent behaviour of basic cake batter. During the first phase, they impaired the melting of fat and led to more equalised curves for the batters treated with the lipases A, G and J. A similar effect had already been described for the isolated reaction of lipases with milk fat [114]. The three batters treated with the lipases A, G and J were shown to be more liquid-like than the control at temperatures higher than 45 °C, as their tanδ were continuously higher than the tanδ of the respective control starting from that temperature.

In pound cake, the lipases also caused a liquefaction of the batters. Additionally, they shifted the maximum peak heights and thereby the structure setting, which is linked to the maximum tanδ [85]. Lower temperatures for the structure setting occurred for batters treated with the lipases A, G, J, K and O as well as for the batter treated with DATEM. This could enable a reduction of baking temperatures and concomitant beneficial effects both on the energy costs needed for baking and heat-induced contaminants. Studies upon that matter however showed an increase in firmness of the resulting pound cakes for the lipases A, G and J, when the baking temperature was decreased [155]. The earlier structure setting does not reduce the needed baking temperature, but results in less time for gas bubble expansion and therefore affects the product firmness. Besides the earlier structure setting, a liquefaction of the batters could also

cause the loss of gas bubbles during baking, resulting in firmer and smaller products. The effects of lipases on the product quality of pound cake were therefore analysed in detail.

In brioche dough, the lipases had no effect on the temperature dependent behaviour.

The first parameter for the product quality of cakes analysed was the product density. Baking improvers are used to increase product volumes and thereby decrease product density. However, the lipases only had slight effects on the product densities of basic cake, pound cake and brioche. In bread, volume increases by up to 58% were reported when lipases were used [60]. Other studies indicated increases from to 8% to 19% after lipase addition to bread dough [48]. The mechanisms stabilizing gas bubbles in bread and cake differ, especially for recipes including eggs. Egg proteins play a crucial role in the maintenance of gas bubble stability in cakes [3]. For cake recipes including eggs, a lack of product density improvement by lipases was also reported by Rodríguez-García et al. [63]. Yet, Guy and Sahi showed a decrease of the product density of cakes with similar recipes with a lipase [62]. Therefore, not the cake recipes but the chosen lipases seem to be the decisive factor. Besides their substrate specificity, possibly also their dosage affects their impact as described for bread [61]. Within the analysed combinations of cake recipes and lipases, only lipase A decreased the product density by 17% in basic cake. This is not in accordance with the results for the effects of lipases on the batter and dough properties, where lipase A did not lead to a decreased batter density of basic cake. The improvements of basic cake batter density by the lipases G and J were not sustained during baking. The effects of lipases were again smaller in recipes with inherent emulsifiers as PC from egg in pound cake and brioche. In pound cake, the product volumes were not diminished by the addition of lipases, although they had caused an earlier structure setting. The lipases A, G and J therefore maintained the volume of pound cake although there was less time for gas bubble expansion during baking. Similar effects have already been described by Rodríguez-García et al. [63], who also observed an earlier structure setting in combination with maintained product volumes when lipases were applied in cake. No effects occurred in baked brioche, as described for brioche dough.

The second marker of product quality analysed was the baking loss, also referred to as water loss during baking. Several lipases were found to slightly negatively impact the baking loss by increasing it compared to the respective controls. In bread, lipases did not affect the baking loss [54]. The water loss during baking is linked to the water-binding capacity of the cake ingredients [156]. Although most lipases did not affect this parameter, for six of a total of 21 lipase-recipe combinations it was shown that lipases can reduce the water-binding capacity of cake batters and doughs.

Besides product density and baking loss, product texture was assessed as the most important marker for product quality. It comprised the parameters firmness, resilience, springiness and cohesiveness. Additionally, the parameters gumminess and chewiness were calculated.

A similar trend for the effect of lipases on the texture of the three cakes as for their effect on the batter and dough properties can be described: The impact of lipase addition decreased from basic cake to pound cake to brioche. Again, the contents of PC originating from egg

can explain the smaller effects in pound cake. For brioche, higher concentrations of lipases in combination with longer incubation times were applied compared to the dough experiments. Still, nearly no effects were observed. It is possible that the chosen dosages were not sufficient and should be further increased. In an accompanying master thesis, slight improvements of the texture of brioche could be achieved with even higher amounts of lipases, although the effects were still smaller than for pound cake [155]. Besides the effects discussed above, the use of yeast could impact the effect of lipases. Yeast fermentation products such as ethanol and succinic acid were shown to negatively affect the product quality of bread [157, 158]. They could also diminish the improving effects of lipases on the product quality of cakes. However, they were also present in the control sample and their effect should be the same in both lipase-treated and untreated samples. Yeast fermentation products could still possibly inhibit lipase activities. As no effects by lipases occurred in brioche dough, either, this seems rather unlikely, though, as no yeast was applied for the characterisation of brioche dough properties. Instead, besides the possibly insufficient lipase activity, an altered substrate specificity of the lipases could play a role. This will be discussed in detail in section 3.3.

Besides the differences between the three recipes, the effects of the lipases also depended on the lipases themselves. In basic cake and pound cake, the three lipases A, G and J were most effective, as already described for the effects on the corresponding batters. They inhibited the staling and reduced both resilience and cohesiveness. Their effects were up to three times higher than the ones caused by other lipases. Similar differences between several lipases have not been reported before for the use of lipases in bread. As an example, in a study of Moayedallaie et al. a range of lipases was found to affect the firmness and staling of breads to similar extents each [48]. The greater differences compared to the literature for bread might be due to different substrate specificity patterns of the lipases. Compared to bread, the range of lipids available for hydrolysis is broader due to more lipid-containing ingredients such as eggs and butter. Further lipid classes can therefore be hydrolysed and lead to textural impacts. The lipases used in the study were developed for the application in bread. Therefore, their reactions with further substrates such as additional classes of glycerophospholipids and the resulting effect on the texture of bakery goods were not assessed before. Lipidomic insights into the reactions of lipases in the recipes are needed to further clarify this phenomenon.

The firmness of basic cake was most improved during storage. For the application of lipases in bread, an inhibition of staling and thus the sustaining of softness as well as the reduction of initial firmness in the products during storage is a known effect [54, 55, 59] which has also already been shown for cake [62]. However, although a similar cake recipe was used, the group of Rodríguez-García et al. could not confirm the findings for cake [63].

The initial firmness of cakes has been linked to the amount and the distribution of air bubbles [63, 87]. In cakes, the air bubbles are coated in and stabilised by fat and, if applicable, by egg constituents [3]. As basic cake does not contain eggs, the effects on the initial firmness caused by lipase K could therefore be due to the effect of the newly released polar lipids on gas bubbles. The polar lipids possibly form joint micelles with other fats at the surfaces and lead to the stabilisation of gas bubble coats. Compared to egg-containing cakes, the effects of polar lipids in basic cake were expected to have a greater impact on the final product properties. Additionally, one main factor determining the initial firmness in bread, namely the crystallisation

of amylose during cooling [54, 59], was also shown to impact the firmness of (eggless) cakes [159]. The interactions of polar lipids as released by the use of lipase K with amylose could also affect the initial firmness in basic cake. The substrate specificity of the lipases is most likely the decisive factor on whether the use of a lipase leads to softer cakes. Not all lipid hydrolysis products can interact with amylose to the same extent. This assumption is supported by the contradictory findings in the aforementioned studies from literature which used different lipases.

The effects of lipases on the long-term firmness of basic cake were greater than on the initial firmness. The long-term firmness of bread depends on amylopectin retrogradation and the migration of water from gluten to starch [59] and is supposedly similar for cakes. The lipids released by the lipases therefore interacted better with amylopectin than with amylose.

In pound cake, the lipases had similar effects on the firmness as in basic cake, albeit to a lesser extent. The initial firmness was only improved by lipase O. The different behaviour of the pound cake batters treated with the lipases G and J as shown in the temperature sweeps therefore did not negatively impact the firmness of pound cake at usual baking conditions. For lipase A however, it led to an increase of initial firmness. This negative effect was not sustained during storage. On the contrary, the lipases A, G, J and M reduced the hardening of the cakes during storage to the greatest extent.

As expected from the results in dough, no softening effects were achieved by the use of any lipase in brioche.

In contrast to the results for the effects of DATEM on the product quality of bread [48], it had no improving effects on the firmness of the cakes analysed in this study. Lipases are therefore better suitable to improve the firmness of cakes than the traditional emulsifier DATEM.

The resilience of bread depends both on the amylose network formed during cooling and the thermoset gluten network formed during baking [160]. For bread, it was shown that lipases reduce the initial resilience and flatten the decrease of resilience during storage [59]. For cakes, no data on the effect of lipases on the resilience was available. In all three analysed recipes, the initial resilience was reduced by lipase addition. The extent of reduction decreased during storage. From the results for the effects of the lipases on cake firmness, it was concluded that they only scarcely interacted with amylose. The impact on resilience was therefore most likely due to an alteration of the gluten network. If eggs are present in the recipe, a cross-linked network between egg proteins and gluten is formed, which is crucial for the quality of pound cake [161]. The cross-linked gluten network in pound cake and the “simple” gluten network in the eggless basic cake probably react differently when exposed to lipase reaction products. This explains that the reduction of resilience in basic cake was greater than in pound cake. The effects of lipases on the resilience were similar to their effects on the batter stickiness and the rheological trends during the amplitude sweeps of cake batters. Therefore, both the stickiness reduction and the liquefaction of cake batters could be linked to the altered properties of the gluten network after lipase addition.

The springiness of the different cakes was only slightly affected by lipase addition. It depends on the interactions of starch with proteins from flour [63] and decreased during the storage of bread [54]. For cake, lipases were reported to have a decreasing effect [63], while in bread,

they maintained the original springiness while decreasing the firmness [54]. Therefore, it was expected that the springiness of the samples remained constant or decreased. The analysed combinations of lipases and cakes did not lead to changes in cake springiness.

The cohesiveness of cakes was affected similarly to the resilience. Overall, the cohesiveness decreased during storage while the impact of the lipases was greatest directly after baking. It might therefore also be linked to the effects of lipase reaction products on the gluten network. A correlation between lipase effects on the resilience and cohesiveness has also been shown in an accompanying master thesis [155].

In the same accompanying master thesis, the sensory profile of lipase-treated basic cakes was described as “sweet, buttery and like flour” [155]. For the basic cakes treated with the lipases A, G and J additionally a “slightly cheesy” flavour was reported. As shown by the results from section 3.1, all lipases release short-chain FA. Short-chain FA and especially C4:0 can cause rancid or cheesy off-flavours. However, short-chain FA are volatile substances and can be lost during incubation times of the batter or during baking. Only about one third of the panellists detected a cheesy off-flavour in basic cake samples treated with lipase J in comparison to two control samples. Instead, the low amounts of butyric acid were described as “buttery”. For pound cake samples with lipase-treatment, the flavours were described as “sweetish, buttery and like flour” while no differences compared to the control samples were reported [155]. This trend was confirmed by the 3 AFC tests conducted in this study. In brioche, the panellists smelled a “sweetish and buttery” flavour [155]. Only lipase O was indicated to lead to a slightly rancid off-flavour of brioche. The selection of lipases due to their lack of specificity towards short-chain FA according to section 3.1 therefore mostly maintained the original flavour of cakes. In individual cases, slightly rancid off-flavours could occur. Before applying the lipases in industrial cake productions, an assessment of the resulting flavour in specific cake recipes is recommended.

The influence of seven lipases on three cake recipes compared to the influence of the traditional emulsifier DATEM was analysed. Both the lipase and the recipe influenced the extent of quality improvement achieved. The results from the assessment of the batter and dough quality and the quality of the resulting products were in good accordance. The presence of intrinsic emulsifiers such as PC from egg impaired the effects caused by the lipases. Especially the lipases A, G and J showed a great potential to ease the machinability of cake batters and improve the texture and storage characteristics of the resulting cakes. Probably, the lipase activities or their substrate specificity patterns are decisive for the extent of improvement. The released polar lipids most likely interact with gluten and amylopectin to cause the textural changes. The effects achieved by the lipases were greater than the ones of DATEM. They therefore represent a possible substitution for traditional emulsifiers in cake recipes while maintaining the original sensory characteristics. To predict the suitability of a lipase, the underlying mechanisms of batter/dough and texture improvement need to be further clarified. Therefore, the released lipids and possible correlations between lipase substrate specificities and effects on the batter or dough and product quality were to be evaluated.

3.3. Lipidomic profile of fine bakery goods after treatment with lipases

The following parts were prepared for publication in *Frontiers in Nutrition* [162].

The lipid compositions in basic cake, pound cake and brioche batters/doughs as affected by lipase addition were analysed by LC-MS/MS using the samples presented in section 3.2. The effects of lipases on the quality of batters, doughs and products were compared using the lipidomic profiles. Based on the lipidomic profiles, two parameters were assessed: the lipase activities and the substrate specificities.

For an estimation of lipase activities, total lipid turnover rates were calculated (see section 5.4.3.2) from the different contents of lipids in untreated and treated batters, doughs and products.

Besides, substrate specificity ratios (short: ratios) were calculated based on the different lipid distributions between treated samples and corresponding control samples (see also section 5.4.3.2). The relative abundance of a lipid was normalised by its relative abundance in a control sample, e.g., the percentage of the total peak area of lipid PC (16:0/18:0) in pound cake batter treated with lipase A divided by the percentage of total peak area of the same lipid in the control batter of pound cake.

Ratios below 1 indicate a preferred release of the lipid and thus a high substrate specificity. Ratios greater than one stand for either the formation of a lysolipid, e.g., LPC, or a lack of hydrolysis, thus a low substrate specificity. The ratio is therefore inversely proportional to the specificity. These ratios were assembled for each combination of lipase and recipe and evaluated with Principal Component Analysis (PCA) to identify patterns. Thereby, possible similarities of substrate specificity patterns and the results from section 3.2 were searched in order to identify key reaction products responsible for the effects on the baking quality of cakes.

Preliminary experiments with a commercially available lipid analytical standard revealed that the chosen setup for LC-MS/MS analysis was suitable for the detection of PC, PE, PS, LPC, LPE, DG, TG and sphingomyelin. In the samples, a total of 22 different lipid classes were identified (Figure 28). Besides the components of the standard, this included also MGDG, MGMG, DGDG, digalactosylmonoacylglycerol (DGMG), PA, bis-methyl glycerophosphate (BisMePA), lysodimethyl phosphatidylethanolamine (LdMePE), glycerophosphoethanol (PEt), PG, LPG, glycerophosphoinositol (4,5)-bisphosphate (PIP2), glycerophosphoinositol (3,4,5)-trisphosphate (PIP3), sphingosine (So) and SPH. All identified lipid classes and their hydrolysis products are summarised in Figure 28.

3.3.1. Basic cake

3.3.1.1. Batter

105 different lipid species were identified in basic cake batter (Table 30). The species belonged to TG (81.0%), DG (7.6%), So (4.8%) , LPC (1.9%) and PE (1.0%). Besides, the glyceroglycolipids DGDG (16:0/18:2), DGDG (18:2/18:2), DGMG (18:2) and MGMG (18:2) were identified. The lipid classes of basic cake batter contained FA with chain lengths varying from 4:0 to 26:0.

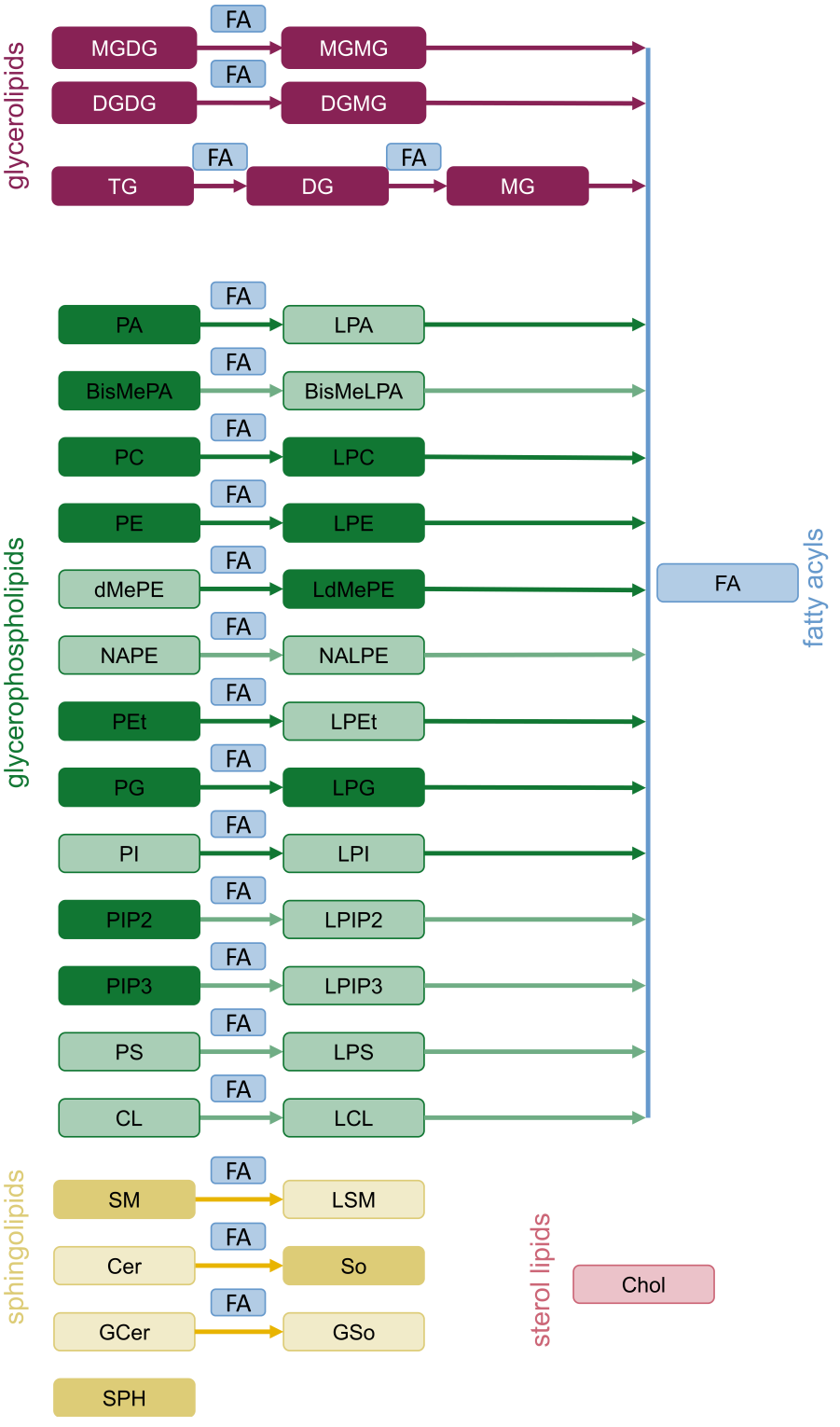


Figure 28: Expected and identified lipid classes in cake samples and their hydrolysis products. Lipids in filled frames were identified in the samples, lipids in darker frames were either expected from literature data on the ingredients or predicted as hydrolysis products. Arrows symbolize the hydrolysis as catalysed by lipases. All abbreviations correspond to the ones used in the text. The prefix L generally indicates the lyso form of a lipid, e.g., PC glycerophosphocholine, LPC lysoglycerophosphocholine. For the complete list of abbreviations, please refer to the abbreviations section. Figure already prepared for publication [162].

The lipases varied in both their turnover rates and the substrate specificities, expressed as ratios.

The total lipid turnover rates ranged from 13.01 mg g^{-1} dry matter (lipase K) to 58.82 mg g^{-1} dry matter (lipase O) (Table 11). Besides lipase O, the lipases G (45.61 mg g^{-1} dry matter) and E (39.25 mg g^{-1} dry matter) led to the greatest changes in total lipid composition. The turnover rates also varied between the different lipid species and classes depending on the lipase. Although lipase O had the highest total lipid turnover rate, towards specific lipids it also had the lowest effect of all lipases. An example for this phenomenon was its effect on the total share of DGMG (18:2), a glyceroglycolipid which is linked to the baking quality of bread as discussed in section 1.4.1. DGMG are released when DGDG are hydrolysed. The share of DGMG was expected to increase after lipase addition. The control contained 0.002% DGMG (18:2) and the lipase-treated samples 0.010%, 0.014%, 0.011%, 0.012%, 0.007%, 0.010% and 0.005% for the lipases A, E, G, J, K, M and O, respectively. All lipases led to an increase as expected, but its extent depended on the lipase. Similar differences between the lipases were also found for further lipid classes, e.g., for DG which were the most abundant lysolipids. DG can both be formed (hydrolysis of TG) and hydrolysed (resulting in the release of MG) by lipases as depicted in Figure 28. They are thus an intermediate product of the hydrolysis of TG, but also occur naturally in the control sample. The control sample contained 0.03% of DG. The share of DG on the total lipids was increased to a maximum of 0.34% (lipase O). The other lipases caused an increase of 0.10% (lipase A), 0.13% (E), 0.16% (G), 0.09% (J) and 0.07% (M). Lipase K did not increase the percentage of DG.

Table 11: Lipid turnover rates in the batters/doughs and final products of basic cake, pound cake and brioche after addition of lipases in mg g^{-1} dry weight (n = 3). Standard deviations are not given because the values were calculated as sums. Data already prepared for publication [162].

		A	E	G	J	K	M	O
Basic cake	batter	17.22	39.25	45.61	18.26	13.01	22.68	58.52
	final product	13.24	13.36	33.72	41.17	133.19	11.91	25.69
Pound cake	batter	100.70	101.11	96.46	94.23	112.90	195.51	179.79
	final product	67.23	67.05	88.64	64.79	126.37	83.83	95.47
Brioche	dough	83.27	42.96	45.58	49.25	52.19	50.33	79.05
	final product	81.00	94.76	62.25	41.69	51.48	57.79	69.64

The effects on the percentages of lipid species or lipid classes can be compared more easily using the substrate specificity ratios. The resulting ratios for all combinations of lipases and lipids in basic cake batter are depicted as a heatmap in Figure 29 based on the values given in Table 30. They show similarities and differences between the lipase reaction patterns. For DGMG (18:2), the ratios vary from 2.4 (lipase O) to 6.4 (lipase E) with an average of 4.6. As expected from the effects on the percentage of DGMG (18:2), the ratios were greater than 1 for all lipases because DGMG were formed in all cases. For the class of DG, the average ratios of the lipases towards DG ranged from 0.9 (lipase K) to 13.6 (lipase O), also indicating a preferred formation of DG when lipase O was applied. For lipase K, the ratio was close to 1.0. The

percentage of DG in basic cake batter samples treated with lipase K was therefore affected to a small extent only. This could be either due to a lack of reaction of DG with lipase K or formation by hydrolysis of TG and loss by further hydrolysis to MG occurring to a similar extent. Whether DG were formed from TG can be concluded from the ratios of lipase K towards TG. If they are below 1, TG were lost and thus DG were created. To account for the large amount of TG, TG were further divided into three groups according to the number of carbon atoms within the FA residues. The first group, low molecular weight TG (LMW-TG), includes all TG with a maximum total number of 39 carbon atoms in the FA. The second group, medium molecular weight TG (MMW-TG), comprises TG with 40 - 54 FA carbon atoms and all TG with more than 54 FA carbon atoms are part of the high molecular weight TG (HMW-TG). For lipase K, the behaviour towards all three groups of TG was similar with an average ratio of 1.0 towards each group. This indicates a lack of specificity of lipase K for all groups of TG and thus also for DG. However, it has to be taken into consideration that the result could also be linked to its overall low activity compared to the other lipases.

For the remaining lipases, the average ratios towards MMW-TG and HMW-TG ranged between 1.0 and 1.3. The overall share of these groups was therefore not diminished by any of the lipases. In contrast to MMW-TG and HMW-TG, LMW-TG were hydrolysed preferentially. This was true for all but the four lightest TG (TG (4:0/8:0/10:0), TG (6:0/8:0/10:1), TG (4:0/10:0/10:0) and TG (4:0/8:0/14:1)), which led to ratios of 2.1 up to 31.9. For the remaining species within LMW-TG, the average ratios were between 0.7 and 0.9 for all lipases except lipase K and thus indicated a preferential release.

To sum up lipase behaviour towards TG, the lipases A, E, G, J, M and O did not hydrolyse MMW-TG or HMW-TG, but interacted preferentially with LMW-TG with more than 26 FA carbon atoms.

The different behaviour of lipase K compared to the other lipases was further proven by PCA. In the PCA loading plot (Figure 29), lipase K was clearly distinguished from the other lipases. As indicated in the score plot, this was due to a different behaviour towards DGMG (18:2), MGMG (18:2), two LMW-TG species (TG (4:0/8:0/10:0) and TG (6:0/8:0/10:1)) and two DG species (DG (18:1/12:0) and DG (18:1/18:2)). Two principal components accounted for 96.2% of total variance between the lipases. This indicated a low level of noise in the measurements and also highlighted the suitability of the method to discriminate lipases according to their reaction patterns.

3.3.1.2. Cake

In baked basic cake, 143 different lipid species were identified (Table 31). Again, the majority was attributable to TG (82.5%). This was in accordance with the results from basic cake batter. Compared to batter, the baked products contained more lipid classes, namely PE, MG, LdMePE, LPC, LPE and LPG. In contrast, DG were not identified in the extracts of baked basic cake.

The total lipid turnover rates in baked basic cake were between 11.19 mg g^{-1} dry matter (lipase M) and 133.19 mg g^{-1} dry matter (lipase K) (Table 11). The lipases K, J and G were most active. Compared to the turnover rates in batter, the values either decreased (by 23.1% for lipase A, 66.0% for lipase E, 26.1% for lipase G, 47.5% for lipase M and 56.1% for lipase O)

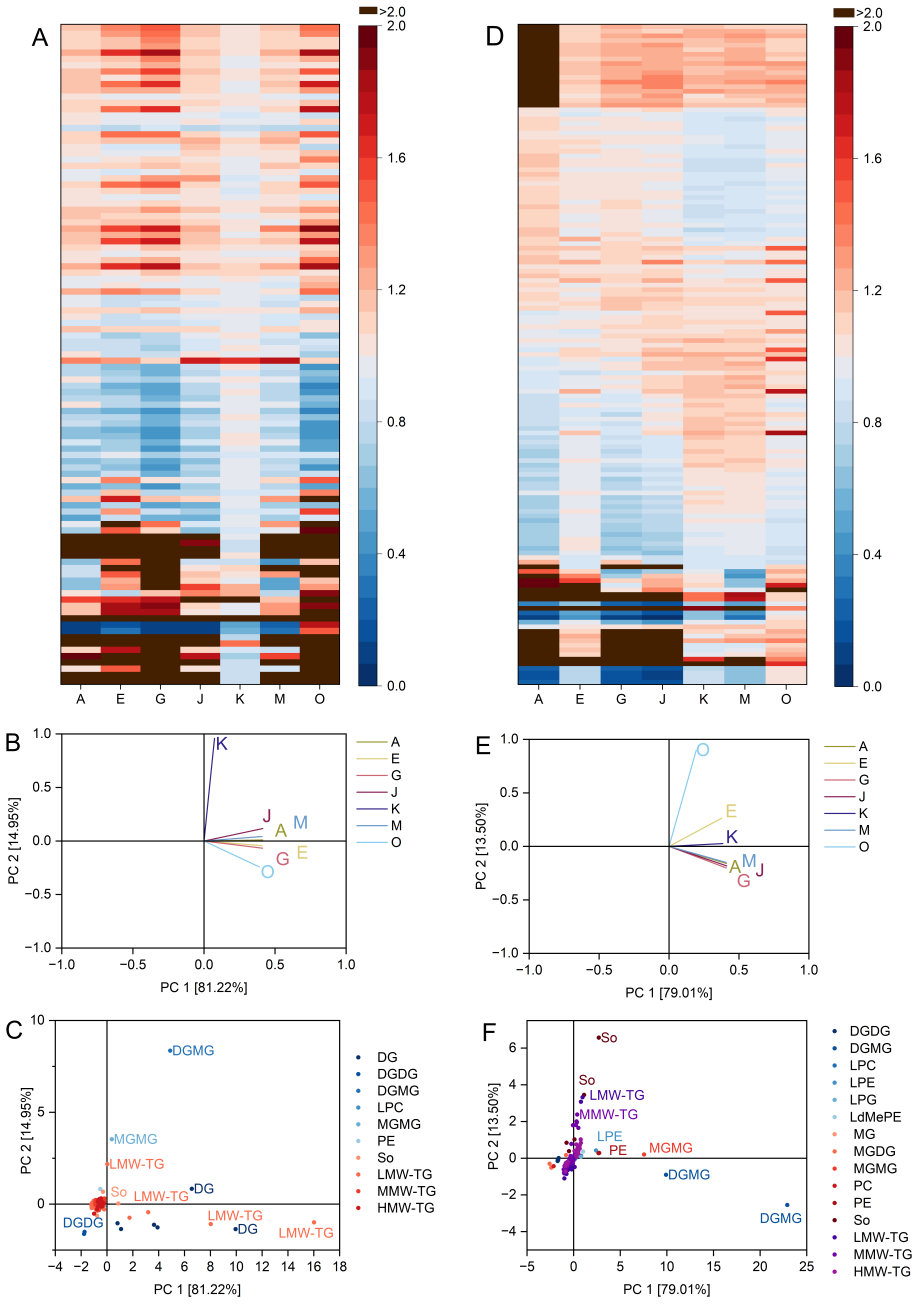


Figure 29: Substrate specificity of the lipases A, E, G, J, K, M and O towards different lipids in basic cake batter (A-C) and in baked basic cake (D-F) (n = 3). For a complete list of substrates, refer to Table 30 for batter and to Table 31 for baked basic cake. A and D: Lipase specificity ratios depicted as heatmap. B and E: Loading plot of the principal component analysis (PCA). C and F: Scores plot of the PCA. For abbreviations please refer to the abbreviations section. Figures already prepared for publication [162].

or increased considerably (by 125.5% for lipase J and 923.8% for lipase K) without clear trend. The extreme increase of the activity of lipase K between batter and baked product caused it to change from least active lipase in batter to most active lipase in the baked product.

Again, the lipases also varied in their reactivity patterns and thus in their ratios (Table 31). The greatest differences were in terms of the specificty towards glyceroglycolipids: The lipases A, G and J had the highest ratios towards DGMG with ratios greater than 13.4. The highest ratio for all lipases towards DGMG was for lipase A towards DGMG (18:3) with 35.6, while lipase O had the lowest ratio of all lipases towards DGMG with 1.4 towards DGMG (18:2). Besides differences concerning glyceroglycolipids, the lipases also affected the formation of lysoglycerophospholipids to different extents. For LPC, the ratios towards LPC (16:0) varied from 1.0 (lipases K and M) up to 4.4 (lipases A and G), for LPE (16:0) from 1.2 (lipase E) up to 4.0 (lipase G), for LPG (16:0) from 1.0 (lipase K) to 3.8 (lipase A) and for LdMePE (18:2) from 1.1 (lipases E, K and M) to 4.0 (lipase G).

The resulting different reactivity patterns are depicted in Figure 29 and were analysed by PCA. For baked basic cake, the two principal components accounted for 92.5% of total variance, indicating a similarly low level of noise as for basic cake batter. The lipases A, G, J and M were clustered in the loading plot. This was mostly due to their specificities towards DGMG, namely DGMG (18:2) and DGMG (18:3). Lipase O was different from the other lipases in terms of its reactivity towards Cer and the concomitant formation of So, especially So (d12:0+pO).

3.3.2. Pound cake

3.3.2.1. Batter

The number of extractable lipids from pound cake batter was 249 (Table 32). They included a broader range of lipid classes compared to basic cake batter due to the additional presence of eggs in the recipe. The majority (60.2%) of lipid species belonged to TG. Besides, the glycerophospholipids PA, PC, PE and PEt accounted for 20.1% of all species and their corresponding lysoforms (LPC and LPE) for another 8.0%. The remaining lipids belonged to BisMePA (7 species), DG (6 species), DGDG (2 species) , DGMG (1 species), MG (1 species), MGDG (1 species), MGMG (1 species), SM (5 species) and SPH (4 species).

The total lipid turnover rates were between 94.23 mg g^{-1} dry matter (lipase J) and 195.51 mg g^{-1} dry matter (lipase M) (Table 11) and thus higher than in basic cake. Besides lipase M, also the lipases O (179.79 mg g^{-1} dry matter) and K (112.90 mg g^{-1} dry matter) were highly effective.

Considering the ratios of the lipases, the high activities of the lipases M, O and K were due to their reaction with different TG (Table 32). Concerning glycerophospholipids and glycero-glycolipids as well as their corresponding lysoforms, the lipases A, G and J were most active. For PC, they reduced the total share from 2.9% (control) to 0.4% (lipase A), 0.5% (lipase G) and 0.6% (lipase J). This corresponds to increases in LPC from 0.1% (control) to 1.4% (lipase A), 1.2% (lipase G) and 1.0% (lipase J) and corresponding average ratios of 27.9, 22.3 and 19.4, respectively. The average ratios towards LPC of the most active lipases M (10.3), O (0.8) and K (5.0) were considerably lower. For the lysoglyceroglycolipids, the average ratios towards DGMG (18:2) ranged from 1.2 (lipase O) to 5.9 (lipase J) and towards MGMG (18:2) from 1.8 (lipase O) to 3.2 (lipase J).

The heatmap of all lipase specificity patterns in pound cake batter is depicted in Figure 30.

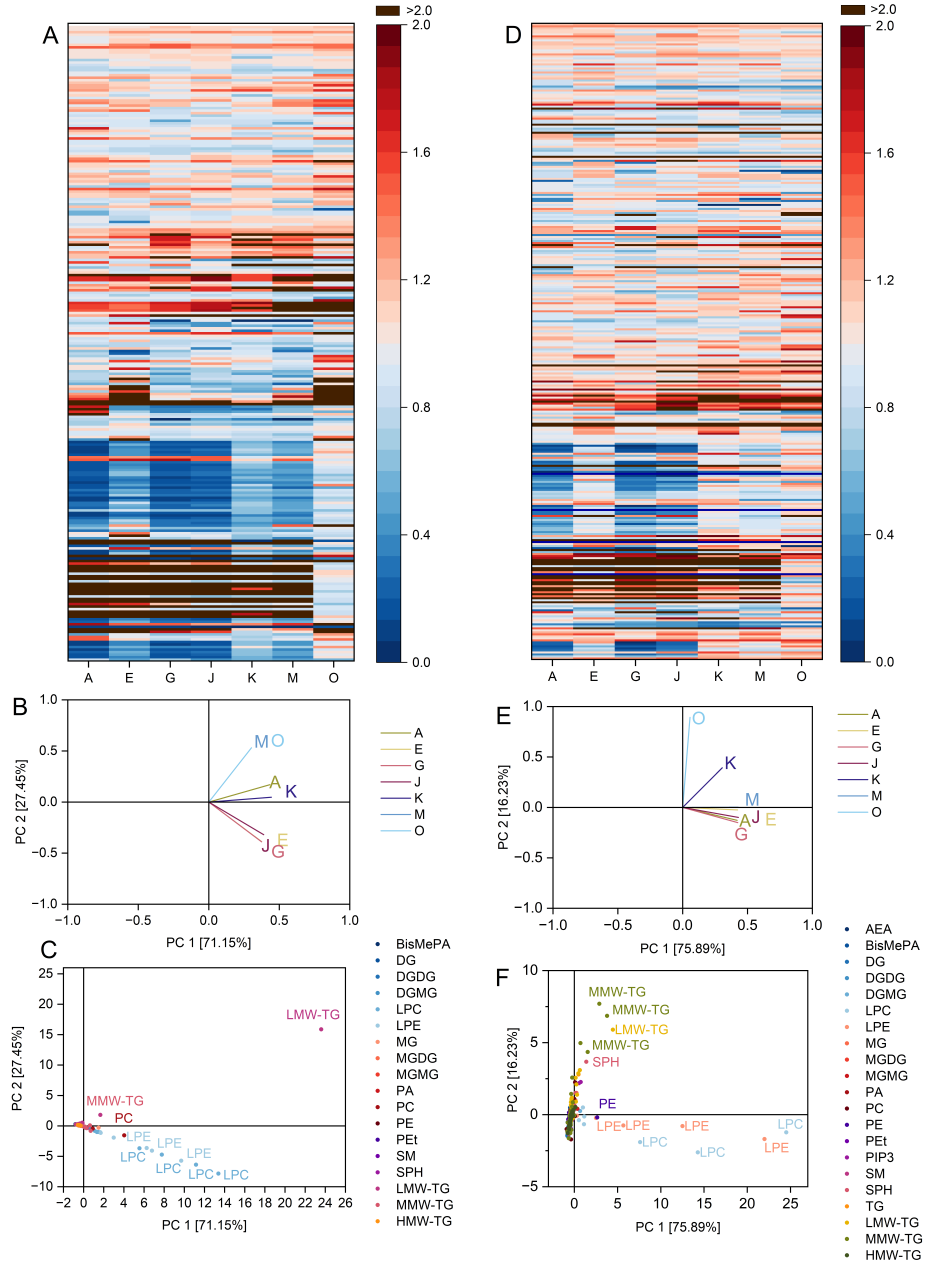


Figure 30: Substrate specificity of the lipases A, E, G, J, K, M and O towards different lipids in pound cake batter (A-C) and in baked pound cake (D-F) (n = 3). For a complete list of substrates, refer to Table 32 for batter and to Table 33 for baked pound cake. A and D: Lipase specificity ratios depicted as heatmap. B and E: Loading plot of the principal component analysis (PCA). C and F: Scores plot of the PCA. For abbreviations please refer to the abbreviations section. Figures already prepared for publication [162].

Besides, Figure 30 also includes the results of a PCA of all ratios within pound cake batter. The PCA captured 98.6% of total variance within the first two components. The loading plot showed three clusters: first the lipases M and O, second the lipases A and K and third the lipases J, E, and G. Considering the scores plot, the similarities between the lipases M and O were mostly due to their reaction towards LMW-TG (4:0/6:0/8:0), while J, E and G had similar ratios concerning four LPC (LPC (18:1), LPC (22:6), LPC (22:5) and LPC (20:4)) and three LPE (LPE (18:1), LPE (22:5) and LPE (20:5)). The lipases A and K were placed in between the two clusters.

3.3.2.2. Cake

In baked pound cake, 316 different lipid species were identified (Table 33). Similar as for pound cake batter, 59.5% of all lipid species belonged to TG. The remaining lipid classes were also as expected based on the results for batter, the only difference between the two batches being the identification of PIP3 in baked products.

The lipid turnover rates ranged from 64.79 mg g^{-1} dry matter (lipase J) to 126.37 mg g^{-1} dry matter (lipase K) (Table 11). They were mostly lower (lipases A, E, G, J, M and O) than in pound cake batter. The turnover rate slightly increased by 11.9% only for lipase K. Besides lipase K, also the lipases O (95.47 mg g^{-1} dry matter) and G (88.64 mg g^{-1} dry matter) had high turnover rates.

As described for pound cake batter, the lipases varied concerning their ratios towards different lipid classes (Table 33). Taking the three most active lipases within baked pound cake (K, O and G) as an example, their average ratios towards lysoglycerophospholipids varied from 1.0 (lipase O) to 6.7 (lipase K) up to 22.6 (lipase G). This corresponds to a complete lack of release of lysoglycerophospholipids after treatment with lipase O, while the share of lysoglycerophospholipids was increased approximately 20-fold by lipase G. For DG, the trend was similar, although less pronounced: the average ratios were 1.3 for lipase G, 1.0 for lipase K and 0.9 for lipase O. Concerning TG, there were only small differences between LMW-TG, MMW-TG and HMW-TG: the lipases G, K and O had average ratios of 1.1, 1.3 and 1.5 towards LMW-TG, 1.2 each towards MMW-TG and 1.0, 1.0 and 1.1 towards HMW-TG, respectively.

The PCA (92.1% of total variance) confirmed visible similarities between the reaction patterns of the lipases A, G and J (Figure 30). Additionally, the specificities of the lipases E and M were also classified as similar to A, G and J based on the release of different LPE and LPC as already stated for pound cake batter. In baked pound cake, the lipid species LPC (20:4), LPC (22:5) and LPC (22:6) as well as LPE (18:1), LPE (22:5), LPE (22:6) and LPE (20:4) were found to be decisive. Lipase O, on the other hand, was characterised by its reaction towards MMW-TG (TG (16:0/16:0/18:2), TG (16:0/18:2/18:2) and TG (6:0/17:1/18:1) and LMW-TG (TG (8:0/10:0/10:0)) and lipase K ranged in between as stated for pound cake batter.

3.3.3. Brioche

3.3.3.1. Dough

202 different lipids were identified in brioche dough (Table 34). As described for basic cake and pound cake, the majority (70.8%) were attributable to TG. In addition to glycerolipids (TG, DG and MG), a range of glycerophospholipids (BisMePA, PC, PE, PEt, PIP2 and PIP3) and corresponding lysoglycerophospholipids (LPC and LPE) were identified. Besides, the brioche dough samples contained glyceroglycolipids (DGDG, DGMG, MGDG, MGMG) and sphingolipids (SM, SPH).

The lipid turnover rates in brioche dough were between 42.96 mg g^{-1} dry matter (lipase E) and 83.27 mg g^{-1} dry matter (lipase A) (Table 11). They ranged between the turnover rates in basic cake and the ones in pound cake systems. Besides lipase A, lipase O had a comparably high lipid turnover rate (79.05 mg g^{-1} dry matter), while the remaining lipases all led to turnover rates between 42.96 mg g^{-1} dry matter (lipase E) and 52.19 mg g^{-1} dry matter (lipase K).

Although the lipid turnover rates in brioche dough were similar to the ones in basic cake and pound cake, the ratios in brioche dough were less prominent and closer to 1.0 (Table 34). For lysoglycerophospholipids, the average ratios varied from 0.5 to a maximum of 1.3, whereas in pound cake, values greater than 20 had occurred. The amount of DGMG (18:2) decreased with ratios of 0.6 to 0.9 (average 0.7), while it had been released with an average ratio of 4.6 in basic cake batter. Correspondingly, the share of DGDG in the samples varied from 0.03% to 0.05% whereas in basic cake batter, values between 0.001% and 0.019% and in pound cake batter values from 0.001% to 0.007% had occurred. The average ratio of all lipases towards HMW-TG was 1.0 ± 0.2, while for LMW-TG and MMW-TG, it also indicated only low variability with average ratios for all lipases of 1.1 ± 0.4 and 1.1 ± 0.3, respectively.

Accordingly, the heatmap of the ratios lacked clear patterns (Figure 31) and the PCA covered only 56.0% of total variance in the first two components, less than in basic cake or pound cake. The lipases were distributed into three quadrants, with only lipase O in the upper left quadrant, the lipases J, E and M in the upper right and the lipases A, G and K in the lower right quadrant. While for basic cake and pound cake, specific lipid classes responsible for the discrimination of certain lipases could be identified, this was not the case for brioche dough. The reaction towards LMW-TG was both characteristic for lipase O and the cluster of the lipases J, E and M. For lipase O, the decisive species was TG (4:0/16:0/18:1) and for the lipases J, E and M these were the species TG (4:0/14:0/18:2) and (4:0/16:0/16:0). The ratios of the lipases A, G and K towards SPH (d18:0) were statistically seen most characteristic. Neither DGMG, as seen for basic cake, nor LPC/LPE as for pound cake, affected the lipase reactivity patterns.

3.3.3.2. Cake

In baked brioche, 192 different lipid species were identified (Table 35). In contrast to basic cake and pound cake, the number of extractable lipids did not increase after baking. Still, the majority of lipid species (70.3%) were TG. Compared to the dough, similar lipid classes were found. Baked brioche contained extractable PG which had not been identified in the dough.

In baked brioche, the lipid turnover rates ranged between 41.69 mg g^{-1} dry matter (lipase J) and 94.76 mg g^{-1} dry matter (lipase E) (Table 11). They resembled the turnover rates in dough. For all lipases besides lipase E, the maximum change was a 36.6% increase in lipid turnover rate from dough to baked product for lipase G. For lipase E, the total change of lipid composition as expressed by the turnover rate was increased by 120.6% in the baked product compared to the dough.

The ratios for the different lipases in baked brioche were similar to the corresponding doughs and therefore less prominent than in basic cake or pound cake (Table 35). Towards DGDG, the average ratios varied from 1.0 (lipase J) to 1.4 (lipase O) and towards MGDG from 1.0 (lipases E, J and K) to 1.3 (lipase O). The share of both was therefore not reduced as expected based on the results for baked basic cake and baked pound cake, where ratios as low as 0.1 towards DGDG and MGDG occurred. For lysoglycerophospholipids, the average ratios were from 0.8 (lipase A) to 1.2 (lipase E), indicating only a slight increase in their percentage. Concerning the group of TG, the average ratio of all lipases towards LMW-TG was 1.1 ± 0.7, towards MMW-TG 1.2 ± 1.0 and towards HMW-TG 1.2 ± 1.2. The variance between the lipases and their ratios towards different MMW-TG and HMW-TG was therefore greater than in dough. However, there were no clear patterns in the ratios.

Consequently, the heatmap (Figure 31) contained few coherent areas and many individual values varying between the lipid classes. The PCA covered only 68.7% of total variance in the first two components. There were no clusters of lipases, all were situated either in the upper right quadrant (lipases O, K and A) or the lower right quadrant (lipases J, G, M and E). In all cases, the ratios towards specific TG were decisive. Of the 49 MMW-TG, the three specific TG (4:0/18:1/18:3), TG (12:0/14:0/18:1) and TG (14:0/16:0/18:1) affected the discrimination and of the 27 HMW-TG, the two TG (16:0/18:0/24:0) and TG (16:0/18:0/20:0) affected the PCA. Similar as reported for brioche dough, no single class leading to a discrimination of the lipases could therefore be identified.

3.3.4. Discussion

A lipidomics LC-MS/MS method was successfully transferred and applied for the analysis of lipase-treated cake samples before and after baking.

First, the identified lipid classes will be compared with the expected lipid classes in the samples, which were reported for the ingredients. Based upon the results, the suitability of the chosen method will be critically discussed. Then, the total lipid turnover rates will be evaluated taking into consideration the results from section 3.1. Finally, a link between the lipase substrate specificities (the molecular level) and the resulting effects on batter and product quality (the macroscopic level) will be established using the results from section 3.2.

The final setup for lipidomic analysis of lipase-treated batters/doughs and cakes and their re-

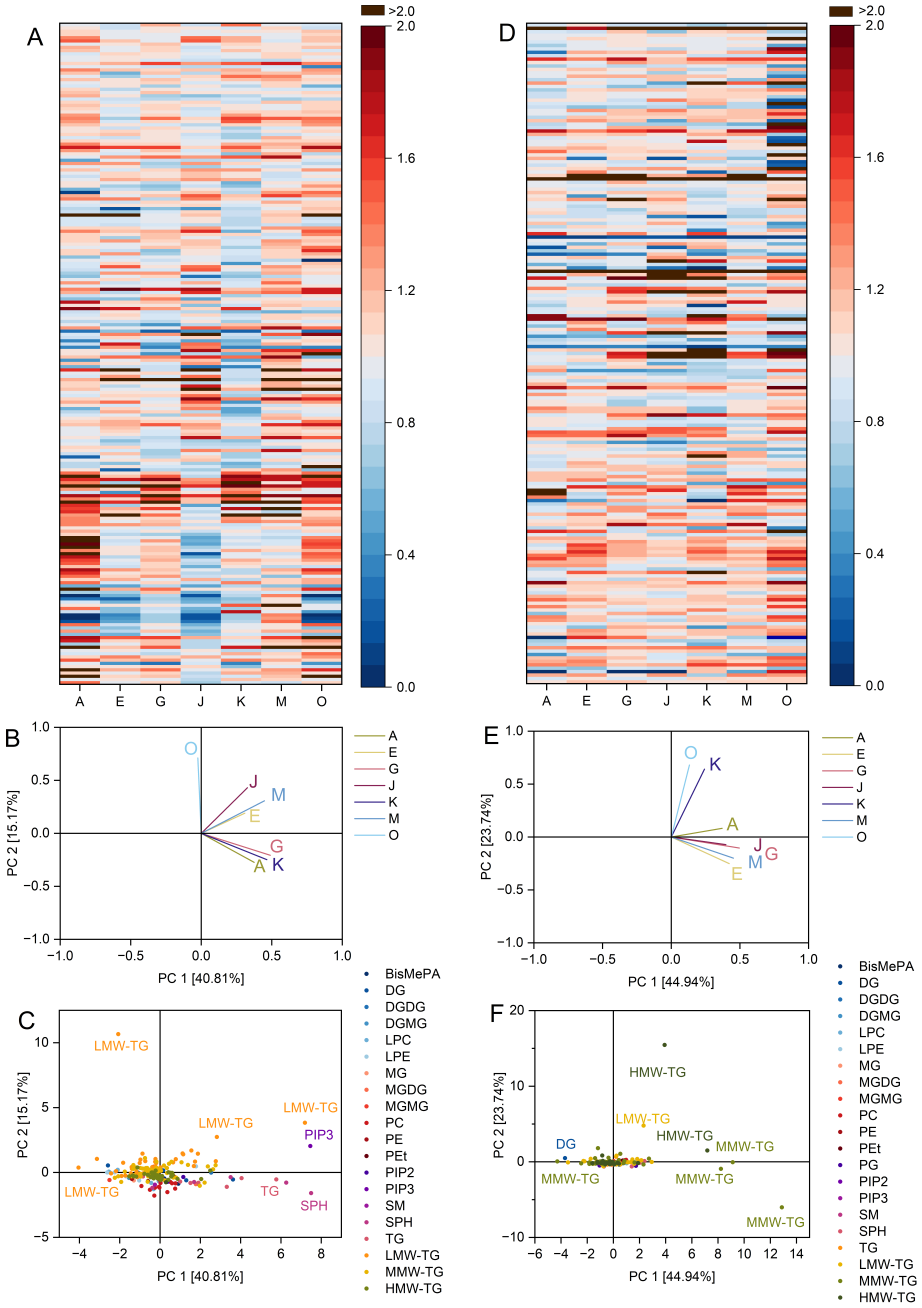


Figure 31: Substrate specificity of the lipases A, E, G, J, K, M and O towards different lipids in brioche dough (A-C) and in baked brioche (D-F) (n = 3). For a complete list of substrates, refer to Table 34 for dough and to Table 35 for baked brioche. A and D: Lipase specificity ratios depicted as heatmap. B and E: Loading plot of the principal component analysis (PCA). C and F: Scores plot of the PCA. For abbreviations please refer to the abbreviations section. Figures already prepared for publication [162].

spective control sample covered 22 different lipid classes.

Of these 22 lipid classes, 8 were found in basic cake batter and 13 in baked basic cake. The lipids in basic cake originate mostly from butter (94% based upon a fat content of 2% for wheat flour [67] and 82% for butter). The lipid distribution of butter is known to be dominated by TG as discussed in section 1.4. A high share of TG (approximately 94% based upon the lipid distributions as discussed in section 1.4) was therefore expected. Concerning the increase of identified lipid classes from batter to cake, similar effects were reported for bread. Baking is known to alter the lipid composition of bread compared to the dough in combination with the use of lipases [45]. Even without lipase application, the extractability of, e.g., lysoglycerophospholipids and glyceroglycolipids, was improved [73]. Therefore, after baking, PE and MGDG were found when neither had been detected in the extracts of basic cake batter. When TG are hydrolysed, DG and MG are formed. Based upon the high share of TG, the comparably low share of DG species in the total number of species after lipase addition was rather unexpected (8.0% in basic cake batter and no identification in baked basic cake). The same applied to MG (no identification in batter and 0.7% in baked basic cake samples). Considering the total change of lipids of treated samples compared to untreated samples, the hydrolysis of TG took place and therefore an increase in DG and MG was anticipated. This expectation was not met by the results which showed only low amounts of DG and MG. Interestingly, a study of lipase treated bread doughs by Schaffarczyk et al. [60] revealed a similar effect: the overall level of DG decreased during proofing with lipases, probably due to their further hydrolysis to MG and then to glycerol. In basic cake samples, either TG were fully hydrolysed to glycerol or the formed DG and MG species were too low in abundance for identification.

In pound cake batter, 16 lipid classes were identified and baked pound cake contained lipid species belonging to 17 different classes. The broader range of lipid classes compared to basic cake batter was caused by the introduction of eggs to the recipe. Based on an egg lipid content of 12% [67], 13% of all lipids in pound cake originated from eggs. An increase in the variability of PC and PE species was therefore expected and found. Besides PC and PE, also the classes of PEt, BisMePA, PIP3 and SPH were identified. They were not reported in the lipidomes of the used ingredients before. However, instead of fresh eggs as analysed in literature, e.g., by Ali et al. [82], pasteurised eggs were used and instead of milk as done by Li et al. [79], butter was applied. Additionally, the ingredients were further processed during sample manufacturing. Both the processes during ingredient manufacturing and sample manufacturing can affect the lipid composition: further lipid classes could be either introduced, released for extraction or formed by the change of already present lipids.

Brioche dough contained lipids belonging to 17 different classes and baked brioche of 18 different classes. Eggs are also part of the brioche recipe. However, due to the low level of eggs relative to the total weight of all ingredients, they only account for 6% of the total dough weight, corresponding to 5% of total lipids. Accordingly, wheat is, besides butter, the main source of lipids in brioche with a share of 8% of the total lipids (compared to 2% in pound cake). The identified lipid classes in pound cake and brioche were still similar. Compared to pound cake batter, brioche dough contained PIP2 and PIP3 instead of PA. Possibly, the effect of yeast in brioche enhanced the extractability of PIP2 and PIP3 compared to the yeast-free pound cake, while the extraction of PA was inhibited. Alternatively, the formation of PA as

a hydrolysis product (e.g. of glycerophospholipids like PC catalysed by a phospholipase of type D as discussed in section 1.2.1) in pound cake but not in brioche would be possible.

Again, baking increased the number of lipid classes compared to the corresponding dough, in this case due to the detection of PG to baked brioche. PG are associated with the cell membranes of animals, plants and microorganisms. They have not been identified in eggs or milk but are part of the lipidome of wheat [71]. Three possible explanations for the identification of PG in baked brioche are therefore put forward: First, PG were also present in basic cake and pound cake, but in lower concentrations and therefore not identified. This assumption is supported by the contribution of wheat lipids to the total lipids in the samples, which were 6% for basic cake, 2% for pound cake and 8% for brioche, approximately. Second, the hydrolysis of PG was more effective in basic cake and pound cake and they were fully degraded. Indeed, LPG were identified in basic cake, but not in pound cake. Third, PG could be formed during yeast fermentation of brioche. Proofing and fermentation were reported to affect the population of glycerophospholipids [73]. As neither basic cake nor pound cake contained yeast, this could therefore also explain the difference. Besides the formation of PG, the fermentation could also affect their extractability. Most likely, a combination of all suggested mechanisms took place.

With regard to the suitability of the method for the lipid classes in the samples, the dominant class of TG was captured extensively in all samples: up to 188 different species could be differentiated (in baked pound cake). For glycerophospholipids, up to 92 different species were detected (also in baked pound cake). However, when comparing the identified lipid classes with the ones reported for the individual ingredients (section 1.4), several lipid classes were missing (Figure 28). There are four main causes for a lack of identification:

1. The database used for identification did not include the lipid class. The search engine “Lipid Search” was originally developed for the analysis of glycerophospholipids and implemented in the analysis of biological samples such as mammalian lipids [163, 164]. It does not comprise NAPE and NALPE, which were reported to occur in bread dough and bread in combination with lipase treatments [73]. Accordingly, neither NAPE nor NALPE were identified in the extracts. As both species are only minor compounds, their lack was not expected to alter the outcome of the study.

2. Further minor compounds could be lacking due to their low abundance in the samples, poor extractability or low ionisation. This could be the case for Chol and its derivatives, which were expected based on the amount of eggs and butter in the recipes. However, neither Chol nor its derivatives were found. The commercially available lipid analytical standard used for method development also contained Chol which could not be identified. Most likely, the ionisation conditions were therefore the cause for missing Chol in the samples. Nonetheless, the ionisation conditions were well suitable for the major lipid classes such as TG and glycerophospholipids.

3. During the interaction of lipases with lipids in batters/doughs and cakes, volatile lipids are formed. They are most likely lost during sample preparation. Long incubation times with lipases were shown to decrease the total amount of FFA as discussed in section 3.1. After incubation, part of the samples were also baked before lyophilisation and milling.

All three processes can further decrease the amount of FFA in the samples. Besides a more gentle procedure, the aforementioned GC method would be better suitable for the analysis of FFA. However, GC methods are not suitable for the analysis of polar lipids such as glycerophospholipids. While these polar lipids remain in the samples where they can exert effects on the rheological and textural properties, the contents of FFA were decreased even without lyophilisation and milling. FFA were therefore not expected to influence the outcome of lipase addition to cakes to a similar extent than other lipids on a macroscopic level. As discussed for NAPE, their lack was not expected to alter the outcome of the study.

4. Besides FFA, lysoforms of lipids are generated when lipases are added. While the lysoforms were not necessarily part of the lipidome of the samples before lipase addition, the original forms may be lost due to their degradation. They could therefore no longer be present in the extracts because they were completely hydrolysed. This could be a possible explanation for the lack of Cer (expected due to the portion of butter in the samples), while So were identified (Figure 28). This loss was anticipated in lipase-treated samples.

Although some minor compounds and volatiles were not covered, the study included the majority of lipid classes which were expected in the samples. It is currently not possible to analyse the complete lipidome of complex samples using a single-run procedure of one analytical technique. The final setup was therefore suitable for the analysis. If needed, further experiments could be conducted in addition to the established method for the comprehensive analysis of cake lipidomes.

For all lipids included in the setup, total lipid turnover rates were calculated. The turnover rates are used as an approximation for lipase activity in the samples. They can only act as an approximation, because first, not all lipid classes were covered and second, the semiquantification of lipids was done by the use of a single internal standard belonging to the class of TG (section 5.4.3). As TG was the most abundant lipid class in all samples, the internal standard was well suitable for most lipids. However, it does not reflect the different ionisation conditions for other lipid classes such as glycerophospholipids and glyceroglycolipids. For a more comprehensive quantification, the introduction of at least one standard per lipid class would be advisable. However, their concentrations would have to be adapted depending on the exact cake recipe and currently commercially available lipid analytical standard mixtures cannot be applied, leading to high costs for single standards.

The lipid turnover rates varied between the different recipes and also between batters/doughs and the corresponding baked products. Based on the share of lipids in the different recipes (14% in basic cake, 24% in pound cake and 13% in brioche), the highest turnover rates were expected in pound cake. With average turnover rates of 125.8 mg g^{-1} dry matter in pound cake batter and 84.8 mg g^{-1} dry matter in baked pound cake, this assumption was proven. In basic cake batter, the average activity was only 30.7 mg g^{-1} dry matter, in baked basic cake 38.9 mg g^{-1} dry matter, in brioche dough it was 57.5 mg g^{-1} dry matter and in baked brioche 65.5 mg g^{-1} dry matter. In basic cake and brioche, the total turnover rates increased from batter/dough to baked product. The extractability of lipids is affected by baking: lipids which were not extractable from batter or dough can be extracted from the baked products as shown

for the variation of lipid classes between corresponding products before and after baking. It is unclear why these effects did not lead to higher lipid turnover rates for pound cake. The turnover rates do not match the effects described for the rheological and textural properties of lipase-treated cakes (section 3.2). There, the highest impact of lipase addition occurred in basic cake, less effects were caused in pound cake and nearly no improvement of either rheological or textural properties was achieved in brioche. The turnover rates therefore do not seem to be decisive for the macroscopic effects. They are more likely linked to the substrate specificities of the lipases. This is in accordance with the results from Gerits et al. [58], who reported that the amount of FFA released by lipases in breads was no indicator for their effect on bread loaf volume.

Besides the recipes, the lipid turnover rates also depended on the individual lipases. In baked basic cake, e.g., the turnover rate of lipase K was 10 times as high as the one of lipase A. Whether a lipase had a rather high or a rather low turnover rate compared to the other lipases, again depended on the sample: in basic cake batter, the lipases O, G and E released most lipids, in baked basic cake, the lipases K, J and G while in pound cake batter, it were the lipases M, O and K and in baked pound cake the lipases K, O and G with the highest turnover rates. In brioche dough, the lipases A, O and K had the highest turnover rates and in baked brioche, the lipases E, A and O. The different samples contained different lipids as substrates for the hydrolysis reaction. Different substrate specificities of the lipases could therefore serve as an explanation for the effect of the recipes. A similar argument is also applicable for the effect of baking: During baking, the accessibility of lipids changes. To interact with lipases, lipids need to be accessible at an oil/water-interphase [24]. If interphases are modified or lost during baking, lipases can no longer catalyse hydrolysis reactions. Besides the lipid accessibility, heating also causes the degradation and loss of heat-sensitive lipids as discussed for FFA. This also leads to differences between batters/doughs and baked products.

The lipid turnover rates did not match the results from section 3.1. In the *p*-nitrophenyl assay, highest activities the lipases J, G and A had the highest activities and for the reaction of the lipases with emulsified butter, the lipases O, A and K released most FFA. The exact lipase activities as presented in section 3.1 were not expected to be reproduced, both due to the approximated values used in the lipidomics approach and the dependency of lipase activity on the accessibility of substrates as discussed in section 1.2.2. However, not even the trends of activities between different lipases were reproducible in the bakery products. Even if 94% of all lipids in basic cake originated from butter, instead of the lipases A, K and O the lipases O, G and E had the highest lipid turnover rates in basic cake batter. Lipase activity trends in complex foodstuff cannot be predicted using simple model systems, even if the same substrates are used.

The seven lipases were classified as phospholipases (A, E, J and M), a galactolipase (K) and a TG lipase (O) by the manufacturers. For lipase G, no information on its preferred substrates were available (Table 7). Despite their classifications, all lipases interacted with a broad range of lipids in the samples. This phenomenon has been described before and led to the description of lipases as variable, as discussed in section 1.2.3. However, the lipases differed in their substrate specificities. Although they interacted with all substrates, they still led to different extents

of hydrolysis and had varying efficiencies in the formation of lysolipids. The relationship between the resulting substrate specificities and the effects of the lipases on a macroscopic level, namely the rheological properties of batters and doughs and the texture of cakes as presented in section 3.2, will be discussed in the following.

To sum up the effects of lipases on the properties of basic cake batter, the lipases A, G and J affected the rheological properties to the greatest extent by increasing the LVE and leading to a liquefaction of the batter as well as a reduction of batter stickiness. The lipases G and J additionally decreased the batter density of basic cake batter. The lipases A, G, J, K and O all had an impact on the onset of flow in the batter. If the different effects of the lipases were caused by their similar specificity patterns, it was therefore expected for the lipases A, G and J to have similar specificity patterns. However, according to the PCA, the only lipase which reacted clearly differently from A, G and J was lipase K. The expectation was therefore not fulfilled: the specificities of the lipases A, G and J were found to be similar to the ones of the lipases O, M and E which all performed less on a macroscopic level. In basic cake batter, all lipases showed a preference for the release of LMW-TG compared to MMW-TG and HMW-TG. Interestingly, this is in accordance with the findings for lipase reactions with emulsified butter from section 3.1. TG with short chain FA seemed to be more easily accessible for hydrolysis in basic cake batter than their long chain counterparts.

On the macroscopic level of baked basic cake, the lipases A, G and J again affected the texture to a similar extent. While all lipases inhibited the staling and reduced resilience and cohesiveness, only the lipases A, G and J led to softer products even after 96 h of storage. Similar as for basic cake batter, the specificities of the lipases A, G and J were therefore expected to be clustered in the PCA. Indeed, they were found to behave in a similar way , especially according to their specificity for the release of DGMG and MGMG. Besides A, G and J, also lipase M exhibited a similar specificity for these two lipid classes and was therefore clustered with A, G and J. The hydrolysis of glyceroglycolipids was also reported to be decisive for the effect of lipases on the texture of bread by two independent studies [58, 61]. A mixture of MGMG, DGDG and NALPE present in bread loafs led to maximum volumes of the products [61]. This indicates possibly similar mechanisms for both the texture of basic cake and the volume of bread loafs. Improved extraction techniques and targeted MS/MS-measurements for basic cake batter could be applied to verify this assumption. Concerning lipase M, its difference to A, G and J on the macroscopic level is probably due to lipids which were not identified with the chosen approach, as, e.g., NALPE.

In pound cake batter, the macroscopic effects were less pronounced than in basic cake batter. Lipase A affected its properties most strongly. Besides, also the lipases G, J and K led to a liquefaction of pound cake batter. Similarities in the substrate specificity patterns of the lipases A, G, J and K could therefore explain their similar behaviour. Indeed, the reaction patterns of the lipases G and J, in combination with the one of lipase E, were found to be similar in the PCA. All three lipases released preferentially LPC and LPE. Two conclusions can be drawn from this finding: First, lipase G, whose preferred substrate type was previously unknown, was possibly also a phospholipase. Both the lipases J and E were stated as such and lipase G also interacted preferentially with glycerophospholipids, supporting this assumption. Second, lysoglycerophospholipids seemed to be linked to the rheological properties of pound

cake batter. This implies a different mechanism for the macroscopic effects of pound cake batter than suggested for basic cake batter. The findings for basic cake batter were in accordance with the mechanisms known for bread, while pound cake clearly differed from this scheme. For bread, a negative correlation of lipase specificity towards glycerophospholipids and bread loaf volume has been stated [58], although synergistic effects of LPC and glyceroglycolipids were suggested [60]. In contrast to bread and basic cake, pound cake contains egg. The gas cells in pound cake, which are linked to its texture as discussed in section 3.2, are therefore mostly stabilised by polar lipids and egg lipoproteins [3]. In bread and supposedly also in eggless basic cake, gluten and non-starch polysaccharides are crucial for gas cell stabilisation [165]. The different mechanisms in pound cake and basic cake require different lipids for an improved gas cell stabilisation. Although the release of lysoglycerophospholipids seemed to be linked to the macroscopic properties of pound cake batter, further mechanisms are needed: instead of the lipases A and K, lipase E was clustered with G and J. An improved extractability of different lipid classes after baking as found for basic cake could solve this question.

The texture of baked pound cake was most improved by the addition of the lipases A, G, J and E. The first three were more effective at reducing staling, but all four lipases reduced the resilience and the cohesiveness of the products. Interestingly, the PCA separated the lipases according to their indicated specificities. The specificities as described by the manufacturers were (partly) proven to be reproducible in pound cake: lipase O, the TG-lipase, interacted mostly with TG, the phospholipases A, E, J and M and the putative phospholipase G with PC and PE and the galactolipase K was situated in between the two. Concerning the link to the macroscopic level, the lipases A, G and J were more similar to each other than to the other phospholipases, also based on their ability to release LPC and LPE. The assumption made for pound cake batter, that the release of lysoglycerophospholipids was decisive for an impact on the macroscopic level, was therefore supported.

For brioche dough, no clustering of lipases was expected based on their missing impact on the rheological properties. In the PCA, the lipases were split into three quadrants. However, the differences were not based upon their reaction with different lipid classes, but on their reaction towards LMW-TG for all lipases. The absence of impact was originally explained by a missing activity of the lipases (section 3.2). Taking the estimated turnover rates in brioche and the results from the analysis of substrate specificity into consideration, it can be stated that lipases are active within brioche dough. However, the lipids they release do not lead to improvements of the rheological properties. The lipids present for hydrolysis in brioche dough are similar to the ones present in pound cake batter, due to similar ingredients for both recipes. However, the substrates specificities towards, e.g., LPC were not comparable. In pound cake batter, LPC were released with substrate specificities which were 20 times as high as in brioche dough. This supports the assumption that the substrate specificity depends not only on the inherent properties of the lipases, but also on the accessibility of the substrates. The same lipases may therefore have different reactivity patterns towards the same lipids in different matrices. This is in accordance with the findings for the FA substrate specificity of lipases in section 3.1. Previous studies stated that PC and TG in bread are only hydrolysed during mixing but not during fermentation [151]. By altering the mixing conditions it might therefore be possible to influence the type of lipids which is hydrolysed by lipases in brioche dough and improve the

outcome on the macroscopic level.

The same as for brioche dough also applies to baked brioche. The lipases affected its macroscopic level and therefore its texture only slightly with a small reduction of resilience and cohesiveness by the lipases E, G and J. In the PCA, the lipases were split according to their indicated specificities as in baked pound cake. Surprisingly, this was not due to their reaction with glycerophospholipids, but caused by their different ratios towards a range of MMW-TG. This effect was therefore considered a coincidence. For brioche, an increase of lipid extractability after baking did not reveal special patterns of lipase specificities. As discussed for brioche dough, the released lipids did not lead to functional effects. The lipids needed for textural improvements seemed to be inaccessbile for hydrolysis.

The results show that an LC-MS/MS method could be successfully used and transferred for the analysis of cake lipidomes with and without lipase treatment in batters/doughs and baked products. A total of 22 different lipid classes was identified. They comprised the major lipid classes of all ingredients used for cake preparation. Baking increased the extractability of several lipid classes such as glyceroglycolipids and lysoglycerophospholipids. Estimated lipase activities based on total lipid turnover rates did not match the lipase activities as determined in section 3.1 using the *p*-nitrophenyl assay or the amounts of released FFA from emulsified butter (also section 3.1).

By calculating substrate specificity ratios, putative key compounds responsible for impacts on the macroscopic level of cakes were identified. For basic cake, the specificities towards glyceroglycolipids and for pound cake, towards lysoglycerophospholipids were found to be a characteristic property of lipases improving both rheological and textural properties of cakes. The results from brioche showed that the same lipases can react with different substrates when used in different media. This is in accordance with the findings from section 3.1 for the FA substrate specificity, which also depended on the accessibility of substrates. The lipidomic insights into lipase reactions in cakes led to a deeper understanding of the mechanisms behind rheological and textural effects of lipase reactions and of lipase reactions in general. To deepen the knowledge about lipase reactions with different substrates and the factors determining lipase substrate specificities, further experiments were carried out (section 3.4.

3.4. Establishment of a new lipase activity assay for use of baking lipases in fine bakery goods

Parts of the following were already published in *Food Chemistry: X* [97].

The seven lipases chosen in section 3.1, used in fine bakery goods (section 3.2) and characterised in terms of their reaction patterns in fine bakery goods (section 3.3) were further characterised by the use of commercially available assays. Then, a new assay for the prediction of lipase reactions with different substrates was developed.

3.4.1. Comparison of three commercially available assays

The activities of the lipases A, E, G, J, K, M and O were determined using three commercially available assays.

3.4.1.1. Assay I

Assay I was based on a coupled enzyme reaction. The calibration was against glycerol, which reacted to a colourimetric product that was measured continuously. The calculation based on the manufacturer's instructions led to lipase activities ranging from -0.06 µmol glycerol $min^{-1}mg^{-1}$ to 2.34 µmol glycerol $min^{-1}mg^{-1}$. Negative lipase activities occurred, because the calculation method suggested by the manufacturer relies on the first and the last measurement point within the calibration range and assumes a linear increase of signal intensity in between. However, this is not necessarily the case for all lipase reactions (Figure 32). Especially lipases with low activities lead to low reaction rates to nearly no reaction at the beginning, as shown exemplarily for lipase K. The signal intensity first decreased before starting to increase linearly approximately 40 min after the first measurement point within the calibration range. Therefore, all curves were checked manually for the area of linear increase of signal intensity and the activities were re-calculated (section 5.5.1).

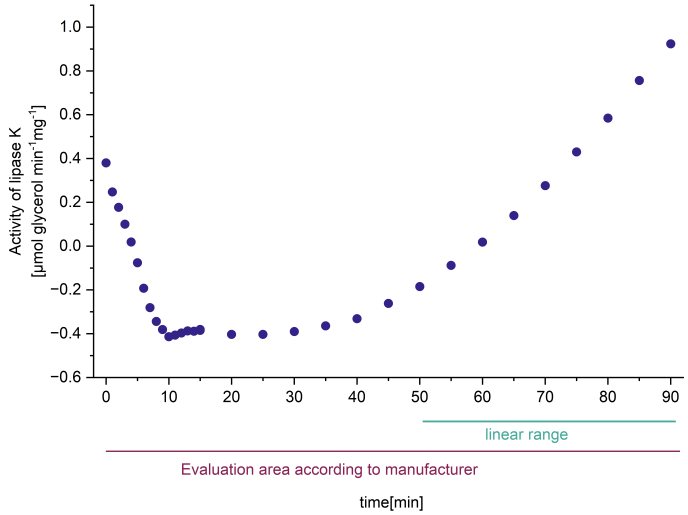
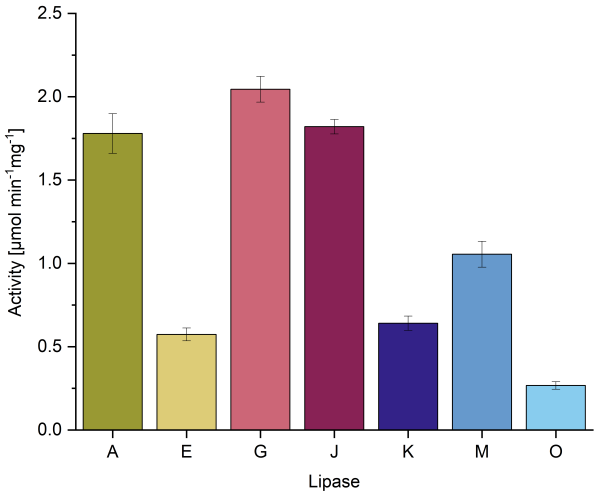
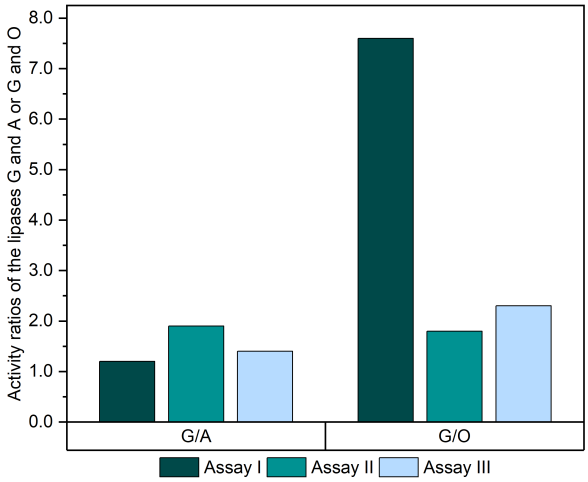


Figure 32: Evaluation area and linear range as measured for lipase K using Assay I.

The new activities ranged from 0.27 µmol glycerol $min^{-1}mg^{-1}$ to 2.05 µmol glycerol $min^{-1}mg^{-1}$ (Figure 33a, Table 36). They were highest for the lipases G and J and lowest for the lipases E and O. To compare the assays, the activity ratios of the lipases G/A and G/O were calculated exemplarily for each of the three commercially available assays (Figure 33b). For Assay I, they were 1.2 for G/A and 7.6 for G/O.



(a) Lipase activities of the lipases A, E, G, J, K, M and O measured with Assay I (n = 3). Data already published in Stemler & Scherf [97].



(b) Activity ratios of the lipases G and A or G and O as determined by the assays I, II and III.

Figure 33: Lipase activities measured with Assay I (a) and activity ratios of the lipases G and A or G and O (b).

3.4.1.2. Assay II

Assay II was also based on a coupled enzyme reaction. The calibration was against 2-nitro-5-thiobenzoate (TNB), which has a yellow colour under the conditions of the assay. The activities calculated as described for Assay I were between 6.68 nmol TNB $min^{-1}mg^{-1}$and 201.96 nmol TNB $min^{-1}mg^{-1}$ (Figure 34, Table 36). The highest activities were again measured for the lipases G and J. The lipases E and K had the lowest activities in this assay. The ratios G/A (1.9) and G/O (1.8) were comparatively similar to each other but did not correspond to the ratios determined in Assay I (Figure 33b).

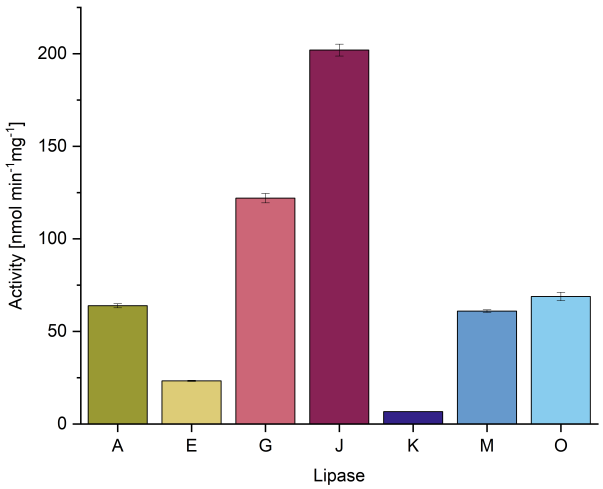


Figure 34: Lipase activity of the lipases A, E, G, J, K, M and O measured with Assay II (n = 3). Data already published in Stemler & Scherf [97].

3.4.1.3. Assay III

In Assay III, the coupled enzymatic reaction of the assay resulted in the release of methylresorufin, which was then detected fluorometrically. The activities based upon the calculation introduced for Assay I ranged from 162.78 nmol methylresorufin $min^{-1}mg^{-1}$to 2244.74 nmol methylresorufin $min^{-1}mg^{-1}$, showing a more than 13fold range between the lipases (Figure 35, Table 36). Again, the highest activities were determined for the lipases G and J while O and K had the lowest. The ratios G/A and G/O were 1.4 and 2.3, respectively (Figure 33b).

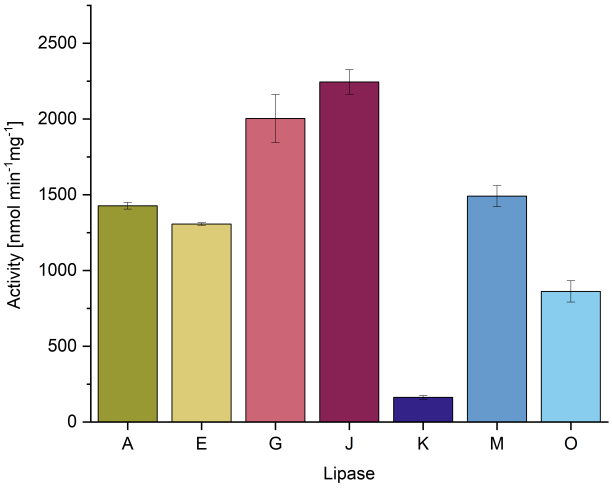


Figure 35: Lipase activity of the lipases A, E, G, J, K, M and O measured with Assay III (n = 3). Data already published in Stemler & Scherf [97].

3.4.1.4. Discussion

Three commercially available assays were used to characterise the lipases A, E, G, J, K, M and O. The assays relied on different substrates and different detection mechanisms. It was therefore not expected to reproduce the exact same lipase activities in the three approaches. However, similarities between the reactivity patterns and also to previous experiments could occur. In the *p*-nitrophenyl assay (section 3.1), the lipases J, G and A had the highest activities of the seven lipases.

For all assays, a new evaluation scheme was applied. It led to more reliable results than the calculations suggested by the manufacturers of the assays. The new evaluation scheme was more time-consuming than the original one, as it included a manual check of linear areas. This was indispensable: the original approach resulted in partly negative lipase activities, although a lipase reaction took place.

The first assay relied on the release of glycerol. The exact reaction remains proprietary, but, most likely, TG were included as substrates as in a similar approach first suggested by McGowan et al. [166]. Lipase O, which showed the highest specificities of all lipases towards TG in fine bakery goods (section 3.3), had the lowest activity of all lipases in this assay. This seems contradictory and needs further clarification.

For the release of glycerol from TG, three consecutive hydrolysis reactions are needed. First, TG have to be hydrolysed to DG, which then react to MG, which release glycerol after the

cleavage of the last FA. If lipases react with TG under the conditions of the assay, they do not necessarily catalyse the following reactions. Regioselective lipases, e.g., which interact only with the *sn* 2 position of TG and selectively release only 1,3-DG, are therefore not covered in the assay. This could explain the low activity of lipase O in this approach and underline the necessity of FFA as a base for the calibration of the newly developed assay. Still, in accordance with the *p*-nitrophenyl assay, the lipases G and J had high activities.

For the second assay, the underlying reaction was assumed to be similar to the one presented by Choi et al. [167] based on the substances included in the kit. In brief, the lipases cleave a thioester of 2,3-dimercapto-1-propanol tributyrate, resulting in free thiol groups. The free thiol groups then react with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, Ellman's reagent) and release the coloured TNB. Again, the lipases G and J had the highest activities of all lipases in accordance with the *p*-nitrophenyl assay. However, the ratios G/A and G/O differed from Assay I: for G/O, the ratio was more than four times as high in Assay I than in Assay II. Thiol esters were not included in the previous experiments and are no typical substrates for lipases in fine bakery goods.

The third assay included methylresorufin for calibration. A putative reaction mechanism can be formulated based on the mechanism for resorufin esters by Beisson et al. [25]: 1,2-O-dilauryl-*rac*-glycero-3-glutaric acid-methylresorufin ester is cleaved to 1,2-O-dilauryl-*rac*-glycerol and glutaric acid-methylresorufin ester, which then reacts to glutaric acid and methylresorufin. The lipases therefore need a specificity for the bulky glutaric acid-methylresorufin ester for this reaction. Similarly bulky FA or FA analogues were not included in previous experiments and, as already stated for the thioester, are no typical substrates for lipases in fine bakery goods. Interestingly, the ratios G/A and G/O resembled to the ones of Assay II. Given the different conditions of the assays, this resemblance was most likely a coincidence. As described for Assay I, Assay II and the *p*-nitrophenyl assay, the lipases G and J had the highest activities of all lipases.

To sum up activity trends, the lipases G and J had rather high activities in all three commercially available assays and the lipases E and K had rather low activities, while the lipases A and M ranged in between (Table 12). For lipase O, the activity was strongly dependent on the assay, as it had the lowest activity of all lipases in Assay I, but the third highest activity of all lipases in Assay II. The activity trends were in accordance with the findings from the total activity in the *p*-nitrophenyl assay from section 3.1, where the order of decreasing activity was: J, G, A, M, E, O, and K. However, in the *p*-nitrophenyl assay, the ratios G/A and G/O were 1.2 and 16.7, respectively, which indicates a much higher range of activities than in the commercially available assays. There were still similarities between lipase activities in all approaches including artificial assays.

These trends from the assays did not match the total amount of released FFA from the model emulsions (also section 3.1, Table 12). In the emulsions, the lipases O (rapeseed oil, butter and wheat germ oil emulsion) and E (margarine emulsion) performed best while the lipase J released rather low amounts of FFA. The reaction conditions in the emulsions and the assays were comparable. In both cases, the lipases were added to substrates emulsified in an aqueous, buffered solution. The main difference between the two setups were the substrates: In the emulsions, the lipases reacted with naturally occurring fats instead of artificial substrates

as used for the assays. These naturally occurring fats included lipids with varying FA chain lengths and degrees of unsaturation. Besides, they differed in terms of their alcohol moiety from the artificial substrates of the assays. Both substrate specificity and FA substrate specificity of the lipases therefore affected their reaction rates in the different setups. This finding leads to the conclusion that a major drawback of activity assays with artificial substrates is also the uniformity of the substrates.

Table 12: Order of lipase activities as determined in emulsions as model systems (section 3.1), batters, doughs and cakes by lipidomics (section 3.3), assays with artificial substrates (sections 3.1 and 3.4.1) and the new assay. DOPC - 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine

Activity determined in ...		lipases in decreasing order of activity						
Emulsions as model systems	rapeseed oil	O	A	M	E	G	J	K
	margarine	E	M	A	K	O	G	J
	butter	O	A	K	G	M	J	E
	wheat germ oil	O	M	K	G	E	A	J
Batters, doughs and cakes	basic cake batter	O	G	E	M	J	A	K
	basic cake	K	J	G	O	E	A	M
	pound cake batter	M	O	K	E	A	G	J
	pound cake	K	O	G	M	A	E	J
	brioche dough	A	O	K	M	J	G	E
	brioche	E	A	O	G	M	K	J
Assays with artificial substrates	*p*-nitrophenyl assay	J	G	A	M	E	O	K
	assay I	G	J	A	M	K	E	O
	assay II	J	G	O	A	M	E	K
	assay III	J	G	M	A	E	O	K
New lipase activity assay (section 3.4.2.2)	olive oil	E	O	M	J	G	A	K
	wheat germ oil	J	A	G	O	E	K	M
	flaxseed oil	J	E	M	O	G	A	K
	coconut oil	O	J	A	E	G	M	K
	butter	O	G	J	A	K	E	M
	triolein	J	O	A	G	E	M	K
	DOPC	J	O	M	E	K	G	A

The trends from the assays could also not be verified in baking trials from section 3.3 and they were no suitable predictor of baking quality. In terms of baking quality, also the applied dosages need to be taken into consideration. Higher amounts of lipases lead to higher reaction rates. Higher reaction rates do not necessarily lead to better baking performances as discussed in section 3.3. Also for the application of lipases in bread, lipase activity assays like the *p*-nitrophenyl assay were not found to be suitable for the prediction of baking performances

[58]. As discussed before, instead of lipase activities, lipase substrate specificities are decisive for an impact on baking quality. The presented assays (Assay I, Assay II, Assay III and the determination of total lipase activity by the *p*-nitrophenyl assay) all rely on specific reactions which are not relevant for baking performance, either because they include artificial substrates or rely on a series of specific reactions (Assay I). These shortcomings were to be overcome with a new assay.

3.4.2. Development of a new lipase activity assay

3.4.2.1. Requirements for the new assay, operating principle and established procedure

For the prediction of lipase reactions in fine bakery goods, a new assay was needed. There were five main requirements for the assay:

1. Its application needed to be easy, both in terms of handling and laboratory equipment required. The assay was meant for companies in the cake industry, which do not necessarily have big research laboratories.

2. The results of the assay should be reproducible. Therefore, either constant conditions or calibrations towards a well-defined standard were needed.

3. A high-throughput assay with continuous monitoring of colourimetric or fluorescence measurements using 96-well plates, should be preferred over end-point determinations.

4. A broad range of natural substrates present in fine bakery goods such as fats frequently used in baking should be included. This applied both to the “original” fats used for baking like butter and to specific lipid classes which were shown to impact the textural properties of fine bakery goods, like PC. A comparison between the specificities towards different lipid classes can only be undertaken if different lipid classes can be applied as substrates at comparable conditions.

5. The quantification should be against released FFA to avoid the use of artificial substrates and to enable the comparability between lipid classes. Therefore, the release of FFA has to be monitored continuously.

The final operating principle of the assay is described in section 5.5.1. The setup was simple, including the emulsification of fats or isolated lipids using GA as an emulsifier and the transfer to 96-well plates for reaction. For spreadable fats like butter, the melt emulsions as established in section 3.1 were used. The emulsification procedure was also applicable for glycerophospholipids as shown for the example of 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine (DOPC, PC (18:1/18:1)). The release of FFA was measured continuously using the fluorescent dye Rhodamine B. For reproducibility, the sensitivity of the assay towards FFA with different chain lengths and different grades of saturation was checked. Rhodamine B interacted with all analysed FFA with similar sensitivities and was therefore suitable for the analysis.

Compared to the original method by Zottig et al. [123] including the improvements suggested by van Gaelen et al. [124], the established setup had the following advantages: First, the sample preparation was optimised for baking lipases. For optimal curves, preliminary experiments revealed that a lipase concentration of 10 mg mL^{-1} was needed. Second, it was applicable

to spreadable fats with a minor modification of the emulsification procedure, the introduction of melt emulsions. Third, the comparability of the results was introduced by using an external calibration. For the calibration, different concentrations of FFA were added to Rhodamine B. To obtain reliable results for the calibration, the setup had to be modified in a way that the light-sensitive dye was added directly before the measurement alongside the lipases. The introduction of calibration curves enabled the use of the assay to compare lipase reaction rates towards substrates belonging to different lipid classes and thus the possible prediction of substrate specificities. The established assay was then applied for a range of substrates.

3.4.2.2. Application of the new lipase activity assay to a range of substrates

The seven lipases previously characterised concerning their impact on the batter/dough and product quality of different fine bakery goods were characterised with the new assay. Their reaction towards olive oil, wheat germ oil, flaxseed oil, coconut oil, butter, glyceryltrioleate (triolein, TG (18:1/18:1/18:1)) and DOPC were analysed. Comparing between the different substrates, the average reaction rates towards flaxseed oil (111.16 mmol $min^{-1}mg^{-1}$) were highest while the reaction rates towards DOPC (average activity 7.06 mmol $min^{-1}mg^{-1}$) were the slowest (Figure 36, Table 37). Of all lipases, lipase J had the highest average activity towards all substrates (81.46 mmol $min^{-1}mg^{-1}$) which was more than four times as high as the average activity of the least active lipase, lipase K (20.00 mmol $min^{-1}mg^{-1}$). The activities of all lipases towards the different substrates ranged from 1.24 mmol $min^{-1}mg^{-1}$ (lipase K towards DOPC) to 196.51 mmol $min^{-1}mg^{-1}$(lipase O towards coconut oil). Between the different substrates, no clear trend was visible. The activity patterns of the lipases depended on the substrates. In olive oil, the lipases E and O had the maximum activities, while in triolein, J and O performed best. In wheat germ oil, the lipases J and A reacted fastest, in flaxseed oil the lipases J and E, in coconut oil the lipases O and J, in butter the lipases O and G and in DOPC the lipases O and J. Accordingly, the ratio G/A varied from 0.8 (flaxseed oil and coconut oil) to 1.5 (olive oil) and G/O from 0.2 (coconut oil) to 2.7 (wheat germ oil).

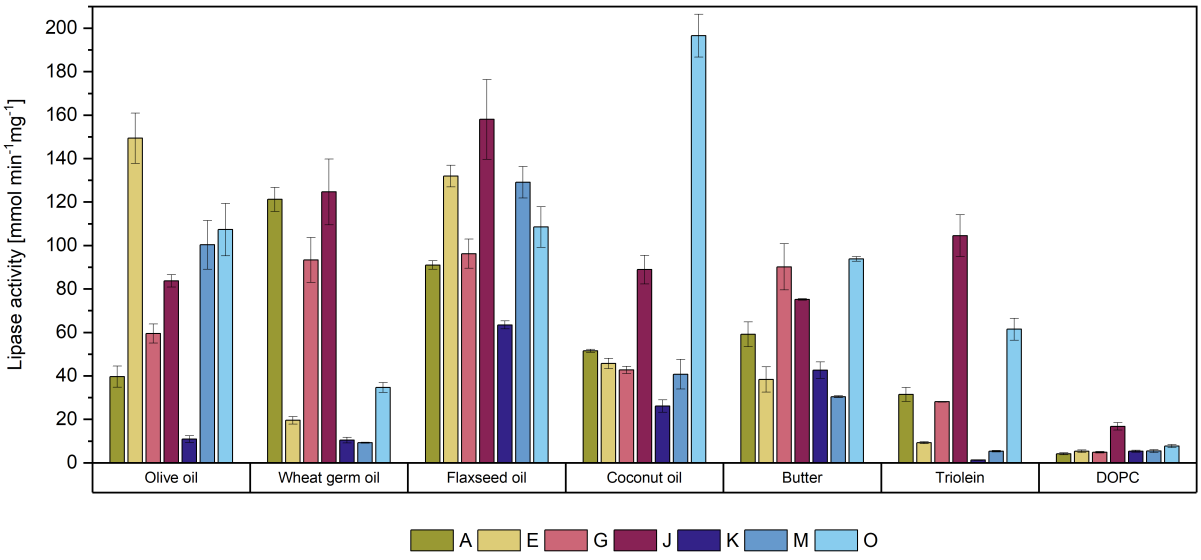


Figure 36: Lipase activity of the lipases A, E, G, J, K, M and O (n = 6) towards different substrates. DOPC - 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine

3.4.2.3. Discussion

The application of the new assay to different substrates and the seven lipases A, E, G, J, K, M and O revealed differences in lipase activity influenced by both the lipases and the substrates.

In terms of average activities of all lipases in a substrate, it has to be taken into consideration that the substrates contained mostly TG and only DOPC belonged to the class of glycerophospholipids. The comparably high difference between the average activities of all lipases towards DOPC and towards the other substrates (7.06 mmol $min^{-1}mg^{-1}$ towards DOPC compared to a minimum of 34.47 mmol $min^{-1}mg^{-1}$ towards triolein) can be explained by the different substrate specificities of the lipases towards TG and towards glycerophospholipids. Considering previous results (section 3.1), it seems however more likely that DOPC was emulsified differently than TG and therefore less accessible for the reaction. DOPC is a polar lipid which is expected to behave differently than the unpolar TG in emulsions.

Of all TG substrates, the average activities towards triolein were lowest, probably due to the lack of a variety of FA chain lengths. All other substrates contained several chain lengths of FA to influence the reaction. Similar as for DOPC, different FA chain length distributions can also affect the properties of the micelle surfaces, both by affecting the surface area and the accessibility of FA at the surface. Olive oil consists mainly of C18:1 (75.5%), C16:0 (11.5%) and C18:2 (7.5%) [67]. The main FA in wheat germ oil is C18:2 (55.7%), but it also contains considerable amounts of C18:1 (13.4%) and C18:3 (7.8%) [129]. Flaxseed oil contains similar FA as wheat germ oil, but with a different distribution: the main FA is C18:3 (58.0%), followed by C18:1 (18.0%) and C18:2 (14.0%) [67]. Coconut oil and butter both contain less unsaturated long-chain FA, given that C16:0 (32.0%) is the main FA in butter and C12:0 (40.0%) in coconut oil. Besides C16:0, butter also contains high amounts of C18:1 (25.0%) and C14:0 (12.4%), whereas coconut oil also contains C14:0 (18.5%) and C18:1 (10.9%) [67, 129]. The broad variety of FA most likely affects both the micelle surface area and the structure of TG at the micelle surface area, leading to different average lipase activities. Besides FA, also further constituents of the oils could play a role. Especially the high content of sterol lipids (e.g. sitosterol and campesterol) and prenol lipids (tocopherols and tocotrienols) [168], which are also present in fat micelles but cannot be hydrolysed by lipases, supposedly affects the interactions between lipases and their substrates.

Besides the difference in average lipase activities, each substrate led to an individual activity pattern (Table 12). Although triolein and olive oil are generally considered as similar to each other, given a share of 75.5% of C18:1 in the FA distribution of olive oil [67], the reactivity patterns towards both were not comparable. In olive oil, the lipases E and O were most active, while in triolein, the lipases J and O had the highest activities and the activity of lipase E ranged between the activities of the lipases G and M. The activity patterns from the new assay were also not in accordance with results for the same fats in other setups, e.g. with the total amount of FFA released from butter or wheat germ oil in section 3.1. In the assay, the lipases O and J were most active towards butter, while in the emulsions tested with GC, O and A released most FFA. For wheat germ oil, in the assay the lipases J and A had the highest activities while in terms of FFA, O and M were most active.

The reactivity pattern for DOPC did not match the results for the reaction of the lipases in bakery goods from section 3.2. In pound cake batter, e.g., the lipases A, G and J had shown

the highest activities towards glycerophospholipids of all lipases while in the assay, this pattern could not be confirmed. Lipase substrates specificities depend on the reaction conditions and are not determined by inherent properties of the lipases. The new assay was therefore not suitable for the prediction of lipase reactions in fine bakery goods.

Similar as for lipase activity [27] and lipase FA substrate specificity (section 3.1), the reaction rates of lipases towards specific lipid classes in complex media cannot be predicted by the use of simplified model systems. Lipase activity in general, as well as in terms of substrate specificity for both FA and lipid classes, is determined by the substrates' accessibility and not by the lipase itself. Each lipid arranges differently in a lipid micelle, according to both its properties and substances used for micelle stabilisation. If the lipid is situated at the surface of the micelle, it can be hydrolysed, depending on the inherent properties of the lipase. The term "substrate specificity" should therefore not only comprise FA substrate specificity and the specificity for certain lipid classes, but also the ability of lipases to deal with differently arranged lipids. How this lipid presentation can be influenced in fine bakery goods should be analysed in further experiments, in order to enlarge possible uses of lipases for the improvement of the baking quality of fine bakery goods.

4. Conclusion and Outlook

Lipases are commonly used as baking improvers for bread and are known to affect, *inter alia*, bread loaf volumes and texture. This work evaluates four prerequisites for their potential application as baking improvers in fine bakery goods like cake.

First, lipases with suitable FA substrate specificities which do not release short-chain FA were identified. Artificial *p*-nitrophenyl esters were used to screen 17 lipases developed for the use in bread for their FA substrate specificities. When analyzing the FA substrate specificities of the lipases during the reaction with fats commonly used in baking like butter and margarine in a model system, however, the results from the *p*-nitrophenyl assay could not be confirmed. Instead, the processes at the micelle surface in the model system seemed to be decisive for the preferences for specific FA. This hypothesis was supported by further experiments, e.g., the modification of the micelle surface by the use of alternative emulsifiers. The conclusion that the individual accessibility of substrates at the surface is crucial for the release of specific FA is in accordance with previous findings on lipase activities, which also depend on the substrate.

To what extent this knowledge can be used to improve further industrial uses of lipases has to be evaluated in further studies. Molecular insights to the processes at micelle surfaces are needed to understand the influence of lipase-inherent properties, like the geometry of the active site, in combination with techniques to modify the presentation of substrates at interfaces. To predict whether a lipase leads to off-flavours when used in fine bakery goods, adapted and fine bakery good-specific model systems are needed.

The second prerequisite was the evaluation of lipase effects in fine bakery goods. Lipases were shown to affect the baking quality in terms of both batter or dough and product quality of three different cake preparations. They eased the machinability of batters and doughs by liquefaction and partly by reduction of stickiness. In the final products, they reduced the firmness and resilience of the products and inhibited staling. The impact depended on both the lipase and the cake recipe. If intrinsic emulsifiers, e.g., from egg in pound cake, were present, the effect caused by lipases was diminished. In the yeast-based cake, little to no changes of baking quality occurred. The effects caused by the lipases were comparable or even greater than the effects of the traditional emulsifier DATEM.

Lipases therefore present an alternative to traditional emulsifiers for the use in fine bakery goods. Especially concerning the application in fine bakery goods, their use is of special interest for the emerging field of vegan bakery. Currently, eggs are replaced by a mixture of proteins and emulsifiers in vegan fine bakery goods. Lipases could act as clean-label substitutes for emulsifiers and thereby meet the consumers' wish for a both sustainable and additive-free diet. The resulting products are clean-label because enzymes in bakery are currently still considered as processing aids. However, studies on possible residual activities of lipases in bakery goods are still lacking. In the ongoing process of food enzyme admission in the European Union, a deeper insight in the fate of lipases during baking would be of interest.

As a third prerequisite, lipase reaction products in the three different cakes were analysed by an in-depth lipidomic study. Therefore, an existing LC-MS/MS method was successfully transferred and applied to lipase-treated cake samples. Out of 22 different lipid classes, putative key compounds for an improvement of baking quality were identified. In the eggless basic cake

recipe, glyceroglycolipids were found to be decisive for effects on the texture. In pound cake, glycerophospholipids could be linked to an improvement of the baking quality. The analysis of the yeast-based brioche revealed that the substrate specificity of lipases depended on the accessibility of substrates. Even if similar substrates were present, as in this case in brioche and pound cake, different substrate specificity patterns for the same lipases resulted. This finding was in accordance with the results for the FA substrate specificity of lipases in model emulsions.

Consequently, further studies on how to alter the accessibility of substrates for lipase reactions should be carried out. This would enhance the use of lipases in yeast-based cakes, where only few improvements of baking quality can be achieved so far. The established lipidomics method offers a new high-throughput approach for molecular insights on lipase reactions in foodstuff and can be used for the analysis of existing as well as future areas of lipase applications.

The fourth prerequisite was the development of a new assay for the prediction of lipase suitability for the use in fine bakery goods. A lipase activity assay based on the quantification of FFA by the fluorescent dye Rhodamine B was therefore established. It enables the characterisation of lipase substrate specificities towards different substrates. However, it is non-competitive and cannot mimic the different accessibilities of substrates in fine bakery goods. A deeper insight in the mechanisms behind lipase substrate specificities is needed in order to introduce the factor of substrate accessibility to the assay.

This work contributed to the understanding of lipase substrate specificities and FA substrate specificities and introduced the accessibility of substrates as an important factor. Additionally, it paved the way for the application of lipases in fine bakery goods by showing their potential as baking improvers and by providing insights into the underlying key reactions.

5. Materials and methods

5.1. Chemicals and materials

5.1.1. Reagents and ingredients

The following reagents and ingredients were used for the experiments:

(9Z)-Octadecenoic acid (oleic acid, C18:1), ≥99%, Merck KGaA, Darmstadt Germany

(9Z)9-Octadecenoic acid 1,2,3-propanetriyl ester (glyceryl trioleate), ≥99%, Merck KGaA, Darmstadt, Germany

(9Z,12Z)-Octadeca-9,12-dienoic acid (linoleic acid, C18:2), ≥98%, Merck KGaA, Darmstadt, Germany

(9Z,12Z,15Z)-Octadeca-9,12,15-trienoic acid (α-linolenic acid, C18:2), ≥98.5%, Merck KGaA, Darmstadt, Germany

1,2,3-Triheptadecanoyl-*sn*-glycerol (TG 17:0/17:0/17:0), >99%, Merck KGaA, Darmstadt, Germany

1,2-Di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphocholine (DOPC; PC(18:1(9Z)/18:1(9Z))), >99%, Avanti Polar Lipids, Birmingham, AL, US

1-Butanol, ≥99.8%, VWR International GmbH, Darmstadt, Germany

2-Amino-2-(hydroxymethyl)propane-1,3-diol-hydrochloride (tris (hydroxymethyl) aminomethane-hydrochloride, Tris-HCl), ≥99%, Carl Roth GmbH + Co. KG, Karlsruhe, Germany

2-Methoxy-2-methylpropane (methyl *tert*-butyl ether (MTBE)), HPLC grade, VWR International GmbH, Darmstadt, Germany

4-Nitrophenol (*p*-nitrophenol), ≥99%, Carl Roth GmbH + Co. KG, Karlsruhe, Germany

Acetonitrile, ≥99.9%, VWR International GmbH, Darmstadt, Germany

Acetonitrile hypergrade for LC-MS, LiChrosolv® hypergrade for LC-MS, Merck KGaA, Darmstadt, Germany

Ammonium formate, ≥99.0%, Thermo Fisher Scientific, Schwerte, Germany

Baking powder, commercial quality, local supermarket

Boron trifluoride-butanol reagent 10% (≈1,3 M) in 1-butanol, Merck KGaA, Darmstadt, Germany

Butanoic acid 4-nitrophenyl ester (butyric acid 4-nitrophenyl ester,4-nitrophenyl butyrate), ≥98%, Merck KGaA, Darmstadt, Germany

Butanoic acid (butyric acid, C4:0), ≥99.5%, Merck KGaA, Darmstadt, Germany

Butter, 82% fat, commercial quality, local supermarket

Calcium chloride ($CaCl_2$), ≥98%, Carl Roth GmbH + Co. KG, Karlsruhe, Germany

Coconut oil, commercial quality, local supermarket

Decanoic acid 4-nitrophenylester (capric acid 4-nitrophenylester, 4-nitrophenyl decanoate), ≥98%, Merck KGaA, Darmstadt, Germany

Decanoic acid (capric acid, C10:0), ≥99.5%, Merck KGaA, Darmstadt, Germany

Diacetyl tartaric acid ester of mono- and diglycerides (DATEM, E472e), commercial quality, Backaldrin The Kornspitz Company, Asten, Austria

Dodecanoic acid 4-nitrophenylester (lauric acid 4-nitrophenylester, 4-nitrophenyl dodecanoate), ≥98%, Merck KGaA, Darmstadt, Germany

Dodecanoic acid (lauric acid, C12:0), ≥99%, Merck KGaA, Darmstadt, Germany

Ethanol, ≥99.8%, VWR International GmbH, Darmstadt, Germany

Ethoxyethane (diethyl ether), ≥99.7%, stabilised VWR International GmbH, Darmstadt, Germany

Extra-white powdered sugar, commercial quality, Nordzucker, Braunschweig, Germany

Flaxseed oil, commercial quality, local supermarket

Formic acid, 98% - 100%, Merck KGaA, Darmstadt, Germany

Formic acid, 99 - 100%, VWR International GmbH, Darmstadt, Germany

Fresh yeast, commercial quality, local supermarket

Gum arabic (GA), spray-dried, Carl Roth GmbH + Co. KG, Karlsruhe, Germany

n-Heptane, ≥99%, VWR International GmbH, Darmstadt, Germany

Hexadecanoic acid 4-nitrophenylester (palmitic acid 4-nitrophenylester, 4-nitrophenyl hexadecanoate), ≥98%, Merck KGaA, Darmstadt, Germany

Hexadecanoic acid (palmitic acid, C16:0), ≥99%, Merck KGaA, Darmstadt, Germany

Hexanoic acid 4-nitrophenylester (caproic acid 4-nitrophenylester, 4-nitrophenyl hexanoate), ≥98%, TCI Deutschland GmbH, Eschborn, Germany

Hexanoic acid (caproic acid, C6:0), ≥99.5%, Merck KGaA, Darmstadt, Germany

Lecithin from egg yolk (EL), GPR RECTAPUR®, VWR International GmbH, Darmstadt, Germany

MAK046 - Lipase Activity Assay Kit I, Merck KGaA, Darmstadt, Germany

MAK047 - Lipase Activity Assay Kit II, Merck KGaA, Darmstadt, Germany

MAK048 - Lipase Activity Assay Kit III, Merck KGaA, Darmstadt, Germany

Margarine, commercial quality, local supermarket

Methanol, HPLC grade, VWR International GmbH, Darmstadt, Germany

Nonadecanoic acid (C19:0), ≥99.5%, Merck KGaA, Darmstadt, Germany

Octadecanoic acid 4-nitrophenylester (stearic acid 4-nitrophenylester, 4-nitrophenyl octadecanoate), ≥90%, Merck KGaA, Darmstadt, Germany

Octadecanoic acid (stearic acid, C18:0, ≥98.5%, Merck KGaA, Darmstadt, Germany

Octanoic acid 4-nitrophenylester (caprylic acid 4-nitrophenylester, 4-nitrophenyl octanoate), ≥90%, Thermo Fisher Scientific, Schwerte, Germany

Octanoic acid (caprylic acid, C8:0), ≥99.5%, Merck KGaA, Darmstadt, Germany

Olive oil, commercial quality, local supermarket

Pasteurised whole egg, commercial quality, local supermarket

Pentanoic acid (valeric acid, C5:0), ≥99.8%, Merck KGaA, Darmstadt, Germany

Propan-2-ol (isopropanol), ≥99.0%, VWR International GmbH, Darmstadt, Germany

Rapeseed oil, commercial quality, local supermarket

Rhodamine B, >98%, Thermo Fisher Scientific, Schwerte, Germany

Sodium chloride (NaCl), ≥99.8%, VWR International GmbH, Darmstadt, Germany

Sodium hydroxide (NaOH), 98.5-100.5%, VWR International GmbH, Darmstadt, Germany

Sodium sulfate anhydrous (Na_2SO_4), ≥99%, Thermo Fisher Scientific, Schwerte, Germany

Splash Lipidomix Mass Spec Standard, Avanti Polar Lipids, Birmingham, AL, US

Sulphuric acid (H_2SO_4), 2.5 mol L^{-1}- 5 N, Carl Roth GmbH + Co. KG, Karlsruhe, Germany

Tetradecanoic acid 4-nitrophenylester (myristic acid 4-nitrophenylester, 4-nitrophenyl tetradecanoate), ≥95%, Merck KGaA, Darmstadt, Germany

Tetradecanoic acid (myristic acid, C14:0), ≥99.5%, Merck KGaA, Darmstadt, Germany

Triton® X-100, extra pure, Carl Roth GmbH + Co. KG, Karlsruhe, Germany

Undecanoic acid (C11:0), ≥99%, Merck KGaA, Darmstadt, Germany

Wheat flour type 405, commercial quality, Good Mills GmbH, Hamburg, Germany

Wheat germ oil, commercial quality, Salandis GbR, Greifswald, Germany

5.1.2. Sample assortment

Baking lipases known for their positive effects on the baking quality of bread were sourced. 17 samples were chosen and kindly donated by ABEnzymes (Darmstadt, Germany), Backaldrin The Kornspitz Company (Asten, Austria), DSM (Heerlen, Netherlands), Kuchenmeister (Soest, Germany) and Novozymes (Bagsvaerd, Denmark). The samples comprised 13 solid lipase preparations and four liquids. The lipases were named randomly A - Q.

5.2. Model systems to characterise lipase substrate specificities

The following parts were already published in *LWT - Food Science and Technology* [125] and *Getreide, Mehl und Brot* [126].

5.2.1. *p*-Nitrophenyl assay

Lipase substrate specificities were analysed using the *p*-nitrophenyl assay according to Glogauer et al. with minor modifications [105]. Solid samples were dissolved at 1 mg mL^{-1} in lipase buffer (50 mmol L^{-1} Tris-HCl, 1 mmol L^{-1} $CaCl_2$, pH 7.5). Liquid samples were used without further dilution. Stock solutions of the *p*-nitrophenyl substrates with chain lengths of 4, 6, 8, 10, 12, 14, 16 and 18 carbon atoms at a concentration of 17.5 mol L^{-1} were prepared in acetonitrile/propan-2-ol (1:4, v:v). They were further diluted with assay buffer (50 mmol L^{-1} Tris-HCl, 1 mmol L^{-1} $CaCl_2$, 0.3% Triton X-100, pH 7.5) to a concentration of 0.175 mmol L^{-1}. *p*-Nitrophenol was used for calibration at concentrations from 0.005 mmol L^{-1} to 0.175 mmol L^{-1} in assay buffer. For each lipase, a 96-well plate with 230 µL of substrate working solutions was prepared to determine its FA substrate specificity in sextuplicate towards each substrate. 20 µL of lipase solution were added to each well and the absorbance at 410 nm was recorded at 30 °C for 60 min using a multiplate reader (Tecan Group, Männedorf, Switzerland). To exclude possible interferences of the reaction due to self-absorption of the substrates or the samples at the selected wavelength, control values consisting of 230 µL substrate working solution and 20 µL lipase buffer as well as 230 µL assay buffer and 20 µL lipase solution were measured and their absorptions were included in the evaluation. The enzymatic activities were expressed as mmol *p*-nitrophenol min^{-1} and checked for linearity using linear regression ($R^2 > 0.98$). All activities of one lipase were recalculated as a percentage of the total activity (sum of all activities) of the corresponding lipase to ensure comparability.

5.2.2. Characterisation of model emulsions

5.2.2.1. Preparation of emulsions

Emulsification procedures were established to serve as model systems for the reaction of lipases with fats frequently used in fine bakery goods. 50 g each of rapeseed oil, butter, margarine and wheat germ oil were weighed in a beaker and mixed with 150 mL of preheated emulsifying solution (50 mmol L^{-1} Tris-HCl, 1 mmol L^{-1} $CaCl_2$, pH 7.5 and either 2.5% Triton X-100 or 3% gum arabic or no emulsifier). The emulsification method used was either stirring, dispersion in an Ultra-Turrax (IKA, Staufen im Breisgau, Germany) or a combination of both. The resulting pre-emulsions were then mixed 1:1 (m:v) with emulsion buffer (50 mmol L^{-1}

Tris-HCl, 1 mmol L^{-1} $CaCl_2$, pH 7.5) in a 10 mL jar and incubated in an overhead shaker at room temperature. Their stability was assessed visually after 24 h.

The final setup was chosen according to stability and similarity to the setup used in the *p*-nitrophenyl assay.

50 g of fat were added to 150 mL of the 50 °C warm Triton X-100 emulsifying solution and stirred for 20 min at room temperature. The emulsion was then dispersed in an Ultra-Turrax (IKA) with a stator diameter of 18 mm for 2.5 min at 12,500 rpm. Emulsion buffer (control samples) or lipase solutions (20 mg mL^{-1}) in lipase buffer (50 mmol L^{-1} Tris-HCl, 1 mmol L^{-1} $CaCl_2$, pH 7.5) were then added to the pre-emulsions as described above. The mixtures were shaken in an overhead shaker for 16 h at room temperature.

5.2.2.2. Particle size distribution

The particle size distributions of the emulsions were assessed in triplicate by laser diffraction using an LS13320 XR (BeckmanCoulter, Brea, CA, USA). Prior to analysis, three additional samples of each fat were incubated for 24 h in an overhead shaker at room temperature. The obtained particle size distributions were compared using their Sauter diameters to draw conclusions about their stability.

5.2.2.3. Petrographic microscopy

The samples (see 5.2.2.2) were further characterised by petrographic microscopy using an Eclipse LV100 ND microscope (Nikon, Minato, Japan) and the software NIS Elements BR (Nikon). The use of polarised light leads to optical highlighting of optically anisotropic materials like fat crystals.

5.2.3. Analysis of fatty acids released from the model emulsions

The established method comprised the FA butyric acid (C4:0), caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and α-linolenic acid (C18:3). Valeric acid (C5:0), undecanoic acid (C11:0) and nonadecanoic acid (C19:0) were used as internal standards. All FA were dissolved in 2% formic acid in diethyl ether (v:v). Unless indicated otherwise, all determinations were carried out in triplicate.

5.2.3.1. Extraction, purification and derivatisation of free fatty acids

The procedure first established by Mannion et al. [120] was used with minor modifications for extraction and purification of FFA. 5 mL of the emulsions (see 5.2.2.1) were mixed with 10 g of anhydrous sodium sulfate, 0.3 mL of 2.5 mol L^{-1} sulfuric acid and 1 mL of internal standard solution (4 mg mL^{-1} of each compound). FFA were extracted with 15 mL of diethyl ether:n-heptane (1:1, v:v) by shaking for 3 min. Phase separation was induced by centrifugation. The organic phase was transferred to a new container and the aqueous phase was re-extracted twice following the same procedure. The organic phases were combined and purified via solid phase extraction (SPE) according to Table 13.

Table 13: Solid phase extraction procedure [125].

Column	aminopropyl column (500 mg, Macherey-Nagel, Düren, Germany)
Conditioning	twice with 5 mL *n*-heptane
	Sample application
Removal of neutral lipids	twice with 5 mL of 20% diethyl ether in *n*-heptane (v:v)
Elution	4 mL of 2% formic acid in diethyl ether (v:v)

The purified extracts were then derivatised based on the method by Mannion et al. [121]. 750 µL of the extract were transferred into an amber glass vial and mixed with 750 µL of a 10% BF_3 solution in butanol and 500 µL of *n*-heptane. For derivatisation, the mixture was heated to 80 °C for 60 min with gentle shaking and let cool to room temperature. According to the method of Iverson & Sheppard [169], the derivatised FFA were washed three times with 10 mL of distilled water. Thus, excess butanol could be removed. The remaining organic supernatant was then used for further analysis.

5.2.3.2. Gas chromatography with flame ionisation detection

The FFA were separated and measured using GC with a flame ionisation detector according to Table 14.

Table 14: Operating conditions of gas chromatography analysis [125].

Instrument	Shimadzu GC 2010-Plus (Shimadzu, Duisburg, Germany)
Detector	Flame ionisation
Column	DB-FATWAX Ultra Inert (30 m × 0.25 mm, film thickness 0.25 µm, Agilent Technologies, Waldbronn, Germany) with precolumn (deactivated fused silica, 5 m x 0.25 mm)
Injection volume	1 µL
Inlet temperature	220 °C
Split ratio	10:1 - 100:1
Carrier gas	helium
Carrier gas velocity	40.0 cm s^{-1}
Temperature program of the column oven	40 °C - 5 °C min^{-1} to 60 °C - hold for 2 min - 10 °C min^{-1} to 220 °C - hold for 10 min
Detector temperature	240 °C
Method duration	32 min

5.2.3.3. Quantification of released fatty acids and calculation of specificity factors

For FFA quantification, external calibrations of the ratio of analyte peak area to internal standard peak area were used (Table 15).

Table 15: Assignment of internal standards to analytes [125].

Internal standard	Analyte
C5:0	C4:0, C6:0
C11:0	C8:0, C10:0, C12:0, C14:0
C19:0	C16:0, C18:0, C18:1, C18:2, C18:3

The calibrations were established over a linear range from 0.001 to 1.7 (C6:0, C8:0, C10:0 and C12:0), 0.001 to 15 (C4:0, C14:0, C18:0 and C18:3) and 0.001 to 100 (C16:0, C18:1 and C18:2).

The intermediate precision of the experiment (same person, same instrument) was calculated per fat type. Three emulsions of each rapeseed oil, margarine, butter and wheat germ oil were prepared on three consecutive days and the released FFA after incubation with lipase A (as described above) were determined.

The release of FFA depends on the total FA composition of fats. Different FA behave differently when released, e.g., concerning their volatility and stability. This is reflected by the FFA composition of fats without lipase treatment. The distribution of FFA after lipase treatment was therefore normalised by the distribution of a control sample without lipase treatment. This ratio was called FA specificity factor. A specificity factor greater than 1 implies the preferred release of a FA. A specificity factor below 1 indicates a lack of specificity for the FA.

5.2.3.4. Time course of the reaction

The reaction of a butter emulsion incubated with lipase A was stopped at 5 different time points (1 h, 2 h, 4 h, 6 h, 8 h) by adding extraction solution to record the time course of the reaction.

5.2.3.5. Impact of the emulsifier

The three lipases A, J and Q were chosen to analyse the influence of the emulsifier on the FA substrate specificity. The emulsifying solution with Triton X-100 was replaced by a GA buffer (50 mmol L^{-1} Tris-HCl, 1 mmol L^{-1} $CaCl_2$, pH 7.5, 20% GA w:v) or an EL buffer (50 mmol L^{-1} Tris-HCl, 1 mmol L^{-1} $CaCl_2$, pH 7.5, 2.5% EL, w:v) and the three lipases were incubated as described in section 5.2.2.1 with all four fat types.

5.2.4. Sensory analysis of lipase-treated emulsions

A sensory panel of 11 members was trained to recognize rancid off-flavours in water and emulsions as described in 5.2.2.1.

5.2.4.1. Determination of odour thresholds

The determination of odour threshold values in the media water, rapeseed oil emulsion, margarine emulsion, butter emulsion and wheat germ oil emulsion was performed according to the standard procedure of the American Society for Testing and Materials [170]. The procedure is based on several, consecutive 3 Alternative Forced Choice tests (3 AFC tests). The panellists

chose the sample with the highest intensity of off-flavour from a sample set of three samples. Two samples of each sample were control samples of the respective matrix (water or the corresponding emulsion). The third sample was spiked with butyric acid as a model substance for rancid off-flavours according to Table 16.

Table 16: Concentrations used for the determination of odour thresholds [126].

c(butyric acid) [mg L^{-1}]/ Matrix	0.25	0.5	1	2	4	8	16	32	64
Water	x	x	x	x	x	x	x		
Rapeseed oil emulsion		x	x	x	x	x	x		
Margarine emulsion		x	x	x	x	x	x		
Butter emulsion		x	x	x	x	x	x		
Wheat germ oil emulsion				x	x	x	x	x	x

5.2.4.2. Description of the flavours of short-chain fatty acids

The panellists were asked to describe the flavours of short-chain fatty acids depending on their concentration. Therefore, they were provided with three aqueous solutions of butyric acid, caproic acid, caprylic acid and capric acid (Table 17).

Table 17: Concentrations of butyric acid (C4:0), caproic acid (C6:0), caprylic acid (C8:0) and capric acid (C10:0) used for the description of the flavours of short-chain fatty acids [126].

c(Fatty acid) [mg mL^{-1}]/ Fatty acid	low	middle	high
C4:0	1	10	50
C6:0	5	10	50
C8:0	0.2	2	10
C10:0	0.5	2	10

5.2.4.3. Sensory analysis

Each panellist received randomly selected five lipases for testing. Each lipase-treated sample was presented with two control samples and was to be identified using a 3 AFC test procedure. The panellists were also asked to describe the criterion by which they could identify the deviating sample (e.g. rancid off-flavour, smell of ..., guessed). The effects of each lipase were analysed by at least three different panellists. Since the 3 AFC test holds the possibility that the correct sample is identified by chance, the Thurstone model was used for evaluation [171]. Therefore, only those samples were considered as statistically significantly different that were correctly identified by at least 67% of the respective panellists.

5.2.5. Statistical analysis

Statistical analysis was performed using Origin 2021b for analysis of variance (ANOVA) with Tukey's test ($p < 0.05$) (OriginLab Corporation, Northampton, MA, USA) and Microsoft Excel built-in functions (means and standard deviations).

5.3. Baking quality of fine bakery goods

The following parts were already published in *LWT - Food Science and Technology* [146] and *Food Chemistry: X* [97].

5.3.1. Cake preparation

Three different cake recipes according to Table 18 were prepared in triplicate. A commercial food processor with planetary mixing (Robert Bosch GmbH, Stuttgart, Germany) equipped with a whisk and a kneading hook was used.

For basic cake and pound cake, first butter, sugar and salt were mixed until creamy (2.5-3 min). Then, for pound cake, eggs were added and blended in for another 3 min. Finally, the remaining ingredients wheat flour, water (if applicable) and baking powder were added and mixed in for another 3 min. Aliquots of 300 g (batter and dough characteristics) or 400 g (product characteristics) were prepared for treatment with lipases.

For brioche, first a pre-dough consisting of flour, pre-heated water (37 °C), yeast (product characteristics only) and pasteurised egg was prepared by thoroughly kneading for 10 min. For the determination of dough characteristics, the dough was left to proof in a proofing cabinet according to Table 18. Then, the remaining flour, butter, sugar and salt were added and the mixture was kneaded for further 5 min before preparing aliquots of 300 g for treatment with lipases. For the determination of product characteristics, the first proofing time was combined with the incubation with lipases. Therefore, aliquots of 240 g pre-dough were prepared and treated with lipases before adding the remaining ingredients.

For lipase addition during the determination of batter and dough characteristics, the lipase dosages as suggested by the manufacturers were applied. These included batter-based dosages of 200 mg kg^{-1} (lipase A), 250 mg kg^{-1} (lipase E), 150 mg kg^{-1} (lipases G and K), 35 mg kg^{-1} (lipase J) and 100 mg kg^{-1} (lipases M and O) for basic cake and pound cake. For brioche, 30% of the dosages were applied on a flour base. As the dosages were not sufficient to affect the product characteristics after baking significantly, all lipase dosages were doubled for the baking experiments. Additional DATEM samples were prepared with a concentration of 280 mg kg^{-1} DATEM in both experiments. The lipases were dissolved in water and added by volume to reduce weighing errors. The control sample and the DATEM sample comprised an equal amount of water to ensure comparability.

The batter and dough aliquots were thoroughly mixed with lipase solutions or water or DATEM and water and incubated either at room temperature for 1 h (basic cake, pound cake and dough characteristics of brioche) or at 37 °C for 2 h (product characteristics of brioche) in plastic boxes. The use of incubation times was first suggested by Gerits et al. [58].

For the determination of dough characteristics of brioche, the remaining ingredients were then added and kneaded in as described above.

The resulting batters and doughs were characterised as described in Section 5.3.2 directly after preparation.

To analyse the product characteristics, eight muffins with a batter/dough weight of 50 g were prepared of each modification (lipase/control/DATEM), proofed for 20 min at 37 °C (brioche) and baked in a preheated hot air oven (UNOX Deutschland GmbH, Büren, Germany) according to Table 18. The product characteristics of the resulting muffins were then analysed as described in Section 5.3.3.

Table 18: Recipes used for basic cake, pound cake and brioche [97][146][162].

	Basic cake	Pound cake	Brioche	
			Pre-dough	Dough
Wheat flour	250 g	200 g	300 g	200 g
Water	200 mL		125 mL	
Butter	100 g	200 g		125 g
Sugar	50 g	200 g		50 g
Salt	2.5 g	2 g		5 g
Pasteurised egg		200 g	50 g	
Baking powder	15 g	0.6 g		
Yeast			35 g	
			First fermentation (pre-dough) 120 min 37 °C	
			Second fermentation 20 min 37 °C	
	Baking 12 min 180 °C	Baking 12 min 180 °C	Baking 12 min 180 °C	

5.3.2. Batter and dough characteristics

Unless indicated otherwise, all analyses were performed on three different batters or doughs. The experiments were performed in the same order on all batters or doughs to enhance the reproducibility.

5.3.2.1. pH values of batters and dough

The pH values of batters and doughs were determined according to the standard procedure from *Standard-Methoden für Getreide, Mehl und Brot* [172] based on the German official test method [173].

In brief, 10 g of dough, batter or pre-dough were homogenised with 5 mL acetone and 40 mL freshly boiled water. The mixture was transferred to a beaker with another 55 mL freshly boiled water. The pH value of the samples was determined using a pH electrode.

5.3.2.2. Density

A measuring cup with a known volume of 60 mL and known weight was used to determine

the dough and batter density. It was completely filled with dough or batter. Then, protruding material was scraped off. The batter or dough weight was measured as the difference between the weight of the filled cup and the weight of the empty cup. The quotient of mass and volume was then calculated as batter or dough density. To improve the reliability of the method, it was performed in triplicate on each of the three doughs or batters.

5.3.2.3. Stickiness

A mini stickiness system (Stable Micro Systems, Godalming, UK) in combination with a TA.XTplus texture analyser equipped with a 5 kg loading cell and a heavy duty platform was used to analyse batter and dough stickiness. The container was filled with dough or batter and the retaining plate was placed on top. A spatula was used to remove protruding material. A mass-time-curve was recorded as a narrow blade (9 cm x 7 cm) was driven 25 mm through the slot of the retaining plate and withdrawn upwards. The area under the curve recorded when withdrawing was used to gauge how sticky the batter or dough was. The software Exponent (version 6.1.16.0, by Stable Micro Systems) was used for data evaluation.

5.3.2.4. Rheological characterisation

Oscillatory tests with a Physica rheometer MCR301 (Anton Paar Group AG, Graz, Austria) equipped with a circulating Viscotherm VT2 cooling water bath and a peltier temperature-controlled hood (H-PTD200, Anton Paar Group AG) were used to assess the rheological properties of batters and doughs. The experiments were conducted at 25 °C. Before each test, approximately 2 g of batter or dough were placed on a plate-plate sensor geometry (25 mm diameter, serrated surface) and the measuring bob was lowered to a gap of 1 mm. Protruding material was removed with the help of a spatula. The sample was left to rest for 3 min under the hood before the measurements were started. The measurements comprised the storage modulus G', the loss modulus G" and the loss factor tan δ. The loss factor is calculated as the quotient G"/G'. The software Rheoplus/32 (V3.40, Anton Paar Group AG) was applied for data evaluation of all measurements.

Three tests, an amplitude sweep, a frequency sweep and a temperature sweep, were performed in the same order on all batters [174, 63, 85, 93].

First, the amplitude sweep was performed. It included a strain ranging from 0.1 to 100% at a fixed frequency of 10 rad s^{-1}. Three parameters were determined from the measurements: first the strain that can be applied to the sample without destroying its structure (LVE), second the structural strength of the sample within the LVE range (G' at the end of LVE) and third the strain where tan δ corresponds to 1 which represents the onset of flow in the sample (cross-over point).

The second test was to analyse the long-term and short-term behaviour of the sample using a frequency sweep from 0.1 to 100 rad s^{-1} and a strain chosen according to the previously determined LVE.

Finally, the temperature-dependent behaviour of the samples was assessed. Therefore, a temperature sweep (fixed frequency of 1 Hz, strain as for the frequency sweep) was carried out. The temperature was increased at a constant rate of 4 °C min^{-1} from 25 °C to 100 °C [85].

5.3.3. Product characteristics

The product characteristics of all samples were measured six times by analysing two muffins from each batter or dough. All samples which were not measured were stored on closed ziplock bags at room temperature.

5.3.3.1. Baking loss

The samples were weighed before and 2 h after baking. The baking loss was then calculated as the difference between both weights.

5.3.3.2. Density

The density was measured as the quotient of muffin weight and muffin volume. For the volume, a VolScan Profiler VSP300 (Stable Micro Systems) with a rotation speed of 0.5 s^{-1} and a vertical step size of 1 mm was used.

5.3.3.3. Texture Profile Analysis

A texture profile analysis of the muffins was carried out directly after cooling (0 h) and 24 h, 48 h and 96 h after baking.

Immediately before the analysis, the tops of the muffins were removed with a knife and the lower parts installed in the centre of the heavy duty platform of a TA.XTplus texture analyser (Stable Micro Systems). The measurement conditions included a 20 mm diameter cylindrical probe which was used for a double compression test (pretest speed 1 mm s^{-1}, test speed and backtest speed 0.8 mm s^{-1}, deformation to 40% of the sample height, 10 s waiting time between the measurements, a release force of 0.049 N and a measurement data rate of 200 measuring points per second). The software Exponent (version 6.1.16.0, by Stable Micro Systems) was used for data evaluation. The evaluation included the calculation of product firmness (maximum peak height of the first compression), springiness (quotient of the time needed for the first compression and the time needed for the second compression), cohesiveness (peak area of the second compression divided by the peak area of the first compression) and resilience (quotient of the peak area of the first compression before the maximum peak height and the remaining peak area of the first peak). Two more parameters were calculated from there on: The gumminess is defined as the product of the multiplication of firmness and cohesiveness. Gumminess multiplied by springiness then results in chewiness.

5.3.3.4. Sensory analysis

In a scientific thesis by J. Wießler, the odour profiles of all samples treated with lipases were described qualitatively by three untrained panellists [155]. These results are discussed in combination with 3 AFC tests carried out by 14 to 17 untrained panellists for muffins treated with the lipases E, J and K as described in section 5.2.4.3.

5.3.4. Statistical analysis

Microsoft Excel, Origin 2021b (OriginLab Corporation) and IBM SPSS Statistics 27 (International Business Machines Corporation, Armonk, NY, USA) were used for statistics. Means and

standard deviations were calculated with Microsoft Excel. An ANOVA with two-sided Dunnett's t-tests ($p < 0.05$) was performed in SPSS to analyse significant differences between treated samples and the respective control samples. For the texture profile analysis, additionally an ANOVA with Tukey's test ($p < 0.05$) was carried out in Origin to detect differences between the means of all values at a certain time.

5.4. Lipidomic profile of fine bakery goods after treatment with lipases

The following parts were prepared for publication in *Frontiers in Nutrition* [162].

5.4.1. Sample preparation

The samples were prepared as described in section 5.3.1. The dosages for the determination of product characteristics were applied for both batter/dough and product samples for better comparability. All samples were prepared in triplicate.

For the batter and dough samples, lipase-treated and control batters and doughs were frozen directly after incubation with lipases (basic cake and pound cake) or after the additional 20 min of proofing time (brioche). For the cake samples, the lipase-treated and control muffins were left to cool for 2 h at room temperature after baking and then frozen. All samples were lyophilised, milled in an ultracentrifugal mill and stored at 20 °C until further analysis.

5.4.2. Lipid extraction

The lipids were extracted by the method of Cutignano et al. [175]. 1 mg of sample was homogenised with 300 µL of methanol. The first extraction step consisted of adding 1 mL of 2-methoxy-2-methylpropane (methyl *tert*-butyl ether, MTBE), sonicating for 5 min and shaking for 1 h at room temperature. Afterwards, the extract was washed with 250 µL of deionised water by shaking for 10 min and centrifugation (10 min, 4 °C, 3,000 *g*). The supernatant was transferred into a pre-weighed glass vial and the lower aqueous phase with the pellet was re-extracted (300 µL MTBE, 5 min sonication, 30 min shaking at room temperature, centrifugation for 10 min at 4 °C with 3,000 *g*). The supernatants were combined and dried under nitrogen. The dried extracts were then stored at -20 °C until further analysis.

5.4.3. Lipid analysis

Before analysis, the lipids were dissolved in 3 mL methanol:propan-2-ol (1:1, v:v).

For quantification, the suitability of a commercially available lipid analytical standard (Splash Lipidomix Mass Spec Standard) was tested in preliminary experiments. The distribution of lipid classes in the samples and the standard did not match and no reliable results could be achieved. Therefore, a single standard from the most abundant lipid class (TG) was chosen as a reference (TG 17:0/17:0/17:0). It was added prior to extraction to a final concentration of 3 µg mL^{-1} .

5.4.3.1. LC-MS/MS method

Two different setups were used for analysis.

The procedure was established for basic cake samples on an Infinity 1290 ultra high-performance liquid chromatography (UHPLC) System (Agilent Technologies) coupled to a Q Exactive Hybrid Quadrupole-Orbitrap (Thermo Fisher Scientific, Waltham, USA) according to Cutignano et al. [176]. A Kinetex Biphenyl column (2.6 µm, 150 x 2.1 mm) (Phenomenex, Bologna, Italy) at a column temperature of 28 °C and flow rate of 0.3 mL min^{-1} was used. The lipids were separated using a binary gradient (solvent A: acetonitrile/water 60:40 (v:v), 10 mM ammonium formate, 0.1% formic acid, solvent B: propan-2-ol/acetonitrile 90:10 (v:v), 2 mM ammonium formate, 0.1% formic acid). The initial concentration of solvent B was 20% which was increased to 40% within the first 4 min, to 50% for 7 min and finally to 99% for 5 min. Afterwards, the initial conditions were set and held for 5.5 min before the next injection. The total runtime was 21 min. For detection, a full MS scan and a top 10 data dependent MS^2 scan in both positive and negative mode with heated electrospray ionisation (HESI) was carried out. The spray voltage was 3.2 kV in positive and 3.0 kV in negative mode, the capillary temperature was set at 320 °C, the auxiliary gas at 350 °C (rate 35), the sweep gas was 0 and the sheath rate was 60. The scan range for the full scan was from 200 to 1200 m z^{-1}, the automatic gain control (AGC) target at 1e5 and the maximum injection time 100 ms. For the data dependent MS^2 scan, the AGC target was 1e5 and the maximum injection time 75 ms. A stepped normalised energy from 16-20 or 20-40 was used in positive and negative mode, respectively. The software LipidSearch (Thermo Scientific, version 4.1.30) was used for data evaluation.

For pound cake and brioche, the procedure was transferred to a Vanquish Flex Binary UHPLC (Thermo Fisher Scientific) coupled to a Q Exactive Plus Orbitrap (Thermo Fisher Scientific). The same column, column temperature, solvents and flow rate as for basic cake were used, but the gradient was adjusted (initial concentration of B 20%, increase up to 40% for 4 min, increase up to 50% for 9 min, increase up to 99% for 7 min, then return to initial conditions and holding the initial conditions for 5.5 min with a total runtime of 25 min). Spray voltage, capillary temperature and settings for the fragmentation were as described above. The auxiliary gas was set to 350 °C (rate 10), the sweep gas to 0 and the sheath gas to rate 35. For the full scan MS, the scan range was extended to 150 to 1800 m z^{-1}, the AGC target set at 1e6 with a maximum injection time of 100 ms. For the MS^2 scan, the AGC target was 2e5 and the acquisition time was shortened to 50 ms. LipidSearch (Thermo Fisher Scientific, version 5.0.63.7) was again used for data evaluation.

The comparability of both systems was assured by comparing the identified lipids for chosen samples of each recipe and in detail for the quality control samples of basic cake. Quality control samples consisted of equal amounts of all 24 samples of one batch (all batter/dough samples of one recipe or all cake samples of one recipe) and were prepared for each batch.

5.4.3.2. Lipid identification and quantification

Data evaluation using the software LipidSearch as specified above included lipid identification and quantification. All samples of one batch including the quality control sample were processed in one alignment. The identified lipids were manually double-checked for reliability of the identification and integration area.

For the following calculation, it was assumed that the extraction and ionisation of a lipid species was similar within all samples of a batch. The relative abundance of each lipid was calculated as the percentage of the peak area of this lipid compared to the total peak areas of all lipids in a sample. The relative abundance was then divided by the relative abundance of the same lipid in the corresponding control sample, giving the substrate specificity ratio in the respective matrix (short: ratio). The ratios of three replicates (3 different batters/doughs of the same recipe treated with the same lipase) were averaged. A ratio below 1 indicates a loss of the lipid compared to the control and therefore a preferred hydrolysis. A ratio greater than 1 stands for either the formation of the lyso-lipid or a lack of hydrolysis of the lipid compared to the other lipids. The ratio is therefore inversely proportional to the specificity.

In addition to the ratios, lipid turnover rates were calculated. The lipid turnover rate was the average change of the total amount of a lipid in µg mg^{-1} dry matter in a lipase-treated sample compared to the respective control sample over the three replicates.

5.4.4. Statistical analysis

Microsoft Excel was used for the calculation of substrate specificity ratios and turnover rates. It was also applied to combine all ratios batch-wise in a new matrix before processing them for PCA with Origin Pro 2022b (Origin Lab Corporation).

5.5. Establishment of a new lipase activity assay for the use of baking lipases in fine bakery goods

5.5.1. Lipase activity assays using commercially available assays

The following parts were already published in *LWT - Food Science and Technology* [125].

For analysis with commercially available assays, the lipases were dissolved in lipase buffer as described in section 5.2.1. All experiments were carried out in triplicate according to the manufacturer's instructions using a Tecan multiplate reader Infinite 200 Pro (Tecan Group). After measurement, the linear range of absorption or fluorescence change over time was manually double-checked. It was adjusted in a way that the coefficient of determination was $\geq$0.95 before calculating lipase activities. Thereby, negative lipase activities could be avoided as discussed in section 3.4.1.

5.5.1.1. Assay I

Assay I was the lipase activity kit MAK046 (Merck KGaA). A lipase concentration of 1 mg mL^{-1} was suitable for analysis. The absorbance was measured at 570 nm as suggested by the manufacturer.

5.5.1.2. Assay II

Assay II was the lipase activity kit MAK047 (Merck KGaA). The lipases E, K, M and O were dissolved at a concentration of 1 mg mL^{-1}, the lipases A, G and J at a concentration of 0.1 mg mL^{-1}. As suggested by the manufacturer, the absorbance was recorded at 412 nm.

5.5.1.3. Assay III

Assay III was the lipase activity kit MAK048 (Merck KGaA). All lipases were dissolved at a concentration of 10 µg mL^{-1} and fluorescence was measured at an excitation wavelength of 529 nm and an emission wavelength of 600 nm as suggested by the manufacturer.

5.5.2. Development of the new lipase activity assay

The new assay was optimised for baking lipases starting with a procedure established by van Gaelen et al. [124] which is based on works of Zottig et al. [123]. All analyses were carried out in sextuplicate. The specificity of all lipases towards one substrate was assessed on a single plate.

Starting from the original method, the following parameters were modified:

(i) the wavelengths used for excitation and emission were checked and adjusted to optimize the sensitivity of the method

(ii) the workflow was changed to increase the repeatability of the experiments

(iii) the amount of lipase and the reaction time was adjusted for baking lipases

(iv) calibrations using the FFA oleic acid (C18:1) were performed after checking the comparability of the sensitivity of rhodamine B towards different FA from the substrates (C18, C18:1, C18:2, C18:3 and C12)

(v) the assay was adapted for more substrates by also introducing melt emulsions for spreadable fats and transferred to glycerophospholipids

The final setup included the substrates olive oil, wheat germ oil, flaxseed oil, coconut oil, butter, glyceryl trioleate (TG 18:1/18:1/18:1) and DOPC (PC 18:1(9Z)/18:1(9Z)). The substrates were emulsified in an emulsion buffer (1.36 g $KH_2PO_4$and 10 g GA in 200 mL distilled water, pH 7) using an Ultra-Turrax (IKA) with a stator diameter of 8 mm for 10 min at 12,500 rpm. For butter, the amount of GA was increased to 20% (w:v). The emulsion buffer was heated to 50 °C and stirred with the substrate for 10 min before homogenizing in the Ultra-Turrax. The lipases were dissolved in emulsion buffer at a concentration of 10 mg mL^{-1}. 150 µL of the resulting lipase solutions were mixed with 75 µL of substrate solution in a black 96-well plate. To monitor the course of the reaction, 10 µL of the fluorescent dye Rhodamine B (1 mg in 10 mL ethanol) were added. A Tecan multiplate reader Infinite 200 Pro (Tecan Group) was used to record the fluorescence (excitation wavelength 355 nm and emission wavelength 580 nm) every 5 min for 4 h. Calibration was performed using oleic acid in a concentration range from 1 mg mL^{-1} to 25 mg mL^{-1} (75 µL calibration solution, 75 µL substrate emulsion of the respective substrate, 75 µL emulsion buffer and 10 µL Rhodamine B solution). Control samples for the absorption of the lipase (75 µL emulsion buffer, 150 µL lipase solution and 10 µL Rhodamine B solution) and the self-fluorescence of the dye (75 µL substrate emulsion, 150 µL emulsion buffer and 10 µL Rhodamine B solution) were prepared for each lipase. The linear ranges of fluorescence change over time were identified manually and the ranges used for evaluation all had a correlation coefficient $R \geq 0.9$. Lipase activities were calculated as nmol oleic acid equivalents released per min per mg of lipase (nmol $min^{-1}mg^{-1}$).

5.5.3. Statistical analysis

Microsoft Excel built-in functions (mean, standard deviation, calculation of slopes, linear regression) were used for data evaluation.

6. References

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A. Appendix

A.1. Model systems to characterize lipase substrate specificities

Table 19: Activity of different lipases towards p-nitrophenyl derivatives with different chain lengths. All results are indicated as mean ± standard deviation (n = 3). Lipase D showed no reaction under the conditions of the assay. Its substrate specificity could therefore not be determined. Data already published in Stemler & Scherf [125].

Lipase	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0
				[$mg^{-1}s^{-1}$]				
A	4.35373 ± 0.38290	4.29232 ± 0.38630	49.90318 ± 1.50504	46.06611 ± 4.22144	43.60334 ± 2.99854	7.81321 ± 0.47170	3.29213 ± 0.03125	2.38529 ± 0.07063
B	1.64263 ± 0.03173	1.68406 ± 0.03494	5.25702 ± 0.25034	4.32672 ± 0.29367	5.11086 ± 0.44308	5.68141 ± 0.06696	3.52214 ± 0.35152	2.56646 ± 0.10497
C	0.27795 ± 0.00959	1.45101 ± 0.12424	2.55771 ± 0.15145	2.06859 ± 0.19887	2.36605 ± 0.12648	1.77595 ± 0.02605	1.43168 ± 0.05336	1.40068 ± 0.02219
E	0.37200 ± 0.00342	1.05525 ± 0.06803	2.62112 ± 0.16552	2.68253 ± 0.24024	2.67248 ± 0.06214	2.61544 ± 0.22578	2.26472 ± 0.10677	2.03189 ± 0.042897
F	0.00360 ± 0.00006	0.00321 ± 0.00005	0.01162 ± 0.00060	0.00948 ± 0.00034	0.01332 ± 0.00054	0.01132 ± 0.00047	0.00745 ± 0.00028	0.00541 ± 0.00026
G	9.93701 ± 0.05396	12.11648 ± 0.026299	33.36071 ± 1.42825	35.30371 ± 2.56673	34.82112 ± 1.53357	32.50318 ± 0.94535	23.02438 ± 0.29757	19.58224 ± 0.72502
H	0.20051 ± 0.00250	0.34245 ± 0.01428	1.52091 ± 0.10784	1.13723 ± 0.02931	1.86874 ± 0.06787	1.38114 ± 0.11324	1.22877 ± 0.03149	0.86491 ± 0.01887
I	27.26203 ± 0.98578	31.04110 ± 1.30749	99.37537 ± 4.13764	69.94160 ± 1.82158	88.75184 ± 3.94448	70.90855 ± 4.02489	54.43665 ± 2.64088	43.19093 ± 1.61518
J	8.75935 ± 0.63912	10.42982 ± 0.23961	44.19906 ± 2.64620	35.36866 ± 2.28323	40.94151 ± 3.16410	32.90420 ± 2.44481	23.25183 ± 0.19161	16.95106 ± 1.34165

Lipase	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0
K	0.07587 ± 0.00386	0.29718 ± 0.01355	1.36110 ± 0.08573	1.10919 ± 0.08279	1.24223 ± 0.10992	1.33139 ± 0.11701	1.04709 ± 0.07312	0.76512 ± 0.02123
L	0.00833 ± 0.00064	0.00945 ± 0.00092	0.00410 ± 0.00033	0.00222 ± 0.00016	0.00094 ± 0.00002	0.00029 ± 0.00001	0.00032 ± 0.00001	0.00012 ± 0.00003
M	0.75092 ± 0.00928	2.44381 ± 0.04416	3.47262 ± 0.02149	3.86624 ± 0.26778	4.45756 ± 0.34213	4.18475 ± 0.13145	3.70840 ± 0.14789	2.17400 ± 0.08985
N	0.00333 ± 0.00021	0.00365 ± 0.00017	0.00504 ± 0.00029	0.00111 ± 0.00008	0.00054 ± 0.00010	0.00023 ± 0.00007	0.00027 ± 0.00005	0.00028 ± 0.00004
O	0.21508 ± 0.00883	1.09705 ± 0.09838	2.24767 ± 0.02375	2.00277 ± 0.03675	2.03225 ± 0.12129	1.55775 ± 0.10841	1.40328 ± 0.08340	1.45185 ± 0.03783
P	0.01070 ± 0.00036	0.03702 ± 0.00305	0.04257 ± 0.00293	0.03161 ± 0.00219	0.02771 ± 0.00191	0.02711 ± 0.00257	0.02166 ± 0.00116	0.01223 ± 0.00102
Q	0.01706 ± 0.00128	0.01506 ± 0.00095	0.02043 ± 0.00128	0.01423 ± 0.00054	0.01703 ± 0.00122	0.02196 ± 0.00209	0.01371 ± 0.00125	0.00903 ± 0.00053

Table 20: Free fatty acids (FFA) in rapeseed oil emulsions treated with different lipases (A to Q) or without lipase addition (control). All results are indicated as mean value ± standard deviation in mg g^{-1} rapeseed oil (n = 3). Data already published in Stemler & Scherf [125].

Lipase	C16:0	C18:0	C18:1	C18:2	C18:3	total
	[mg g^{-1} rapeseed oil]					
Control	0.15 ± 0.03	0.10 ± 0.02	0.32 ± 0.03	0.10 ± 0.01	0.04 ± 0.01	0.70
A	5.53 ± 0.51	1.75 ± 0.30	51.85 ± 8.08	22.17 ± 4.00	9.68 ± 1.75	90.98
B	0.72 ± 0.06	0.28 ± 0.02	8.03 ± 0.13	3.49 ± 0.07	0.95 ± 0.02	13.47
C	6.78 ± 0.15	2.84 ± 0.08	68.60 ± 0.50	24.32 ± 0.67	10.48 ± 0.33	113.02
D	0.10 ± 0.00	0.07 ± 0.00	0.28 ± 0.01	0.08 ± 0.00	0.03 ± 0.00	0.57
E	4.42 ± 0.44	0.48 ± 0.057	44.87 ± 6.01	19.26 ± 2.75	8.52 ± 1.24	77.56
F	0.20 ± 0.02	0.06 ± 0.00	1.61 ± 0.06	0.65 ± 0.02	0.20 ± 0.02	2.73
G	2.04 ± 0.15	0.81 ± 0.04	22.57 ± 1.43	10.37 ± 0.60	4.49 ± 0.22	40.27
H	0.12 ± 0.01	0.04 ± 0.00	1.08 ± 0.13	0.35 ± 0.04	0.12 ± 0.01	1.71
I	1.37 ± 0.13	0.55 ± 0.06	15.16 ± 1.18	7.53 ± 0.76	3.33 ± 0.30	27.94
J	1.93 ± 0.18	0.76 ± 0.07	21.85 ± 1.67	9.31 ± 0.43	4.02 ± 0.13	37.87
K	0.44 ± 0.07	0.13 ± 0.02	6.95 ± 0.74	3.88 ± 0.42	1.16 ± 0.20	12.55
L	0.14 ± 0.00	0.10 ± 0.01	0.35 ± 0.02	0.12 ± 0.00	0.04 ± 0.00	0.74
M	4.24 ± 0.20	0.96 ± 0.02	46.27 ± 1.09	21.02 ± 0.61	9.38 ± 0.30	81.86
N	0.12 ± 0.00	0.09 ± 0.01	0.32 ± 0.01	0.10 ± 0.01	0.03 ± 0.00	0.67
O	7.62 ± 0.12	3.09 ± 0.06	78.42 ± 1.02	27.81 ± 0.38	12.35 ± 0.17	129.29

Lipase	C16:0	C18:0	C18:1	C18:2	C18:3	total
P	7.24 ± 0.16	2.48 ± 0.24	64.29 ± 2.15	17.53 ± 0.19	7.77 ± 0.30	99.31
Q	3.01 ± 0.48	0.18 ± 0.03	27.34 ± 4.03	9.66 ± 1.02	4.36 ± 0.46	44.55

Table 21: Free fatty acids (FFA) in margarine emulsions treated with different lipases (A to Q) or without lipase addition (control). All results are indicated as mean value ± standard deviation in mg g^{-1} margarine (n = 3). Data already published in Stemler & Scherf [125].

Lipase	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	total
	[mg g^{-1} margarine]							
Control	0.07 ± 0.00	0.01 ± 0.00	0.40 ± 0.01	0.33 ± 0.01	0.20 ± 0.01	0.12 ± 0.01	0.01 ± 0.00	1.15
A	4.10 ± 0.37	1.57 ± 0.19	38.93 ± 3.08	3.98 ± 0.41	222.77 ± 13.50	74.26 ± 6.02	28.29 ± 2.92	373.90
B	2.91 ± 0.23	1.27 ± 0.10	35.15 ± 3.91	4.49 ± 0.56	102.50 ± 14.30	40.91 ± 5.36	16.67 ± 2.28	203.90
C	5.29 ± 0.36	2.17 ± 0.17	543.00 ± 3.24	11.153 ± 0.76	225.11 ± 11.44	73.14 ± 3.48	26.62 ± 0.91	397.49
D	0.06 ± 0.01	0.01 ± 0.00	0.35 ± 0.04	0.26 ± 0.05	0.18 ± 0.02	0.17 ± 0.03	0.01 ± 0.00	1.05
E	4.11 ± 0.26	1.42 ± 0.06	36.20 ± 2.70	6.52 ± 0.53	234.53 ± 18.92	79.40 ± 5.60	30.10 ± 2.04	392.27
F	0.08 ± 0.01	0.09 ± 0.00	1.45 ± 0.06	0.56 ± 0.03	3.94 ± 0.11	1.05 ± 0.02	0.13 ± 0.01	7.31
G	3.47 ± 0.18	1.46 ± 0.03	38.50 ± 2.11	5.59 ± 0.29	141.29 ± 11.71	55.92 ± 4.60	22.25 ± 1.68	268.48
H	1.20 ± 0.11	0.60 ± 0.05	11.03 ± 1.27	1.62 ± 0.18	26.14 ± 2.65	11.83 ± 1.39	3.29 ± 0.55	55.71
I	5.36 ± 0.32	2.51 ± 0.20	57.02 ± 5.12	8.88 ± 0.73	203.26 ± 15.53	66.71 ± 4.90	23.94 ± 1.55	367.68
J	2.11 ± 0.22	1.06 ± 0.06	21.16 ± 2.27	3.43 ± 0.36	98.98 ± 10.52	33.02 ± 3.48	12.49 ± 1.10	172.24

Lipase	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	total
K	3.20 ± 0.27	1.39 ± 0.13	31.27 ± 2.79	5.15 ± 0.49	188.56 ± 15.88	68.83 ± 4.89	26.28 ± 1.89	324.70
L	0.01 ± 0.00	0.01 ± 0.00	0.33 ± 0.03	0.28 ± 0.02	0.23 ± 0.04	0.20 ± 0.03	0.02 ± 0.00	1.09
M	3.59 ± 0.15	1.52 ± 0.08	36.08 ± 1.85	6.03 ± 0.27	223.37 ± 10.25	79.41 ± 4.62	29.80 ± 2.00	379.79
N	0.01 ± 0.00	0.01 ± 0.00	0.36 ± 0.04	0.30 ± 0.02	0.22 ± 0.03	0.20 ± 0.03	0.01 ± 0.00	1.12
O	4.41 ± 0.84	1.96 ± 0.39	46.43 ± 7.10	7.15 ± 1.01	171.74 ± 23.18	56.50 ± 7.50	20.51 ± 2.70	308.70
P	4.15 ± 0.67	2.02 ± 0.33	53.00 ± 8.98	9.73 ± 1.24	144.49 ± 23.53	40.96 ± 6.66	14.95 ± 2.23	269.30
Q	2.18 ± 0.09	0.91 ± 0.07	26.54 ± 1.33	3.84 ± 0.06	91.87 ± 3.83	27.19 ± 1.10	10.44 ± 0.68	162.96

Table 22: Free fatty acids (FFA) in butter emulsions treated with different lipases (A to Q) or without lipase addition (control). All results are indicated as mean value ± standard deviation in mg g^{-1} butter (n = 3). Data already published in Stemler & Scherf [125].

Lipase	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	total
	[mg g^{-1} butter]										
Control	0.05 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.04 ± 0.00	0.08 ± 0.01	0.42 ± 0.04	0.18 ± 0.02	0.23 ± 0.03	0.02 ± 0.00	1.04
A	15.27 ± 1.13	5.96 ± 0.44	4.54 ± 0.29	7.83 ± 1.03	7.15 ± 0.54	20.91 ± 1.36	78.10 ± 4.63	23.47 ± 1.37	66.58 ± 5.30	6.06 ± 0.62	235.87
B	12.40 ± 2.17	5.07 ± 0.93	4.36 ± 0.61	6.78 ± 1.06	6.75 ± 0.97	19.21 ± 2.37	68.30 ± 12.08	21.03 ± 3.61	60.60 ± 9.49	4.70 ± 0.66	209.19
C	14.38 ± 0.77	6.54 ± 0.43	5.10 ± 0.17	8.70 ± 0.37	8.27 ± 0.42	22.56 ± 1.10	87.35 ± 3.24	27.27 ± 0.98	72.13 ± 1.98	6.69 ± 0.24	258.99
D	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.14 ± 0.01	0.47 ± 0.03	0.19 ± 0.01	0.40 ± 0.03	0.04 ± 0.00	1.35
E	6.43 ± 1.04	3.15 ± 0.30	2.57 ± 0.23	3.68 ± 0.31	3.17 ± 0.28	6.78 ± 0.59	31.23 ± 2.70	7.15 ± 0.50	42.35 ± 3.85	3.68 ± 0.47	110.18
F	0.20 ± 0.02	0.10 ± 0.01	0.09 ± 0.00	0.15 ± 0.01	0.06 ± 0.01	0.50 ± 0.03	1.24 ± 0.06	0.41 ± 0.01	1.34 ± 0.07	0.17 ± 0.01	4.25
G	11.77 ± 0.83	5.26 ± 0.28	3.29 ± 0.28	5.27 ± 0.39	4.98 ± 0.32	13.83 ± 0.45	50.97 ± 3.04	14.77 ± 0.71	63.39 ± 3.74	5.69 ± 0.83	179.21
H	6.33 ± 0.62	2.76 ± 0.29	1.44 ± 0.26	2.13 ± 0.41	2.30 ± 0.29	6.17 ± 0.65	25.77 ± 4.85	6.45 ± 1.25	29.56 ± 3.07	2.06 ± 0.39	84.97
I	16.64 ± 0.22	6.70 ± 0.20	5.23 ± 0.47	8.00 ± 0.61	6.85 ± 0.17	18.33 ± 0.67	88.38 ± 2.60	24.68 ± 0.75	72.12 ± 6.17	7.53 ± 0.35	254.47
J	6.93 ± 0.26	2.70 ± 0.28	2.58 ± 0.13	3.48 ± 0.21	3.96 ± 0.20	8.27 ± 0.39	34.32 ± 2.69	9.14 ± 0.77	36.24 ± 0.70	3.34 ± 0.25	110.95
K	14.01 ± 0.20	5.43 ± 0.17	4.36 ± 0.20	6.08 ± 0.23	5.34 ± 0.21	13.25 ± 0.62	60.40 ± 1.11	16.02 ± 0.17	77.55 ± 10.40	6.42 ± 0.37	208.86
L	0.03 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.05 ± 0.00	0.08 ± 0.02	0.19 ± 0.00	0.66 ± 0.02	0.25 ± 0.01	0.60 ± 0.01	0.08 ± 0.00	1.96
M	11.86 ± 0.75	4.99 ± 0.14	3.22 ± 0.01	5.27 ± 0.10	4.75 ± 0.08	12.08 ± 0.09	39.78 ± 2.53	10.72 ± 0.62	60.91 ± 5.48	5.29 ± 0.12	158.87
N	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.04 ± 0.00	0.06 ± 0.01	0.15 ± 0.01	0.50 ± 0.01	0.20 ± 0.01	0.55 ± 0.02	0.10 ± 0.00	1.63
O	15.19 ± 0.83	6.45 ± 0.57	5.00 ± 0.55	8.34 ± 0.93	8.43 ± 0.88	24.50 ± 2.28	81.58 ± 6.90	23.97 ± 1.69	71.85 ± 5.75	5.82 ± 1.03	251.14

Lipase	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	total
P	11.95 ± 0.97	5.08 ± 0.44	3.06 ± 0.35	3.94 ± 0.12	4.52 ± 0.60	13.46 ± 1.73	64.77 ± 7.60	20.51 ± 2.13	53.97 ± 4.65	4.80 ± 0.38	186.07
Q	2.34 ± 0.18	1.57 ± 0.10	0.96 ± 0.14	1.56 ± 0.16	2.04 ± 0.17	4.04 ± 0.30	18.73 ± 2.20	5.74 ± 0.66	18.75 ± 1.00	1.34 ± 0.24	57.05

Table 23: Free fatty acids (FFA) in wheat germ oil emulsions treated with different lipases (A to Q) or without lipase addition (control). All results are indicated as mean value ± standard deviation in mg g^{-1} wheat germ oil (n = 3).

Lipase	C16:0	C18:0	C18:1	C18:2	C18:3	total
	[mg g^{-1} wheat germ oil]					
Control	17.28 ± 1.17	0.77 ± 0.13	13.70 ± 2.89	48.28 ± 3.06	6.90 ± 0.65	86.93
A	74.60 ± 14.09	2.94 ± 0.42	62.96 ± 11.19	228.92 ± 44.15	31.86 ± 5.96	401.28
B	79.34 ± 1.28	3.00 ± 0.06	60.83 ± 0.76	218.52 ± 2.36	30.85 ± 0.31	392.54
C	103.68 ± 4.38	4.04 ± 0.23	83.25 ± 3.50	309.34 ± 14.35	42.73 ± 2.03	543.04
D	10.71 ± 0.95	0.38 ± 0.05	12.26 ± 0.67	31.48 ± 2.80	3.64 ± 0.42	58.47
E	75.32 ± 9.64	3.55 ± 0.46	66.86 ± 7.57	238.72 ± 30.11	32.89 ± 4.33	417.34
F	52.29 ± 5.57	2.55 ± 0.27	45.33 ± 2.45	169.37 ± 18.76	25.08 ± 2.81	294.62
G	78.80 ± 7.53	3.02 ± 0.28	69.20 ± 5.88	250.88 ± 23.16	34.14 ± 2.85	436.05
H	43.86 ± 1.47	1.80 ± 0.25	33.69 ± 1.18	124.29 ± 4.75	18.39 ± 0.66	222.03
I	91.74 ± 6.19	3.41 ± 0.37	76.22 ± 4.09	279.89 ± 16.07	37.95 ± 2.00	489.22
J	81.08 ± 1.72	2.88 ± 0.16	61.11 ± 3.33	225.00 ± 12.00	30.50 ± 1.70	400.57
K	95.59 ± 7.20	4.21 ± 0.32	79.35 ± 4.68	324.41 ± 18.97	45.07 ± 2.05	548.63
L	15.13 ± 0.90	0.51 ± 0.03	8.38 ± 0.53	45.80 ± 2.96	5.85 ± 0.46	75.67
M	101.50 ± 13.79	4.26 ± 0.45	84.13 ± 10.57	352.03 ± 48.58	46.39 ± 6.11	588.31
N	14.61 ± 0.27	0.55 ± 0.03	15.98 ± 0.23	49.85 ± 1.24	5.91 ± 0.14	86.90
O	133.05 ± 0.59	5.34 ± 0.34	104.53 ± 2.50	391.56 ± 7.64	53.06 ± 0.82	687.54

Lipase	C16:0			C18:0			C18:1			C18:2			C18:3			total
P	118.51	±	4.15	4.81	±	0.32	97.67	±	4.36	362.86	±	14.90	49.48	±	1.97	633.34
Q	97.08	±	2.84	3.60	±	0.22	48.39	±	0.70	155.81	±	2.22	23.79	±	0.41	328.67

Table 24: Free fatty acids (FFA) in butter emulsions treated with lipase A for different time periods. All results are indicated as mean value ± standard deviation in mg g^{-1} butter (n = 2). Data already published in Stemler & Scherf [125]

Time [min]	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	total
	[mg g^{-1} butter]										
60	3.40 ± 0.42	1.31 ± 0.24	0.82 ± 0.15	1.50 ± 0.26	2.28 ± 0.35	6.83 ± 0.73	18.82 ± 3.09	7.52 ± 0.85	16.80 ± 1.53	1.00 ± 0.04	60.27
120	3.97 ± 0.09	1.80 ± 0.10	1.21 ± 0.14	2.15 ± 0.28	2.99 ± 0.36	8.18 ± 0.79	26.26 ± 3.60	9.83 ± 1.05	21.69 ± 1.90	1.45 ± 0.10	79.53
240	4.43 ± 0.88	1.26 ± 0.22	0.91 ± 0.18	1.44 ± 0.25	2.32 ± 0.44	7.02 ± 1.03	17.71 ± 3.44	7.50 ± 0.85	16.36 ± 1.31	0.96 ± 0.11	59.91
360	3.79 ± 0.66	1.62 ± 0.30	0.66 ± 0.13	1.33 ± 0.21	2.00 ± 0.33	5.98 ± 0.83	16.22 ± 3.15	6.87 ± 1.01	15.97 ± 2.75	0.98± 0.16	79.04
480	2.93 ± 0.16	0.92 ± 0.10	0.58 ± 0.10	1.03 ± 0.19	1.56 ± 0.28	5.23 ± 0.90	12.99 ± 2.57	6.24 ± 0.93	14.24 ± 1.89	0.76 ± 0.11	46.49

Table 25: Free fatty acids (FFA) in rapeseed oil/margarine/butter/wheat germ oil emulsions with the emulsifiers gum arabic (GA) and egg yolk lecithine (EL) treated with the lipases A, J or Q or without lipase addition (control). All results are indicated as mean value ± standard deviation (n = 3). Data partly already published in Stemler & Scherf [125].

fat + emulsifier	Lipase	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	total
		[mg g^{-1} rapeseed oil/margarine/butter/wheat germ oil]											
Rapeseed oil + GA	Control							0.06 ± 0.00	0.05 ± 0.01	0.14 ± 0.02	0.02 ± 0.00	0.01 ± 0.00	0.28
	A							1.65 ± 0.23	0.63 ± 0.12	27.22 ± 3.15	5.45 ± 0.87	2.02 ± 0.30	36.95
	J							6.11 ± 0.60	1.92 ± 0.11	79.91 ± 6.91	25.01 ± 1.92	8.44 ± 0.75	121.39
	Q							1.02 ± 0.03	0.31 ± 0.05	16.84 ± 0.65	2.53 ± 0.12	0.94 ± 0.06	21.64
Rapeseed oil + EL	Control							0.19 ± 0.03	0.14 ± 0.03	1.64 ± 0.09	0.09 ± 0.02	0.03 ± 0.00	2.08
	A							16.56 ± 1.93	7.19 ± 1.11	102.90 ± 10.91	33.45 ± 3.28	12.45 ± 2.29	172.55
	J							9.30 ± 0.31	3.31 ± 0.18	58.74 ± 2.13	18.88 ± 0.83	6.19 ± 0.40	96.42
	Q							3.80 ± 0.77	1.43 ± 0.27	29.05 ± 5.53	6.64 ± 1.14	2.49 ± 0.50	43.42
Margarine + GA	Control					0.04 ± 0.00	0.01 ± 0.00	0.24 ± 0.01	0.19 ± 0.01	0.12 ± 0.02	0.03 ± 0.01	0.04 ± 0.01	0.65

fat + emulsifier	Lipase	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	total
	A					2.32 ± 0.46	1.19 ± 0.23	29.40 ± 4.98	3.76 ± 0.66	78.56 ± 12.18	24.26 ± 3.94	7.17 ± 1.36	146.66
	J					2.13 ± 0.40	1.07 ± 0.18	24.66 ± 2.54	3.52 ± 0.07	63.63 ± 6.29	21.29 ± 1.59	6.16 ± 0.63	122.46
	Q					0.69 ± 0.11	0.36 ± 0.06	7.06 ± 0.95	1.01 ± 0.19	24.86 ± 3.31	4.57 ± 0.69	1.49 ± 0.23	40.04
Margarine + EL	Control					0.02 ± 0.00	0.02 ± 0.00	0.73 ± 0.03	0.47 ± 0.05	0.41 ± 0.04	0.21 ± 0.02	0.03 ± 0.00	1.88
	A					1.60 ± 0.23	0.77 ± 0.10	19.35 ± 2.86	3.72 ± 0.57	52.41 ± 7.38	16.40 ± 2.43	5.31 ± 0.83	99.55
	J					5.35 ± 0.13	2.65 ± 0.08	70.52 ± 2.74	12.06 ± 0.08	169.64± 7.41	57.06 ± 2.99	18.14 ± 2.61	335.42
	Q					0.58 ± 0.05	0.34 ± 0.03	7.96 ± 1.01	1.55 ± 0.22	17.80 ± 1.71	4.30 ± 0.47	1.35 ± 0.10	33.88
Butter + GA	Control	0.06 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.08 ± 0.01	0.11 ± 0.02	0.40 ± 0.05	0.16 ± 0.02	0.38 ± 0.04	0.04 ± 0.00		1.29
	A	5.00 ± 0.58	2.90 ± 0.47	1.47 ± 0.26	2.57 ± 0.51	3.34 ± 0.47	7.06 ± 1.03	24.35 ± 4.24	7.67 ± 1.43	23.03 ± 3.17	1.58 ± 0.27		78.97
	J	9.42 ± 1.87	5.09 ± 0.85	3.46 ± 0.70	6.11 ± 1.11	7.39 ± 1.46	17.16 ± 2.83	53.50 ± 7.91	18.93 ± 2.95	46.52 ± 8.03	4.04 ± 0.64		171.62

fat + emulsifier	Lipase	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	total
	Q	2.44 ± 0.36	1.90 ± 0.37	1.42 ± 0.26	2.17 ± 0.39	2.91 ± 0.51	5.62 ± 0.73	21.87 ± 4.19	7.26 ± 1.45	22.78 ± 2.73	1.55 ± 0.31		69.93
Butter + EL	Control	0.08 ± 0.01	0.01 ± 0.00	0.02 ± 0.00	0.03 ± 0.01	0.06 ± 0.01	0.12 ± 0.02	0.50 ± 0.07	0.16 ± 0.02	0.40 ± 0.07	0.05 ± 0.01		1.43
	A	8.18 ± 1.44	3.56 ± 0.35	3.02 ± 0.25	5.27 ± 0.54	7.00 ± 0.75	16.21 ± 1.91	75.51 ± 7.34	22.49 ± 2.14	50.42 ± 5.57	6.62 ± 0.75		198.27
	J	21.05 ± 1.73	9.08 ± 0.37	7.29 ± 0.34	13.42 ± 0.54	17.75 ± 0.62	41.31 ± 1.41	167.77 ± 6.50	50.26 ± 1.83	109.50± 5.11	17.22 ± 0.90		454.64
	Q	10.58 ± 1.03	4.34 ± 0.22	3.90 ± 0.41	5.89 ± 0.63	8.21 ± 0.91	18.40 ± 2.01	82.85 ± 10.80	28.74 ± 3.00	58.24 ± 6.65	6.86± 0.60		228.01
Wheat germ oil + GA	Control							4.17± 0.31	0.09 ± 0.01	2.96 ± 0.25	16.39 ± 0.85	1.47 ± 0.11	25.09
	A							31.58 ± 3.45	1.41 ± 0.27	31.65 ± 2.66	104.61 ± 10.65	14.87 ± 1.30	184.13
	J							36.10 ± 6.47	1.41 ± 0.21	33.52 ± 4.76	120.40 ± 20.30	16.38 ± 2.74	207.81
	Q							26.00 ± 4.84	0.87 ± 0.10	17.52 ± 1.83	49.64 ± 7.67	6.34 ± 1.21	100.37
Wheat germ oil + EL	Control							14.40 ± 0.96	0.63 ± 0.12	7.62 ± 0.79	39.00 ± 2.37	4.79 ± 0.33	66.44
	A							77.83 ± 11.87	7.64 ± 1.48	64.40 ± 8.88	213.60 ± 32.29	27.79 ± 4.01	391.26

fat + emulsifier	Lipase	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	total
	J							110.13 ± 18.82	13.68 ± 1.78	80.92 ± 16.81	285.06 ± 51.23	37.26 ± 6.50	527.03
	Q							7.96 ± 1.61	0.69 ± 0.03	3.77 ± 0.62	15.45 ± 1.24	2.19 ± 0.22	30.06

A.2. Lipases as improvers for the baking quality of fine bakery goods

Table 26: Density, baking loss and firmness of differently modified basic cake, pound cake and brioche samples over time. Control – untreated sample, DATEM – addition of DATEM, A-O – addition of respective lipase. Values are given as mean (n = 6) ± standard deviation. Data already published in Stemler & Scherf [97].

		Density [g mL^{-1}]	Water loss [%]	Firmness [N]			
				0 h	24 h	48 h	96 h
Basic cake	Control	0.58 ± 0.04	12.68 ± 0.14	4.87 ± 0.62	9.47 ± 0.50	10.94 ± 0.52	11.01 ± 0.41
	DATEM	0.60 ± 0.03	13.23 ± 0.16	5.23 ± 0.91	8.31 ± 1.18	11.20 ± 1.18	14.35 ± 0.71
	A	0.48 ± 0.04	14.01 ± 0.20	3.29 ± 0.47	3.96 ± 0.82	4.50 ± 0.24	4.56 ± 0.82
	E	0.61 ± 0.04	12.20 ± 0.11	4.02 ± 0.99	7.37 ± 0.94	7.370 ± 0.86	10.23 ± 1.85
	G	0.59 ± 0.03	13.03 ± 0.22	3.34 ± 0.76	3.33 ± 0.14	3.67 ± 0.36	3.79 ± 0.59
	J	0.58 ± 0.03	12.51 ± 0.43	2.97 ± 0.79	3.10 ± 0.49	3.79 ± 0.40	3.67 ± 0.57
	K	0.60 ± 0.04	13.50 ± 0.15	2.81 ± 0.68	6.77 ± 0.38	8.71 ± 1.11	9.31 ± 1.44
	M	0.62 ± 0.02	12.96 ± 0.37	3.54 ± 0.64	6.56 ± 0.34	8.69 ± 0.22	9.95 ± 1.59
	O	0.59 ± 0.00	13.03 ± 0.22	3.07 ± 0.73	7.10 ± 1.41	9.12 ± 0.10	10.63 ± 0.87
Pound cake	Control	0.63 ± 0.02	8.81 ± 0.25	9.27 ± 0.77	14.91 ± 1.20	16.26 ± 2.13	20.20 ± 2.55
	DATEM	0.65 ± 0.01	8.86 ± 0.25	10.91 ± 1.65	14.99 ± 2.21	17.78 ± 2.87	21.54 ± 3.51
	A	0.61 ± 0.02	9.64 ± 0.25	12.30 ± 1.20	13.98 ± 0.11	14.29 ± 0.90	15.56 ± 1.48
	E	0.65 ± 0.01	8.42 ± 0.37	9.46 ± 1.19	12.92 ± 1.41	13.21 ± 1.79	18.31 ± 1.72
	G	0.63 ± 0.01	9.44 ± 0.13	11.13 ± 0.67	13.61 ± 1.23	12.13 ± 0.52	15.17 ± 0.96
	J	0.62 ± 0.01	9.61 ± 0.22	9.90 ± 0.42	13.60 ± 1.31	12.85 ± 0.43	15.21 ± 1.16
	K	0.63 ± 0.01	9.11 ± 0.08	7.40 ± 1.25	14.14 ± 1.71	14.53 ± 1.67	17.58 ± 1.90

		Density [g mL^{-1}]			Water loss [%]			Firmness [N]											
								0 h			24 h			48 h			96 h		
	M	0.64	±	0.01	9.33	±	0.24	7.43	±	0.63	13.76	±	1.15	12.70	±	1.27	15.40	±	1.44
	O	0.66	±	0.01	9.02	±	0.11	6.91	±	0.74	13.16	±	0.55	13.80	±	2.11	16.24	±	2.25
Brioche	Control	0.49	±	0.01	10.61	±	0.22	6.92	±	0.08	15.98	±	0.12	23.97	±	0.54	31.99	±	1.01
	DATEM	0.53	±	0.03	10.69	±	0.31	9.13	±	1.42	19.13	±	3.37	26.16	±	1.84	35.88	±	3.47
	A	0.51	±	0.01	10.41	±	0.27	7.26	±	0.37	16.01	±	0.51	22.32	±	0.77	32.75	±	2.41
	E	0.47	±	0.03	11.81	±	0.27	8.74	±	1.04	16.67	±	1.56	23.21	±	1.82	32.88	±	3.53
	G	0.46	±	0.03	11.53	±	0.49	8.35	±	1.15	14.68	±	2.87	22.63	±	4.07	29.56	±	3.99
	J	0.48	±	0.01	11.35	±	0.16	7.28	±	0.80	14.39	±	0.89	21.36	±	2.57	29.65	±	3.68
	K	0.47	±	0.02	10.74	±	0.30	6.13	±	0.60	17.06	±	1.68	22.74	±	0.47	27.15	±	0.04
	M	0.49	±	0.03	11.06	±	0.21	6.41	±	0.81	16.41	±	0.50	23.75	±	3.29	28.17	±	2.51
	O	0.49	±	0.03	11.11	±	0.16	6.81	±	1.10	17.98	±	0.22	25.01	±	0.94	32.25	±	2.36

Table 27: Springiness and cohesiveness of differently modified basic cake, pound cake and brioche samples over time. Control – untreated sample, DATEM – addition of DATEM, A-O – addition of respective lipase. Values are given as mean (n = 6) ± standard deviation. Data already published in Stemler & Scherf [97].

		Springiness [1]												Cohesiveness [1]											
		0 h			24 h			48 h			96 h			0 h			24 h			48 h			96 h		
Basic cake	Control	0.77	±	0.07	0.76	±	0.03	0.70	±	0.01	0.64	±	0.06	0.53	±	0.02	0.38	±	0.01	0.31	±	0.03	0.27	±	0.02
	DATEM	0.76	±	0.02	0.78	±	0.08	0.74	±	0.04	0.79	±	0.11	0.52	±	0.04	0.39	±	0.03	0.38	±	0.02	0.32	±	0.02
	A	0.74	±	0.11	0.74	±	0.10	0.67	±	0.08	0.59	±	0.03	0.32	±	0.03	0.23	±	0.01	0.24	±	0.02	0.24	±	0.02
	E	0.76	±	0.09	0.75	±	0.11	0.64	±	0.01	0.69	±	0.03	0.45	±	0.03	0.35	±	0.01	0.31	±	0.02	0.27	±	0.02
	G	0.74	±	0.05	0.66	±	0.05	0.75	±	0.05	0.62	±	0.07	0.31	±	0.02	0.27	±	0.02	0.28	±	0.03	0.27	±	0.01
	J	0.63	±	0.09	0.80	±	0.11	0.72	±	0.01	0.65	±	0.14	0.28	±	0.01	0.28	±	0.03	0.27	±	0.01	0.28	±	0.01
	K	0.73	±	0.01	0.73	±	0.07	0.70	±	0.06	0.62	±	0.02	0.52	±	0.03	0.38	±	0.01	0.32	±	0.04	0.26	±	0.02
	M	0.72	±	0.03	0.66	±	0.05	0.70	±	0.01	0.64	±	0.10	0.51	±	0.03	0.39	±	0.02	0.33	±	0.01	0.27	±	0.03
	O	0.83	±	0.02	0.78	±	0.06	0.81	±	0.06	0.72	±	0.09	0.57	±	0.02	0.41	±	0.05	0.31	±	0.02	0.26	±	0.02
Pound cake	Control	0.94	±	0.02	0.94	±	0.02	0.91	±	0.02	0.89	±	0.01	0.64	±	0.06	0.55	±	0.04	0.51	±	0.02	0.47	±	0.01
	DATEM	0.93	±	0.02	0.93	±	0.03	0.91	±	0.01	0.90	±	0.02	0.61	±	0.06	0.52	±	0.03	0.51	±	0.02	0.47	±	0.03
	A	0.91	±	0.04	0.93	±	0.02	0.89	±	0.01	0.88	±	0.03	0.54	±	0.01	0.49	±	0.00	0.47	±	0.02	0.47	±	0.02
	E	0.92	±	0.01	0.90	±	0.01	0.88	±	0.03	0.85	±	0.01	0.52	±	0.02	0.48	±	0.01	0.43	±	0.00	0.43	±	0.02
	G	0.86	±	0.02	0.90	±	0.02	0.89	±	0.01	0.86	±	0.03	0.48	±	0.01	0.48	±	0.00	0.45	±	0.01	0.44	±	0.01
	J	0.88	±	0.02	0.89	±	0.02	0.87	±	0.00	0.85	±	0.00	0.48	±	0.02	0.46	±	0.02	0.45	±	0.02	0.44	±	0.03
	K	0.91	±	0.01	0.90	±	0.01	0.89	±	0.02	0.89	±	0.01	0.60	±	0.01	0.51	±	0.02	0.46	±	0.02	0.45	±	0.01

		Springiness [1]												Cohesiveness [1]											
		0 h			24 h			48 h			96 h			0 h			24 h			48 h			96 h		
	M	0.91	±	0.03	0.91	±	0.01	0.86	±	0.05	0.87	±	0.01	0.59	±	0.01	0.51	±	0.02	0.48	±	0.01	0.46	±	0.03
	O	0.93	±	0.01	0.90	±	0.01	0.88	±	0.03	0.86	±	0.01	0.60	±	0.01	0.50	±	0.02	0.45	±	0.02	0.43	±	0.01
Brioche	Control	0.84	±	0.07	0.84	±	0.05	0.92	±	0.02	0.94	±	0.01	0.63	±	0.02	0.41	±	0.02	0.36	±	0.02	0.34	±	0.02
	DATEM	0.82	±	0.08	0.88	±	0.05	0.93	±	0.04	0.92	±	0.04	0.58	±	0.03	0.39	±	0.01	0.39	±	0.02	0.35	±	0.00
	A	0.75	±	0.02	0.88	±	0.02	0.92	±	0.00	0.90	±	0.02	0.60	±	0.03	0.40	±	0.03	0.35	±	0.01	0.33	±	0.01
	E	0.81	±	0.02	0.95	±	0.03	0.89	±	0.02	0.91	±	0.03	0.54	±	0.03	0.39	±	0.02	0.37	±	0.01	0.35	±	0.02
	G	0.78	±	0.03	0.90	±	0.02	0.92	±	0.01	0.91	±	0.03	0.54	±	0.00	0.40	±	0.02	0.36	±	0.02	0.35	±	0.01
	J	0.74	±	0.02	0.89	±	0.06	0.93	±	0.01	0.92	±	0.01	0.57	±	0.02	0.38	±	0.02	0.35	±	0.03	0.33	±	0.01
	K	0.86	±	0.07	0.92	±	0.05	0.91	±	0.01	0.90	±	0.02	0.63	±	0.02	0.38	±	0.01	0.35	±	0.02	0.33	±	0.01
	M	0.78	±	0.03	0.92	±	0.02	0.91	±	0.03	0.93	±	0.02	0.62	±	0.02	0.37	±	0.02	0.34	±	0.02	0.32	±	0.03
	O	0.75	±	0.02	0.88	±	0.03	0.92	±	0.02	0.90	±	0.03	0.60	±	0.03	0.39	±	0.01	0.35	±	0.00	0.34	±	0.00

Table 28: Gumminess and chewiness of differently modified basic cake, pound cake and brioche samples over time. Control – untreated sample, DATEM – addition of DATEM, A-O – addition of respective lipase. Values are given as mean (n = 6) ± standard deviation. Data already published in Stemler & Scherf [97] .

		Gumminess [N]												Chewiness [N]									
		0 h			24 h			48 h			96 h			0 h			24 h			48 h			96 h		
Basic cake	Control	2.55	±	0.48	3.63	±	0.15	3.42	±	0.50	3.00	±	0.28	1.94	±	0.27	2.77	±	0.11	2.44	±	0.38	1.93	±	0.34
	DATEM	2.69	±	0.51	3.27	±	0.71	4.19	±	0.38	4.54	±	0.13	2.04	±	0.36	2.50	±	0.32	3.12	±	0.33	3.59	±	0.41
	A	1.06	±	0.14	0.94	±	0.23	1.11	±	0.15	1.13	±	0.27	0.80	±	0.20	0.75	±	0.22	0.77	±	0.19	0.68	±	0.12
	E	1.80	±	0.45	2.64	±	0.44	2.29	±	0.38	2.86	±	0.78	1.18	±	0.31	1.78	±	0.42	1.49	±	0.27	1.99	±	0.50
	G	1.11	±	0.23	0.90	±	0.08	1.03	±	0.21	1.04	±	0.22	0.66	±	0.08	0.60	±	0.02	0.77	±	0.17	0.65	±	0.13
	J	0.77	±	0.16	0.86	±	0.09	1.02	±	0.11	1.04	±	0.20	0.52	±	0.10	0.69	±	0.12	0.74	±	0.09	0.66	±	0.13
	K	1.39	±	0.31	2.56	±	0.12	2.87	±	0.61	2.50	±	0.58	1.00	±	0.23	1.80	±	0.27	2.27	±	0.31	1.24	±	0.20
	M	1.83	±	0.42	2.58	±	0.29	2.84	±	0.14	2.76	±	0.66	1.26	±	0.34	1.73	±	0.25	2.01	±	0.11	1.87	±	0.44
	O	1.76	±	0.40	2.91	±	0.69	2.87	±	0.15	2.73	±	0.27	1.42	±	0.34	2.12	±	0.47	2.33	±	0.11	1.94	±	0.15
Pound cake	Control	5.86	±	0.15	8.24	±	1.36	8.28	±	1.29	9.54	±	1.33	5.49	±	0.22	7.75	±	1.11	7.50	±	1.19	8.49	±	1.16
	DATEM	6.58	±	0.57	7.84	±	1.22	9.14	±	1.58	10.23	±	2.05	6.13	±	0.63	7.32	±	1.27	8.31	±	1.40	9.24	±	1.98
	A	6.61	±	0.67	6.89	±	0.10	6.79	±	0.49	7.34	±	0.91	5.98	±	0.36	6.42	±	0.19	6.02	±	0.48	6.47	±	0.98
	E	4.94	±	0.39	6.19	±	0.78	5.74	±	0.82	7.85	±	1.05	4.56	±	0.40	5.56	±	0.72	5.07	±	0.79	6.69	±	0.99
	G	5.36	±	0.45	6.53	±	0.61	5.46	±	0.37	6.73	±	0.58	4.62	±	0.50	5.89	±	0.60	4.84	±	0.35	5.75	±	0.48
	J	4.77	±	0.27	6.35	±	0.83	5.77	±	0.44	6.68	±	0.88	4.20	±	0.32	5.66	±	0.79	5.04	±	0.39	5.65	±	0.73
	K	4.41	±	0.72	7.26	±	1.23	6.71	±	0.91	7.90	±	0.96	4.02	±	0.67	6.54	±	1.07	5.98	±	0.88	7.04	±	0.89

		Gumminess [N]												Chewiness [N]											
		0 h			24 h			48 h			96 h			0 h			24 h			48 h			96 h		
	M	4.41	±	0.37	7.05	±	0.90	6.10	±	0.58	7.10	±	1.13	4.01	±	0.21	6.37	±	0.75	5.29	±	0.78	6.15	±	0.87
	O	4.18	±	0.47	6.61	±	0.29	6.27	±	1.07	7.04	±	1.05	3.89	±	0.47	5.98	±	0.25	5.54	±	1.08	6.07	±	0.97
Brioche	Control	4.33	±	0.17	6.58	±	0.34	8.51	±	0.52	10.73	±	0.40	3.65	±	0.41	5.53	±	0.11	7.81	±	0.35	9.69	±	0.44
	DATEM	5.28	±	0.62	7.59	±	1.51	10.14	±	1.04	12.68	±	1.18	4.28	±	0.25	6.58	±	0.94	9.39	±	0.80	11.51	±	1.30
	A	4.33	±	0.32	6.39	±	0.61	7.81	±	0.15	10.89	±	1.06	3.26	±	0.30	5.64	±	0.56	7.17	±	0.13	9.82	±	1.03
	E	4.73	±	0.47	6.46	±	0.70	8.66	±	0.86	11.85	±	2.00	3.83	±	0.37	6.13	±	0.84	7.70	±	0.61	10.57	±	1.65
	G	4.49	±	0.58	5.77	±	1.01	8.12	±	1.80	10.32	±	1.50	3.48	±	0.30	5.19	±	0.98	7.48	±	1.70	9.54	±	1.68
	J	4.12	±	0.42	5.51	±	0.32	7.44	±	1.35	9.91	±	1.76	3.05	±	0.33	4.93	±	0.56	6.94	±	1.30	9.05	±	1.71
	K	3.84	±	0.27	6.45	±	0.80	8.14	±	1.54	10.42	±	2.23	3.28	±	0.09	5.95	±	1.03	7.22	±	1.50	9.50	±	2.00
	M	4.18	±	0.64	6.70	±	1.17	8.17	±	1.54	10.66	±	2.65	3.25	±	0.44	6.13	±	1.00	7.70	±	1.55	10.16	±	2.43
	O	4.06	±	0.43	7.05	±	0.27	8.78	±	0.41	11.07	±	1.05	3.05	±	0.32	6.18	±	0.43	8.11	±	0.42	9.96	±	0.70

Table 29: Resilience of differently modified basic cake, pound cake and brioche samples over time. Control – untreated sample, DATEM – addition of DATEM, A-O – addition of respective lipase. Values are given as mean (n = 6) ± standard deviation. Data already published in Stemler & Scherf [97].

		Resilience [1]											
		0 h			24 h			48 h			96 h		
Basic cake	Control	0.21	±	0.01	0.14	±	0.00	0.12	±	0.01	0.11	±	0.01
	DATEM	0.20	±	0.02	0.15	±	0.02	0.15	±	0.01	0.13	±	0.01
	A	0.08	±	0.00	0.06	±	0.01	0.06	±	0.01	0.06	±	0.01
	E	0.15	±	0.01	0.12	±	0.00	0.10	±	0.00	0.10	±	0.01
	G	0.07	±	0.00	0.07	±	0.01	0.07	±	0.01	0.07	±	0.01
	J	0.07	±	0.01	0.07	±	0.01	0.06	±	0.00	0.07	±	0.01
	K	0.20	±	0.02	0.13	±	0.00	0.12	±	0.02	0.10	±	0.01
	M	0.19	±	0.01	0.13	±	0.01	0.11	±	0.01	0.09	±	0.01
	O	0.24	±	0.01	0.16	±	0.03	0.11	±	0.01	0.09	±	0.01
Pound cake	Control	0.29	±	0.05	0.22	±	0.04	0.19	±	0.02	0.17	±	0.01
	DATEM	0.26	±	0.05	0.20	±	0.03	0.20	±	0.02	0.17	±	0.02
	A	0.18	±	0.00	0.16	±	0.00	0.15	±	0.01	0.15	±	0.01
	E	0.19	±	0.02	0.16	±	0.01	0.14	±	0.01	0.14	±	0.01
	G	0.16	±	0.01	0.16	±	0.00	0.14	±	0.01	0.15	±	0.01
	J	0.16	±	0.01	0.15	±	0.01	0.14	±	0.01	0.14	±	0.01
	K	0.25	±	0.00	0.19	±	0.02	0.16	±	0.01	0.15	±	0.00

		Resilience [1]											
		0 h			24 h			48 h			96 h		
	M	0.23	±	0.01	0.18	±	0.02	0.16	±	0.01	0.15	±	0.01
	O	0.25	±	0.01	0.18	±	0.01	0.15	±	0.01	0.15	±	0.01
Brioche	Control	0.25	±	0.02	0.14	±	0.01	0.12	±	0.01	0.12	±	0.01
	DATEM	0.21	±	0.02	0.13	±	0.01	0.14	±	0.01	0.12	±	0.00
	A	0.22	±	0.02	0.13	±	0.01	0.12	±	0.00	0.11	±	0.01
	E	0.20	±	0.01	0.13	±	0.01	0.13	±	0.00	0.12	±	0.01
	G	0.19	±	0.01	0.13	±	0.01	0.12	±	0.01	0.12	±	0.01
	J	0.21	±	0.01	0.12	±	0.01	0.12	±	0.01	0.11	±	0.01
	K	0.25	±	0.02	0.12	±	0.00	0.12	±	0.01	0.11	±	0.00
	M	0.24	±	0.01	0.12	±	0.01	0.12	±	0.01	0.11	±	0.01
	O	0.23	±	0.03	0.13	±	0.01	0.12	±	0.00	0.12	±	0.00

A.3. Lipidomic profile of fine bakery goods after treatment with lipases

Table 30: Substrate specificity ratios for the lipases A, E, G, J, K, M and O towards different lipids in basic cake batter (n = 3). Data already prepared for publication ([162]).

	A	E	G	J	K	M	O
DG(18:1/12:0)	8.967	9.681	12.706	7.898	0.855	5.901	25.071
DG(18:1/14:0)	4.231	4.529	6.842	3.406	0.800	2.617	11.807
DG(16:0/16:0)	1.054	1.481	2.611	1.009	0.839	0.872	12.472
DG(16:1/18:1)	2.993	3.477	4.762	2.682	0.817	2.091	7.853
DG(16:0/18:1)	1.983	2.633	3.793	1.777	0.683	1.550	7.465
DG(18:0/16:0)	1.117	1.747	2.928	1.132	0.896	0.835	14.685
DG(18:1/18:2)	6.773	6.205	8.841	6.077	1.412	3.844	16.777
DG(18:1/18:1)	4.185	5.968	5.277	3.551	0.756	3.411	12.973
DGDG(16:0/18:2)	0.098	0.272	0.112	0.097	0.559	0.308	1.506
DGDG(18:2/18:2)	0.082	0.189	0.102	0.091	0.530	0.212	1.788
DGMG(18:2)	4.528	6.434	5.170	5.694	3.447	4.712	2.445
LPC(16:0)	1.148	1.830	1.835	1.062	0.993	1.279	2.017
LPC(18:2)	1.167	1.834	1.909	1.111	0.988	1.314	1.897
MGMG(18:2)	1.570	1.653	1.800	2.055	2.058	1.136	2.190
PE(18:2/18:2)	1.061	0.791	1.153	1.375	1.245	0.787	1.875
So(d12:0+pO)	0.608	1.314	3.091	1.598	1.210	0.468	1.321
So(d14:0+pO)	0.657	1.264	2.462	1.121	0.988	0.492	1.147
So(d16:1)	0.813	1.509	2.360	1.163	1.006	1.274	2.335
So(d16:0)	0.802	2.079	3.025	1.045	1.097	3.033	7.255
So(d16:0+pO)	0.642	1.463	2.121	0.846	0.824	0.436	1.176
TG(4:0/8:0/10:0)	10.806	23.417	15.851	10.742	1.038	11.512	31.881
TG(6:0/8:0/10:1)	6.417	12.166	8.759	5.983	0.836	6.852	16.798
TG(4:0/10:0/10:0)	2.112	5.006	3.695	1.878	0.862	2.407	7.005
TG(4:0/8:0/14:1)	3.277	6.594	4.478	2.896	0.966	3.361	9.325
TG(4:0/10:0/12:0)	0.690	1.501	1.127	0.657	0.907	0.957	1.952
TG(4:0/10:0/14:1)	0.967	2.208	1.430	0.915	0.947	1.331	3.348
TG(4:0/10:0/14:0)	0.501	0.847	0.581	0.535	0.934	0.714	0.885

	A	E	G	J	K	M	O
TG(4:0/8:0/18:1)	0.745	1.277	0.818	0.682	0.920	0.931	1.592
TG(4:0/12:0/14:0)	0.545	0.749	0.490	0.584	0.961	0.727	0.666
TG(4:0/10:0/18:2)	1.136	1.704	1.162	1.129	0.960	1.306	2.007
TG(4:0/10:0/18:1)	0.756	1.164	0.776	0.758	0.931	0.925	1.339
TG(4:0/14:0/14:0)	0.596	0.657	0.458	0.672	0.967	0.751	0.515
TG(6:0/10:0/18:2)	1.001	1.337	1.009	0.993	0.945	1.076	1.503
TG(4:0/12:0/18:1)	0.655	0.832	0.589	0.706	0.968	0.795	0.813
TG(4:0/14:0/16:0)	0.762	0.601	0.466	0.741	0.951	0.828	0.403
TG(4:0/14:0/17:1)	0.639	0.712	0.525	0.666	0.985	0.778	0.602
TG(4:0/14:0/18:2)	0.838	0.966	0.730	0.806	0.929	0.875	0.880
TG(4:0/14:0/18:1)	0.629	0.676	0.461	0.664	0.955	0.718	0.531
TG(4:0/16:0/16:0)	0.881	0.696	0.574	0.932	1.022	0.973	0.489
TG(4:0/15:0/18:1)	0.628	0.629	0.455	0.704	1.007	0.779	0.441
TG(4:0/16:0/17:1)	0.613	0.551	0.411	0.699	0.992	0.724	0.422
TG(4:0/16:0/17:0)	0.864	0.698	0.580	0.826	0.985	0.880	0.464
TG(4:0/16:0/18:2)	0.735	0.779	0.579	0.766	1.001	0.827	0.622
TG(4:0/16:0/18:1)	0.625	0.593	0.430	0.632	0.977	0.700	0.386
TG(6:0/16:0/16:0)	1.034	0.766	0.678	0.974	0.996	0.945	0.540
TG(4:0/17:1/18:1)	0.744	0.789	0.556	0.707	0.945	0.785	0.662
TG(4:0/17:0/18:1)	0.714	0.642	0.515	0.800	1.008	0.784	0.441
TG(6:0/16:0/17:1)	0.739	0.560	0.489	0.776	0.974	0.798	0.334
TG(6:0/16:0/17:0)	1.005	0.788	0.725	0.973	1.043	0.975	0.575
TG(4:0/18:1/18:1)	0.755	0.732	0.554	0.777	0.930	0.774	0.646
TG(6:0/16:0/18:1)	0.778	0.644	0.546	0.826	1.035	0.850	0.430
TG(4:0/16:0/20:0)	1.360	1.300	1.107	1.694	1.629	1.739	1.084
TG(16:0/10:0/14:0)	1.020	0.839	0.812	1.026	0.987	0.972	0.696
TG(6:0/17:1/18:1)	0.825	0.831	0.689	0.875	1.026	0.934	0.816
TG(6:0/18:1/18:1)	0.832	0.806	0.734	0.890	0.984	0.835	0.867
TG(16:0/8:0/18:1)	0.894	0.726	0.757	0.968	1.051	0.891	0.648
TG(16:0/10:0/16:0)	1.144	1.072	1.096	1.071	0.986	1.083	1.066
TG(8:0/17:1/18:1)	0.886	0.864	0.856	0.913	0.979	0.874	1.029

	A	E	G	J	K	M	O
TG(15:0/10:0/18:1)	1.002	0.850	0.889	0.989	0.991	0.902	0.859
TG(15:0/12:0/16:0)	1.110	1.127	1.186	1.062	1.010	1.066	1.242
TG(8:0/18:1/18:1)	0.939	0.849	0.882	0.916	0.969	0.866	1.008
TG(16:0/10:0/18:1)	0.946	0.830	0.859	0.954	0.983	0.917	0.764
TG(16:0/12:0/16:0)	1.202	1.345	1.298	1.086	0.996	1.049	1.418
TG(10:0/17:1/18:1)	0.972	1.003	0.993	0.978	0.986	0.932	1.254
TG(10:0/18:1/18:1)	0.973	0.971	1.032	0.988	1.018	0.936	1.184
TG(16:0/12:0/18:1)	1.078	1.026	1.140	1.074	1.074	1.122	1.043
TG(16:0/14:0/16:0)	1.210	1.626	1.752	1.193	1.099	1.261	1.865
TG(18:1/12:0/18:2)	1.062	1.134	1.208	1.066	1.056	1.039	1.411
TG(16:0/14:1/18:1)	1.061	0.984	1.043	1.003	0.989	0.983	1.171
TG(16:0/14:0/18:1)	1.120	1.055	1.142	1.088	1.020	1.021	1.075
TG(16:0/16:0/16:0)	1.192	1.598	1.676	1.185	1.081	1.136	1.765
TG(16:0/16:0/18:1)	1.161	1.208	1.314	1.135	0.993	1.125	1.300
TG(18:0/16:0/16:0)	1.243	1.557	1.732	1.234	1.066	1.347	1.868
TG(18:1/14:0/18:2)	1.073	1.144	1.165	1.075	1.036	0.998	1.326
TG(18:1/14:0/18:1)	1.145	1.096	1.101	1.023	0.974	1.009	1.093
TG(16:0/17:0/18:1)	1.152	1.197	1.300	1.146	1.027	1.138	1.293
TG(16:0/18:1/18:2)	1.010	1.033	1.096	1.048	1.005	1.020	1.122
TG(16:0/18:1/18:1)	1.014	0.965	0.994	1.052	0.960	1.041	0.925
TG(18:0/16:0/18:1)	1.063	1.089	1.159	1.064	0.892	1.089	1.117
TG(18:0/16:0/18:0)	1.082	1.402	1.529	1.130	0.973	1.093	1.530
TG(16:0/18:1/18:3)	0.970	1.116	1.288	1.294	0.913	0.976	1.277
TG(17:0/18:1/18:1)	0.974	0.887	0.961	1.078	0.973	1.056	0.902
TG(18:0/17:0/18:1)	1.096	1.082	1.152	1.094	0.988	1.168	1.077
TG(18:1/18:1/18:2)	0.964	1.035	1.106	1.059	0.957	1.034	1.370
TG(18:1/18:1/18:1)	1.054	0.954	0.990	1.085	1.005	1.065	1.169
TG(18:0/18:1/18:1)	1.058	0.912	0.916	1.168	1.054	1.070	0.784
TG(18:0/18:0/18:1)	1.131	1.144	1.134	1.255	1.078	1.237	1.036
TG(18:0/18:0/18:0)	1.114	1.419	1.431	1.107	0.970	1.199	1.500
TG(18:2/18:2/18:2)	0.804	0.786	0.932	0.786	0.835	0.753	0.770

	A	E	G	J	K	M	O
TG(19:1/18:1/18:1)	1.005	1.000	0.983	1.054	0.949	0.978	1.114
TG(19:0/18:1/18:1)	1.025	0.977	0.945	0.984	0.901	0.937	0.833
TG(16:0/16:0/23:0)	1.119	1.465	1.621	1.063	0.966	1.130	1.786
TG(20:1/18:1/18:1)	1.070	1.125	1.105	1.082	1.036	1.019	1.195
TG(20:0/18:1/18:1)	1.024	0.971	1.000	1.011	1.032	0.950	0.917
TG(16:0/18:1/22:0)	1.150	1.208	1.250	1.088	0.991	1.121	1.276
TG(16:0/16:0/24:0)	1.164	1.456	1.635	1.106	0.970	1.174	1.730
TG(16:0/18:1/23:1)	1.144	1.062	1.208	1.123	1.019	1.067	1.060
TG(18:0/16:0/23:1)	1.185	1.351	1.564	1.141	1.034	1.154	1.484
TG(18:1/18:1/22:0)	1.064	1.091	1.237	1.088	0.995	1.062	1.007
TG(16:0/18:1/24:0)	1.097	1.191	1.375	1.107	0.960	1.090	1.259
TG(26:0/16:0/16:0)	1.221	1.620	1.853	1.157	1.018	1.220	1.839
TG(25:1/16:0/18:1)	1.097	1.141	1.307	1.127	1.035	1.108	1.132
TG(18:1/18:1/24:0)	1.085	1.170	1.354	1.082	1.007	1.087	1.128
TG(26:0/16:0/18:1)	1.106	1.294	1.410	1.141	1.005	1.124	1.347
TG(26:0/16:0/18:1)	1.153	1.361	1.479	1.177	1.018	1.136	1.426

Table 31: Substrate specificity ratios for the lipases A, E, G, J, K, M and O towards different lipids in baked basic cake (n = 3). Data already prepared for publication ([162]).

	A	E	G	J	K	M	O
DGDG(16:0/18:2)	0.203	0.783	0.185	0.146	0.808	0.649	1.023
DGDG(18:2/18:3)	0.198	0.775	0.146	0.113	0.850	0.646	1.051
DGDG(18:2/18:2)	0.168	0.779	0.148	0.123	0.814	0.628	1.031
DGDG(18:1/18:2)	0.253	0.787	0.209	0.161	0.787	0.627	1.013
DGMG(18:3)	35.605	4.232	33.368	27.559	2.968	5.805	1.603
DGMG(18:2)	18.318	2.563	16.363	13.449	1.653	2.793	1.374
LPC(16:0)	4.375	1.116	4.371	2.681	0.963	1.001	1.205
LPC(18:3)	2.912	1.157	2.865	2.079	0.917	0.943	1.139
LPC(18:2)	3.149	1.115	3.097	2.187	0.903	0.923	1.122
LPE(16:0)	6.478	1.172	6.319	3.804	1.266	1.269	1.352
LPG(16:0)	3.800	1.207	3.185	2.497	0.996	1.155	1.162

	A	E	G	J	K	M	O
LdMePE(18:2)	3.734	1.097	3.956	2.691	1.120	1.064	1.252
MG(24:2)	0.964	1.030	1.029	1.122	1.152	1.195	1.094
MGDG(16:0/18:2)	0.560	0.952	0.594	0.459	0.987	0.882	1.092
MGDG(18:2/18:2)	0.096	0.406	0.090	0.054	0.656	0.333	1.012
MGDG(18:1/18:2)	0.234	0.502	0.269	0.179	0.653	0.444	0.961
MGMG(18:2)	10.474	2.509	10.320	8.173	1.911	2.646	1.357
PC(22:0/12:2)	0.359	0.646	0.262	0.185	0.691	0.487	0.961
PE(18:2/18:2)	2.895	2.065	2.971	2.600	1.453	1.788	1.100
PE(18:1/18:2)	3.001	2.034	3.063	2.732	1.464	1.804	1.112
So(d12:0+pO)	2.079	3.101	1.021	1.361	0.978	0.944	2.342
So(d14:0+pO)	1.977	2.054	0.915	1.221	1.120	0.645	1.723
So(d16:1)	1.985	1.562	1.124	1.203	1.029	0.643	1.222
So(d16:0)	2.091	1.349	0.714	1.145	1.012	0.424	1.079
So(d16:0+pO)	1.479	1.099	0.569	0.709	0.976	0.500	1.217
TG(4:0/8:0/10:0)	4.103	0.888	3.864	2.056	0.904	0.923	1.069
TG(4:0/8:0/12:0)	1.243	0.921	1.174	0.822	0.890	0.933	0.821
TG(4:0/10:1/12:0)	1.028	0.931	0.963	0.802	0.899	0.920	0.913
TG(4:0/10:0/12:0)	0.724	0.999	0.673	0.667	0.915	0.940	0.860
TG(4:0/8:0/15:0)	0.650	1.018	0.638	0.668	0.905	0.945	0.855
TG(4:0/8:0/16:1)	0.728	1.009	0.723	0.751	0.960	0.994	0.886
TG(4:0/10:0/14:0)	0.680	1.022	0.667	0.701	0.957	0.982	0.889
TG(4:0/10:0/15:0)	0.679	0.981	0.664	0.733	0.932	0.958	0.873
TG(4:0/8:0/18:1)	0.691	0.976	0.696	0.756	0.955	0.987	0.875
TG(4:0/10:0/16:0)	0.687	0.951	0.688	0.746	0.985	0.987	0.886
TG(4:0/10:0/17:1)	0.664	0.971	0.763	0.778	1.020	1.003	0.860
TG(4:0/12:0/15:0)	0.682	0.946	0.692	0.743	0.984	0.994	0.908
TG(4:0/10:0/18:1)	0.788	1.008	0.772	0.823	1.026	1.022	0.923
TG(4:0/12:0/16:0)	0.732	0.987	0.728	0.798	1.042	1.029	0.947
TG(4:0/12:0/17:1)	0.725	0.987	0.745	0.822	1.089	1.032	0.970
TG(4:0/14:0/15:0)	0.711	0.978	0.715	0.784	1.005	1.009	0.932
TG(4:0/12:0/18:2)	0.961	1.020	0.998	0.998	1.095	1.060	0.959

	A	E	G	J	K	M	O
TG(6:0/10:0/18:1)	0.743	0.944	0.755	0.817	0.985	1.017	0.918
TG(4:0/14:0/17:1)	0.747	0.994	0.787	0.876	1.053	1.012	0.934
TG(4:0/15:0/16:0)	0.726	0.987	0.730	0.830	1.046	1.063	0.962
TG(4:0/14:0/18:2)	0.859	0.956	0.882	0.919	1.039	1.006	0.917
TG(4:0/14:0/18:1)	0.775	0.975	0.778	0.855	1.047	1.086	0.949
TG(4:0/16:0/16:0)	0.716	0.990	0.802	0.874	1.152	1.127	1.023
TG(4:0/15:0/18:2)	0.824	1.048	0.962	0.953	1.108	1.172	1.009
TG(4:0/15:0/18:1)	0.730	0.976	0.825	0.875	1.117	1.099	0.993
TG(4:0/16:0/17:1)	0.690	0.982	0.791	0.873	1.194	1.108	1.005
TG(6:0/15:0/16:0)	0.786	0.960	0.821	0.909	1.096	1.072	0.951
TG(4:0/16:1/18:1)	0.803	0.970	0.895	0.925	1.124	1.111	0.997
TG(4:0/16:0/18:1)	0.775	0.952	0.824	0.909	1.156	1.121	0.960
TG(4:0/14:0/20:0)	0.883	1.190	0.909	1.116	1.257	1.180	1.847
TG(6:0/16:0/16:0)	0.795	0.987	0.915	1.016	1.171	1.123	1.056
TG(4:0/17:1/18:1)	0.804	0.922	0.850	0.957	1.163	1.154	0.975
TG(4:0/17:0/18:1)	0.834	0.999	0.950	1.021	1.154	1.122	1.036
TG(6:0/16:0/17:1)	0.815	0.930	0.796	1.032	1.121	1.158	1.084
TG(6:0/16:0/17:0)	0.834	0.932	0.891	0.987	1.086	1.081	0.997
TG(4:0/18:1/18:2)	0.859	1.031	0.974	1.027	1.176	1.127	0.925
TG(4:0/18:1/18:1)	0.803	0.928	0.891	0.975	1.113	1.094	0.999
TG(6:0/16:0/18:1)	0.842	0.943	0.904	1.016	1.156	1.147	1.026
TG(4:0/16:0/20:0)	0.953	1.213	0.938	1.174	1.186	1.123	1.784
TG(16:0/10:0/14:0)	0.873	0.995	1.042	1.127	1.192	1.216	1.085
TG(6:0/17:1/18:1)	0.872	0.966	0.943	1.092	1.166	1.141	1.029
TG(6:0/17:0/18:1)	0.867	0.968	1.042	1.132	1.196	1.205	1.054
TG(16:0/8:0/17:1)	0.878	0.814	0.882	0.954	1.052	1.131	1.099
TG(15:0/10:0/16:0)	0.956	0.993	1.048	1.166	1.149	1.165	1.054
TG(6:0/18:1/18:1)	0.902	0.970	1.050	1.149	1.174	1.205	1.018
TG(4:0/18:1/20:0)	0.870	1.047	0.907	1.115	1.135	1.117	1.641
TG(16:0/8:0/18:1)	1.024	1.115	1.103	1.220	1.211	1.219	1.144
TG(6:0/16:0/20:0)	0.905	1.101	1.032	1.135	1.082	1.102	1.499

	A	E	G	J	K	M	O
TG(16:0/10:0/16:0)	0.976	0.958	1.060	1.065	1.082	1.105	1.052
TG(8:0/17:1/18:1)	1.023	1.014	1.064	1.165	1.206	1.173	1.125
TG(15:0/10:0/18:1)	0.958	0.994	1.081	1.096	1.100	1.107	1.035
TG(16:0/10:0/17:1)	0.961	0.876	1.018	1.037	1.051	1.087	1.285
TG(15:0/12:0/16:0)	1.011	1.030	1.090	1.119	1.066	1.060	1.040
TG(8:0/18:1/18:1)	0.962	1.011	1.060	1.118	1.109	1.102	1.018
TG(16:0/10:0/18:1)	0.989	0.991	1.064	1.074	1.063	1.038	1.024
TG(20:0/8:0/16:0)	0.884	0.988	0.983	1.007	1.020	1.008	1.505
TG(16:0/12:0/16:0)	1.087	1.043	1.155	1.094	1.006	1.049	1.038
TG(10:0/17:1/18:1)	1.055	1.105	1.138	1.131	1.104	1.087	1.051
TG(15:0/12:0/18:1)	1.067	1.039	1.158	1.139	1.069	1.102	1.073
TG(15:0/14:0/16:0)	1.108	0.992	1.101	1.130	1.011	1.063	1.047
TG(10:0/18:1/18:1)	1.054	1.001	1.126	1.163	1.077	1.119	1.045
TG(16:0/12:0/18:1)	1.072	0.953	1.038	1.054	1.010	1.018	1.028
TG(20:0/10:0/16:0)	1.033	1.238	1.108	1.254	1.016	1.093	1.530
TG(16:0/14:0/16:0)	1.142	1.071	1.133	1.085	0.963	0.965	1.029
TG(11:0/18:1/18:1)	1.075	1.034	1.063	1.085	1.000	0.983	1.035
TG(15:0/14:0/18:1)	1.043	1.014	1.060	1.046	0.961	0.946	0.988
TG(16:0/12:0/19:0)	1.141	1.220	1.124	1.379	1.122	1.170	1.455
TG(15:0/16:0/16:0)	1.141	1.018	1.120	1.055	0.933	0.942	1.000
TG(16:0/14:1/18:1)	1.099	1.039	1.112	1.072	1.007	0.989	1.005
TG(20:0/10:0/18:1)	1.199	1.163	1.097	1.168	1.079	1.092	1.530
TG(16:0/14:0/18:1)	1.089	0.970	1.079	1.038	0.924	0.940	0.992
TG(20:0/14:0/14:0)	1.124	1.235	1.062	1.129	0.974	0.922	1.066
TG(16:0/16:0/16:0)	1.164	0.958	1.080	0.936	0.867	0.897	0.927
TG(18:0/15:0/16:0)	1.164	0.968	1.066	0.989	0.797	0.858	0.928
TG(14:0/17:1/18:2)	1.102	1.004	1.037	1.043	0.961	0.926	0.969
TG(15:0/16:1/18:1)	1.080	0.960	1.047	0.971	0.903	0.903	0.914
TG(15:0/16:0/18:1)	1.086	0.960	1.022	0.967	0.848	0.875	0.884
TG(16:0/16:0/18:1)	1.086	1.019	1.066	0.980	0.843	0.865	0.941
TG(18:0/16:0/16:0)	1.173	1.041	1.113	1.009	0.836	0.870	0.935

	A	E	G	J	K	M	O
TG(18:1/14:0/18:2)	1.085	0.964	1.078	0.998	0.898	0.921	0.911
TG(18:1/14:0/18:1)	1.095	1.007	1.066	1.005	0.861	0.904	0.965
TG(16:0/17:1/18:1)	1.085	1.016	1.058	1.004	0.874	0.910	0.932
TG(16:0/17:0/18:1)	1.102	1.025	1.027	0.974	0.853	0.901	0.940
TG(15:0/18:1/18:2)	1.061	1.017	1.055	0.992	0.896	0.863	0.928
TG(16:0/18:1/18:2)	1.129	1.044	1.051	1.000	0.903	0.928	0.941
TG(16:0/18:1/18:1)	1.163	1.039	1.058	1.016	0.879	0.874	0.986
TG(18:0/16:0/18:1)	1.080	0.978	0.981	0.977	0.842	0.849	0.969
TG(18:0/16:0/18:0)	1.190	1.053	1.021	1.007	0.864	0.866	1.000
TG(16:1/18:1/18:2)	1.175	1.043	1.058	0.950	0.840	0.855	0.930
TG(18:1/17:1/18:1)	1.137	0.986	1.034	1.042	0.897	0.897	0.997
TG(17:0/18:1/18:1)	1.112	1.081	1.028	0.997	0.929	0.875	1.006
TG(18:0/17:0/18:1)	1.073	1.034	0.979	0.973	0.891	0.838	0.942
TG(18:1/18:1/18:2)	1.115	1.067	1.019	1.033	0.935	0.872	0.967
TG(18:1/18:1/18:1)	1.054	1.006	1.000	1.002	0.965	0.874	0.972
TG(18:0/18:1/18:1)	1.012	0.941	0.984	0.992	0.907	0.869	0.986
TG(18:0/18:0/18:1)	1.095	1.018	1.059	1.065	0.926	0.931	1.005
TG(18:0/18:0/18:0)	1.129	0.960	1.012	1.025	0.868	0.914	0.973
TG(18:2/18:2/18:2)	1.117	1.052	1.056	0.958	0.908	0.850	0.884
TG(19:1/18:1/18:1)	1.007	0.896	0.980	0.998	0.911	0.905	1.017
TG(19:1/18:0/18:1)	1.061	0.955	1.034	1.084	0.963	0.969	1.032
TG(16:0/16:0/23:1)	2.322	1.157	1.267	1.282	1.169	1.150	1.197
TG(18:0/16:0/21:0)	2.266	1.108	1.238	1.244	1.113	1.115	1.264
TG(20:1/18:1/18:1)	2.130	1.066	1.159	1.299	1.029	1.177	1.190
TG(20:0/18:1/18:1)	2.249	1.119	1.229	1.227	1.273	1.204	1.239
TG(16:0/18:1/22:0)	2.266	1.104	1.234	1.343	1.190	1.188	1.226
TG(18:0/16:0/22:0)	2.488	1.167	1.358	1.374	1.203	1.273	1.337
TG(18:1/18:1/21:1)	2.312	1.125	1.260	1.336	1.240	1.260	1.262
TG(16:0/18:1/23:1)	2.319	1.159	1.264	1.282	1.147	1.213	1.146
TG(16:0/18:1/23:0)	2.271	1.159	1.237	1.233	1.138	1.197	1.208
TG(18:1/18:1/22:1)	2.332	1.169	1.276	1.263	1.256	1.299	1.286

	A	E	G	J	K	M	O
TG(18:1/18:1/22:0)	2.179	1.110	1.187	1.242	1.158	1.230	1.115
TG(16:0/18:1/24:0)	2.272	1.149	1.241	1.224	1.201	1.265	1.146
TG(18:0/16:0/24:0)	2.225	1.161	1.213	1.222	1.116	1.211	1.121
TG(18:1/18:1/23:1)	2.218	1.144	1.209	1.301	1.133	1.196	1.109
TG(18:0/18:1/23:1)	2.146	1.110	1.169	1.191	1.176	1.222	1.104
TG(18:1/18:1/24:0)	2.265	1.124	1.236	1.309	1.262	1.269	1.217
TG(26:0/16:0/18:1)	2.172	1.096	1.186	1.105	1.265	1.216	1.184
TG(26:0/18:1/18:1)	2.259	1.117	1.237	1.137	1.181	1.116	1.122

Table 32: Substrate specificity ratios for the lipases A, E, G, J, K, M and O towards different lipids in pound cake batter (n = 3). Data already prepared for publication ([162]).

	A	E	G	J	K	M	O
BisMePA(34:3)	0.145	0.399	0.171	0.188	0.574	0.380	1.015
BisMePA(34:2)	0.188	0.500	0.193	0.265	0.652	0.526	0.986
BisMePA(36:4)	0.239	0.546	0.251	0.291	0.622	0.528	1.009
BisMePA(36:3)	0.114	0.342	0.136	0.163	0.601	0.393	0.907
BisMePA(36:2)	0.267	0.559	0.370	0.376	1.008	0.724	1.287
BisMePA(38:7)	0.119	0.363	0.122	0.167	0.605	0.416	0.977
BisMePA(40:7)	0.095	0.347	0.135	0.166	0.624	0.419	0.920
DG(O-42:6)	1.523	0.957	0.806	0.841	0.732	0.855	1.116
DG(18:1/18:3)	0.532	0.936	0.635	0.636	0.735	0.639	1.318
DG(18:2/18:2)	2.952	2.232	1.785	1.579	0.791	1.006	2.925
DG(18:1/18:2)	3.989	3.801	3.184	3.008	1.083	1.396	4.216
DG(16:0/24:6)	0.122	0.114	0.107	0.058	0.050	0.061	0.142
DG(24:6/18:0)	1.852	1.494	1.516	1.319	1.361	1.647	2.174
DGDG(16:0/18:2)	0.262	0.580	0.289	0.282	0.692	0.560	1.050
DGDG(18:2/18:2)	0.160	0.338	0.222	0.180	0.553	0.413	0.968
DGMG(18:2)	5.469	5.795	5.174	5.946	2.827	4.208	1.197
LPC(14:0)	5.119	5.201	4.182	2.827	1.760	2.950	0.865
LPC(16:1)	12.538	9.450	10.520	9.132	2.508	4.872	0.822
LPC(16:0)	0.975	1.058	0.840	0.781	0.788	0.829	0.880

	A	E	G	J	K	M	O
LPC(17:1)	15.518	13.610	12.715	11.523	3.185	6.246	0.889
LPC(18:3)	1.783	1.639	1.186	0.900	0.814	0.989	0.607
LPC(18:2)	14.288	11.306	11.963	10.321	3.101	5.723	0.834
LPC(18:1)	47.379	25.581	38.768	33.997	7.193	15.804	0.818
LPC(18:0)	0.821	0.921	0.719	0.630	0.698	0.710	0.894
LPC(19:1)	13.521	10.166	12.262	10.038	2.970	4.596	0.913
LPC(20:4)	66.947	41.163	50.408	40.098	10.233	22.297	0.697
LPC(20:1)	4.466	2.950	4.308	5.523	1.596	2.814	0.872
LPC(22:6)	96.879	74.057	77.315	68.462	17.113	35.943	0.887
LPC(22:5)	82.118	67.842	64.615	58.363	13.556	30.510	0.814
LPE(16:0)	0.749	0.967	0.845	0.720	0.834	0.730	0.989
LPE(18:2)	13.105	11.244	12.487	11.503	3.638	6.652	0.955
LPE(18:1)	20.102	16.988	19.935	17.802	5.195	10.068	1.045
LPE(18:0)	0.824	0.950	0.811	0.822	0.893	0.788	1.069
LPE(20:4)	62.068	52.760	55.051	49.201	13.635	26.968	1.117
LPE(22:6)	44.001	36.309	39.271	35.615	9.989	20.418	0.808
LPE(22:5)	42.803	35.500	32.926	32.030	10.001	19.280	0.878
MG(18:2)	1.660	1.407	1.186	1.373	0.890	1.018	0.768
MG(O-17:3)	8.149	4.054	3.560	2.788	4.098	14.918	5.109
MGDG(18:2/18:2)	0.066	0.056	0.084	0.060	0.277	0.118	0.348
MGMG(18:2)	2.908	2.361	2.901	3.219	2.312	2.129	1.768
PA(22:5/18:1)	0.109	0.667	0.243	0.295	0.749	0.596	1.187
PC(P-39:10)	0.127	0.346	0.106	0.124	0.529	0.361	0.782
PC(O-34:2)	0.993	1.690	1.177	1.337	1.183	1.087	0.517
PC(O-39:10)	0.199	0.371	0.184	0.224	0.692	0.495	0.812
PC(30:2)	27.832	45.159	22.076	15.673	5.074	14.153	1.041
PC(30:1)	14.759	14.616	11.755	9.324	0.000	5.983	0.187
PC(30:0)	0.403	0.980	0.730	0.801	0.643	0.395	0.977
PC(32:3)	0.969	2.071	0.897	0.941	0.637	0.791	0.770
PC(32:2)	1.001	1.407	0.991	0.825	0.762	0.896	0.864
PC(32:1)	0.319	0.597	0.292	0.288	0.618	0.471	0.881

	A	E	G	J	K	M	O
PC(32:0)	0.617	0.975	0.635	0.661	0.710	0.753	0.992
PC(33:2)	0.503	1.289	0.282	0.273	0.522	1.522	3.009
PC(33:1)	0.348	0.625	0.315	0.250	0.453	0.388	0.861
PC(34:4)	0.378	0.795	0.305	0.316	0.552	0.447	0.768
PC(34:3)	0.249	0.432	0.165	0.242	0.586	0.513	0.810
PC(16:0/18:2)	0.133	0.249	0.091	0.116	0.381	0.320	0.996
PC(16:0/18:1)	0.096	0.307	0.142	0.178	0.630	0.341	0.849
PC(34:0)	0.667	0.910	0.603	0.617	0.657	0.597	0.653
PC(35:2)	0.208	0.521	0.258	0.240	0.605	0.497	0.885
PC(35:1)	0.303	0.594	0.264	0.334	0.667	0.522	0.880
PC(36:6)	0.324	0.817	0.313	0.301	0.721	0.512	1.015
PC(36:5)	0.133	0.343	0.129	0.157	0.536	0.457	0.766
PC(16:0/20:4)	0.108	0.188	0.080	0.084	0.494	0.290	0.680
PC(18:1/18:2)	0.262	0.691	0.267	0.307	0.603	0.535	0.873
PC(18:0/18:2)	0.168	0.478	0.236	0.244	0.583	0.495	0.821
PC(18:0/18:1)	0.282	0.589	0.266	0.314	0.702	0.545	0.922
PC(38:7)	0.135	0.316	0.120	0.154	0.509	0.261	0.490
PC(16:0/22:6)	0.144	0.472	0.201	0.221	0.760	0.445	1.342
PC(21:3/17:2)	0.097	0.375	0.171	0.199	0.334	0.334	0.874
PC(18:0/20:4)	0.138	0.281	0.198	0.215	0.487	0.487	0.715
PC(40:9)	0.137	0.393	0.132	0.152	0.628	0.400	0.798
PC(40:8)	0.225	0.628	0.207	0.272	0.467	0.362	0.616
PC(40:7)	0.260	0.641	0.249	0.300	0.595	0.484	0.801
PC(40:6)	0.179	0.511	0.191	0.236	0.473	0.494	0.840
PC(40:5)	0.156	0.435	0.183	0.200	0.624	0.445	0.915
PE(16:0/18:2)	0.141	0.388	0.178	0.184	0.665	0.450	0.984
PE(16:0/18:1)	0.137	0.413	0.217	0.268	0.738	0.565	1.003
PE(18:2/18:2)	1.513	1.781	1.823	1.505	1.128	1.474	0.882
PE(18:2/18:2)	1.452	1.288	1.414	1.479	0.921	1.127	1.003
PE(20:4/16:0)	0.173	0.442	0.203	0.210	0.552	0.440	0.983
PE(18:0/18:2)	0.119	0.416	0.163	0.224	0.695	0.558	1.212

	A	E	G	J	K	M	O
PE(18:0/18:1)	0.112	0.439	0.217	0.299	0.751	0.571	1.100
PE(16:0/22:6)	0.125	0.396	0.154	0.189	0.641	0.471	0.998
PE(20:4/18:1)	0.160	0.577	0.209	0.255	0.641	0.510	1.015
PE(18:0/20:4)	0.099	0.280	0.140	0.175	0.565	0.370	0.900
PEt(10:0/12:1)	0.438	2.035	0.710	0.724	0.758	0.858	3.023
PEt(10:0/18:2)	1.010	1.330	0.870	0.967	0.838	0.771	1.412
PEt(10:0/18:1)	0.894	0.867	0.781	0.850	0.758	0.656	0.937
PEt(10:0/22:1)	0.871	0.905	0.804	0.910	0.866	0.864	0.889
PEt(10:0/24:1)	1.084	0.949	0.925	0.997	0.942	0.939	1.027
SM(d32:1)	0.989	1.000	1.052	1.003	0.924	0.969	1.029
SM(d34:1)	0.687	1.115	0.500	0.607	0.542	0.541	0.939
SM(d34:0)	0.722	0.892	0.691	0.710	0.667	0.644	0.750
SM(d36:4)	0.773	0.729	0.000	0.468	0.374	0.000	0.502
SM(d36:1)	0.860	1.028	0.837	0.842	0.742	0.723	0.861
SPH(d14:0)	2.033	0.871	0.741	0.762	0.886	1.035	1.173
SPH(d18:0)	1.616	0.682	0.375	0.428	0.519	0.640	0.540
SPH(d14:1)	2.047	0.449	0.343	0.361	0.287	0.627	1.114
SPH(d16:1)	1.729	0.402	0.307	0.330	0.453	0.540	0.757
TG(4:0/6:0/8:0)	53.850	204.297	27.629	24.169	9.983	71.654	483.859
TG(4:0/6:0/10:0)	4.975	21.599	2.828	2.258	1.318	6.849	48.263
TG(4:0/4:0/14:1)	1.639	2.521	0.970	1.256	1.040	1.240	3.609
TG(4:0/6:0/12:0)	1.434	5.143	1.011	0.997	0.811	1.811	11.078
TG(4:0/6:0/14:1)	1.277	5.842	1.170	1.100	1.014	1.330	9.458
TG(4:0/6:0/14:0)	0.679	1.542	0.669	0.576	0.491	0.482	2.904
TG(4:0/4:0/18:2)	1.241	3.951	0.846	0.802	0.582	1.305	6.420
TG(4:0/4:0/18:1)	1.081	2.311	0.913	0.624	0.614	0.872	3.906
TG(4:0/6:0/16:0)	0.549	0.671	0.460	0.488	0.538	0.244	1.003
TG(4:0/6:0/17:1)	0.727	1.400	0.642	0.667	0.785	0.681	2.213
TG(4:0/6:0/18:2)	0.752	2.166	0.657	0.864	0.823	0.976	2.670
TG(4:0/6:0/18:1)	0.795	1.096	0.718	0.739	0.826	0.406	1.110
TG(4:0/8:0/16:0)	0.798	0.634	0.422	0.789	0.531	0.622	0.790

	A	E	G	J	K	M	O
TG(4:0/8:0/17:1)	0.670	0.970	0.696	0.671	0.618	0.538	1.006
TG(4:0/8:0/18:2)	1.037	1.716	0.560	0.720	0.982	0.771	1.613
TG(4:0/8:0/18:1)	1.027	0.949	1.001	1.035	0.897	0.860	0.985
TG(4:0/10:0/16:0)	0.715	0.963	0.765	1.322	0.839	0.897	1.038
TG(4:0/10:0/18:2)	1.042	1.556	1.282	0.998	1.195	0.990	1.631
TG(4:0/10:0/18:1)	0.960	0.694	0.880	1.144	1.035	0.743	1.451
TG(4:0/14:0/14:1)	0.724	0.807	0.763	0.940	0.938	0.864	1.615
TG(4:0/12:0/16:0)	0.783	0.214	0.755	0.864	0.916	0.833	0.737
TG(4:0/12:0/16:0)	0.656	0.136	0.618	0.968	0.879	0.269	0.662
TG(4:0/11:0/18:1)	1.089	1.174	0.969	0.393	0.837	0.455	0.878
TG(4:0/15:0/14:1)	0.714	0.787	0.803	0.919	0.878	0.825	0.628
TG(8:0/11:0/14:0)	0.815	0.741	0.738	0.851	0.717	0.659	0.787
TG(4:0/12:0/18:2)	0.908	1.178	0.776	0.767	0.842	0.747	0.998
TG(4:0/12:0/18:1)	0.810	0.887	0.881	0.957	0.888	0.775	0.969
TG(4:0/14:0/16:0)	1.039	0.997	1.027	1.065	1.036	0.925	0.970
TG(4:0/14:0/16:0)	1.494	1.415	1.438	1.631	1.558	1.405	2.096
TG(4:0/15:0/16:1)	0.890	0.832	0.289	0.972	0.273	0.654	0.696
TG(6:0/14:0/15:1)	0.954	1.101	0.849	1.027	0.960	0.866	1.124
TG(4:0/14:0/18:3)	0.924	0.909	0.633	0.428	0.570	0.464	0.789
TG(8:0/10:0/18:2)	0.688	1.669	0.478	0.463	0.877	0.905	1.370
TG(4:0/14:0/18:1)	0.858	0.804	0.083	0.945	0.058	0.060	0.582
TG(4:0/14:0/18:1)	0.875	0.778	0.927	0.984	0.936	0.800	0.812
TG(4:0/12:0/20:0)	1.237	1.740	3.396	2.082	3.415	2.870	2.138
TG(6:0/14:0/16:0)	1.015	1.004	1.004	0.983	0.838	0.992	0.923
TG(4:0/16:0/16:0)	1.533	1.522	1.659	1.841	1.555	2.297	2.595
TG(4:0/16:0/16:0)	1.574	1.439	1.666	1.759	209.349	205.124	2.407
TG(6:0/14:0/16:0)	1.574	1.439	1.666	1.759	1.539	2.154	2.407
TG(6:0/14:0/16:0)	1.610	1.575	1.667	1.783	1.908	2.231	2.443
TG(4:0/16:0/17:1)	0.802	0.838	0.861	1.271	1.340	0.706	1.854
TG(4:0/16:0/17:0)	0.951	0.990	0.978	0.989	0.908	0.901	0.476
TG(4:0/16:0/18:2)	0.913	0.721	0.910	0.767	0.888	0.785	0.918

	A	E	G	J	K	M	O
TG(4:0/16:0/18:1)	0.811	0.836	0.769	0.877	0.735	0.668	0.678
TG(4:0/16:0/18:1)	1.409	1.286	1.441	1.602	1.437	2.030	2.000
TG(4:0/16:0/18:0)	1.189	1.529	2.318	1.613	2.372	3.383	1.739
TG(4:0/16:0/18:0)	1.324	1.189	1.019	1.145	0.894	1.123	0.869
TG(10:0/12:0/16:0)	0.865	1.108	1.307	1.051	0.809	1.452	0.940
TG(4:0/16:0/18:0)	1.604	1.671	1.576	1.893	1.555	2.045	2.249
TG(6:0/16:0/16:0)	1.604	1.671	1.576	1.893	1.555	2.045	2.249
TG(4:0/17:0/18:2)	2.175	2.118	2.102	2.194	1.572	1.753	2.402
TG(4:0/17:0/18:1)	1.088	0.932	0.930	1.068	1.001	0.947	1.047
TG(6:0/16:0/17:0)	1.028	0.807	1.005	0.970	0.958	0.821	0.759
TG(6:0/16:0/18:2)	0.643	0.819	0.645	0.890	0.668	0.730	0.870
TG(10:0/14:0/16:1)	0.886	1.147	1.432	1.019	3.200	2.129	4.389
TG(4:0/18:0/18:1)	1.556	0.840	0.861	0.988	0.848	0.761	0.903
TG(6:0/16:0/18:1)	0.859	0.883	1.068	0.452	0.415	0.904	0.621
TG(4:0/16:0/20:0)	1.143	1.568	2.178	1.461	3.142	3.508	1.755
TG(6:0/16:0/18:0)	1.138	1.181	1.062	1.114	1.206	1.472	0.907
TG(6:0/17:1/18:1)	1.319	1.186	1.308	1.168	1.386	1.202	1.151
TG(6:0/17:0/18:1)	0.942	1.045	1.167	1.049	1.044	1.295	0.995
TG(6:0/17:0/18:1)	0.886	1.329	1.462	1.186	1.384	1.533	1.319
TG(4:0/20:0/18:2)	2.159	1.050	1.774	1.242	4.050	2.255	2.329
TG(16:1/6:0/20:1)	1.234	1.167	1.594	1.337	1.469	1.279	1.139
TG(4:0/20:0/18:1)	0.902	0.855	1.731	1.039	6.714	1.598	0.859
TG(8:0/16:0/18:1)	1.356	1.565	1.708	1.735	1.116	1.445	1.449
TG(6:0/16:0/20:0)	1.127	1.331	2.077	1.440	2.171	10.735	5.297
TG(12:0/14:0/16:0)	1.194	1.203	1.292	1.297	1.315	1.289	1.238
TG(12:0/14:0/16:0)	1.165	1.307	1.374	1.284	1.233	1.158	1.183
TG(6:0/18:1/19:1)	0.991	1.008	1.024	1.008	1.061	1.163	1.214
TG(8:0/17:1/18:1)	0.842	0.928	0.963	1.046	0.984	0.940	1.080
TG(10:0/16:0/17:0)	0.984	0.359	1.133	1.156	1.069	1.126	1.073
TG(10:0/16:1/18:1)	0.953	0.774	0.981	0.871	1.074	1.071	1.039
TG(10:0/16:0/18:2)	0.955	0.774	0.976	0.869	1.068	1.055	1.036

	A	E	G	J	K	M	O
TG(10:0/16:0/18:1)	0.571	0.805	0.926	0.838	0.925	0.831	0.899
TG(10:0/16:0/18:1)	0.572	0.805	0.925	0.838	0.930	0.831	0.899
TG(14:0/14:0/16:0)	0.759	0.989	1.085	1.068	1.023	0.696	0.192
TG(10:0/16:0/18:0)	1.169	1.148	0.713	1.264	1.222	1.171	0.789
TG(10:0/17:1/18:1)	0.696	1.036	1.343	1.038	1.111	1.066	1.251
TG(14:0/14:0/18:3)	0.914	0.819	1.174	0.858	1.012	0.644	1.259
TG(14:0/14:1/18:1)	0.850	0.891	0.790	0.756	0.966	0.746	1.058
TG(12:0/16:0/18:1)	1.102	1.182	1.206	1.000	1.083	1.070	1.203
TG(12:0/16:0/18:1)	1.098	1.181	1.199	1.000	1.082	1.070	1.199
TG(14:0/16:0/16:0)	1.143	1.209	0.990	1.162	1.108	1.048	1.315
TG(14:0/16:0/17:0)	1.521	1.566	1.430	1.552	1.311	1.499	1.750
TG(12:0/18:1/18:2)	0.722	0.772	1.175	1.126	0.648	1.146	1.324
TG(16:0/14:1/18:1)	1.017	0.701	0.926	0.934	0.930	0.928	0.960
TG(14:0/16:0/18:1)	1.190	1.220	1.170	1.167	0.993	1.156	1.203
TG(14:0/16:0/18:1)	1.095	1.141	1.170	1.125	0.940	1.155	1.201
TG(14:0/16:0/18:0)	1.209	1.062	0.927	1.088	1.234	1.090	1.664
TG(14:1/18:1/18:2)	1.196	1.250	1.034	1.183	1.149	0.754	1.094
TG(14:0/18:1/18:2)	0.961	0.687	0.906	0.760	0.726	0.946	0.953
TG(16:0/16:1/18:1)	1.064	0.981	1.009	1.044	0.832	0.818	1.046
TG(16:0/16:0/18:1)	1.119	1.129	0.909	0.949	1.039	0.929	1.082
TG(16:0/16:0/18:1)	1.097	1.067	0.974	0.970	1.143	1.092	1.282
TG(16:0/16:0/18:0)	1.441	1.520	1.220	1.487	1.242	1.564	2.274
TG(16:0/17:1/18:1)	1.379	1.085	0.979	1.166	1.077	1.076	1.060
TG(16:0/17:0/18:1)	1.110	1.055	0.997	1.141	1.087	1.319	1.087
TG(16:0/18:2/18:3)	0.948	0.920	0.746	1.016	0.888	0.779	0.986
TG(16:1/18:1/18:2)	0.801	0.799	0.770	0.734	0.847	0.570	0.730
TG(16:0/18:1/18:2)	0.905	0.782	0.766	0.697	0.731	0.756	0.687
TG(16:0/18:1/18:1)	0.985	0.883	0.859	0.899	0.741	0.886	1.048
TG(16:0/18:1/18:1)	0.894	0.868	0.921	0.919	0.993	0.893	0.756
TG(16:0/18:1/18:1)	0.894	0.868	0.921	0.919	0.993	0.893	0.756
TG(16:0/18:0/18:1)	1.595	1.329	1.100	1.393	1.278	1.163	1.528

	A	E	G	J	K	M	O
TG(16:0/18:0/18:0)	0.950	1.028	0.750	0.974	0.853	0.703	1.114
TG(18:0/17:1/18:1)	1.073	0.923	1.051	0.993	0.748	1.087	0.961
TG(17:0/18:0/18:1)	1.263	1.121	0.980	1.022	0.881	0.929	1.003
TG(17:0/17:0/19:0)	1.684	0.945	0.979	1.104	1.259	1.238	1.666
TG(18:1/18:2/18:3)	0.863	0.880	0.830	0.982	0.793	0.887	0.859
TG(18:0/18:2/18:3)	0.957	0.889	0.746	0.807	0.818	0.583	1.063
TG(18:1/18:1/18:2)	0.873	0.919	0.523	0.807	0.829	0.751	0.985
TG(18:0/18:1/18:2)	0.946	0.942	0.931	0.889	0.893	0.928	0.983
TG(18:0/18:1/18:1)	0.822	0.873	0.923	0.673	0.908	0.906	0.787
TG(18:0/18:0/18:1)	0.920	0.926	0.811	1.160	0.979	1.126	1.521
TG(16:0/18:0/20:0)	1.317	1.435	0.832	0.985	1.124	1.052	0.883
TG(18:1/18:1/19:1)	1.150	1.125	0.778	0.788	0.902	1.002	0.650
TG(18:0/18:1/19:1)	0.986	1.291	1.196	0.997	1.147	1.374	1.535
TG(16:0/21:0/18:1)	1.361	1.199	1.185	0.872	1.116	1.377	1.461
TG(16:0/16:0/23:0)	1.299	1.192	1.214	1.311	0.986	1.328	1.576
TG(16:0/18:1/22:5)	0.882	1.021	0.952	1.074	0.973	0.747	0.739
TG(18:1/18:1/20:1)	1.109	0.789	0.945	0.973	0.647	0.936	1.119
TG(18:0/18:1/20:1)	1.039	0.916	1.041	1.005	0.997	1.087	0.999
TG(16:0/22:0/18:1)	1.321	1.130	0.817	1.499	1.571	1.332	1.634
TG(16:0/18:0/22:0)	1.242	1.200	1.047	1.023	1.267	1.202	1.097
TG(18:0/18:2/21:2)	1.224	1.112	0.962	1.125	1.102	1.097	1.630
TG(18:0/21:1/18:2)	1.168	1.122	1.077	1.173	1.007	1.282	1.325
TG(16:0/18:1/23:1)	1.037	0.709	0.944	0.799	0.786	0.917	0.973
TG(16:0/23:0/18:1)	1.304	1.278	1.188	1.291	1.108	1.215	1.245
TG(16:0/18:0/23:0)	0.972	1.164	1.003	1.097	0.974	0.985	1.402
TG(18:0/18:1/22:5)	0.974	0.867	0.894	0.800	0.774	0.877	0.675
TG(18:0/18:1/22:4)	0.767	0.679	0.771	0.802	0.716	0.653	0.609
TG(22:0/18:1/18:2)	0.799	0.899	0.845	1.016	0.787	0.813	1.006
TG(16:0/18:1/24:1)	0.862	1.030	1.028	1.066	0.826	1.064	1.023
TG(16:0/24:0/18:1)	0.990	1.046	0.990	1.009	1.013	1.027	1.021
TG(16:0/18:0/24:0)	0.725	0.956	1.008	1.104	1.036	1.029	1.096

	A	E	G	J	K	M	O
TG(18:1/18:1/23:1)	1.021	1.011	1.071	1.067	0.643	1.054	0.999
TG(18:0/18:1/23:1)	1.047	1.043	1.082	1.044	0.829	0.842	1.090
TG(16:0/25:0/18:1)	1.003	1.129	1.034	1.111	1.058	1.026	1.103
TG(16:0/18:0/25:0)	1.064	1.108	1.022	1.122	1.047	1.121	1.214
TG(24:0/18:1/18:2)	1.493	1.429	1.489	1.314	1.351	1.322	1.450
TG(16:0/26:0/18:2)	1.070	1.395	1.476	1.369	1.377	1.431	1.405
TG(16:0/26:0/18:1)	0.986	1.120	1.080	1.017	0.936	1.062	1.132
TG(16:0/18:0/26:0)	0.990	1.089	0.962	1.053	0.957	1.093	1.123
TG(18:0/18:1/25:1)	0.985	1.054	1.048	1.014	0.947	1.015	1.073
TG(18:0/26:0/17:1)	1.033	1.075	1.051	1.058	1.073	1.109	1.065
TG(26:0/18:1/18:1)	1.177	1.276	1.299	1.365	1.298	1.333	1.316
TG(18:0/26:0/18:1)	1.011	1.111	1.098	1.089	1.035	1.073	1.101
TG(18:0/26:0/18:1)	1.064	1.012	1.135	1.078	1.090	1.130	1.159

Table 33: Substrate specificity ratios for the lipases A, E, G, J, K, M and O towards different lipids in baked pound cake (n = 3). Data already prepared for publication ([162]).

	A	E	G	J	K	M	O
BisMePA(34:3)	0.786	0.947	0.636	0.686	1.228	1.081	1.113
BisMePA(34:2)	0.339	0.642	0.262	0.397	1.153	0.826	1.253
BisMePA(36:4)	1.030	1.121	1.084	1.379	1.541	1.321	1.383
BisMePA(36:3)	0.304	0.669	0.253	0.366	0.992	0.761	0.798
BisMePA(36:2)	0.241	0.847	0.132	0.310	0.836	0.849	1.046
BisMePA(36:2)	0.182	0.540	0.214	0.292	1.223	1.009	1.421
BisMePA(38:7)	0.234	0.848	0.099	0.205	1.010	0.829	1.119
BisMePA(40:7)	0.205	1.315	0.065	0.202	1.167	1.066	1.463
DG(P-22:4/18:3)	1.451	1.213	1.627	1.621	1.663	1.655	1.638
DG(P-24:4/18:3)	1.037	1.143	1.072	1.228	1.062	1.280	1.067
DG(P-26:4/18:3)	1.110	1.032	0.957	1.195	0.914	1.132	0.932
DG(P-30:4/18:2)	1.454	1.587	1.091	1.530	1.216	1.489	1.047
DG(P-30:4/20:4)	1.229	1.265	1.156	1.293	1.094	1.311	1.089
DG(P-50:7)	1.008	1.056	0.977	1.020	0.995	0.945	1.014

	A	E	G	J	K	M	O
DG(O-30:6)	1.482	2.128	2.009	2.681	2.376	1.907	1.259
DG(O-26:8/16:2)	0.921	0.601	0.491	0.464	0.307	0.389	0.331
DG(O-42:6)	0.397	0.301	0.440	0.426	0.386	0.549	0.893
DG(O-24:7/21:3)	0.979	0.671	0.475	0.451	0.329	0.353	0.305
DG(O-28:6/19:0)	1.098	1.015	0.924	1.176	0.981	0.963	1.033
DG(6:0/12:1)	0.985	1.338	1.294	1.904	2.094	1.342	1.336
DG(18:1/18:3)	0.746	0.737	0.733	0.847	0.829	0.786	0.911
DG(18:2/18:2)	1.014	0.858	0.808	0.582	0.844	0.536	0.887
DG(18:1/18:2)	1.665	0.985	2.255	2.112	1.365	1.070	1.409
DG(16:0/24:6)	0.217	0.648	0.723	0.445	0.459	0.695	0.686
DG(24:6/18:0)	0.943	0.467	1.486	0.885	0.773	0.564	0.212
DGDG(16:0/18:2)	0.669	0.896	0.702	0.652	0.939	0.693	1.133
DGDG(18:2/18:2)	0.944	1.037	0.683	1.047	0.934	0.837	1.036
DGMG(18:2)	4.240	2.749	4.258	4.820	2.259	2.707	1.210
LPC(14:0)	0.932	1.136	0.734	0.734	0.742	0.932	0.868
LPC(16:1)	6.045	2.176	7.791	6.861	1.422	1.951	0.968
LPC(16:0)	1.422	1.254	1.412	1.566	1.236	1.224	1.112
LPC(17:1)	5.779	3.231	8.293	7.781	2.334	2.362	1.410
LPC(18:3)	1.441	1.132	1.186	1.064	1.047	0.992	0.868
LPC(18:2)	2.345	1.403	2.620	2.501	1.333	1.170	1.010
LPC(18:1)	10.864	2.723	12.976	10.464	1.615	2.336	0.841
LPC(18:0)	1.279	1.087	1.484	1.355	1.317	1.103	1.225
LPC(19:1)	7.520	3.901	7.587	5.640	2.151	3.450	1.034
LPC(20:4)	50.258	10.930	59.046	48.601	3.678	8.926	0.529
LPC(20:1)	1.556	0.950	1.765	1.510	0.967	0.612	0.783
LPC(22:6)	83.774	19.770	112.270	89.123	5.555	15.312	0.611
LPC(22:5)	188.100	26.140	143.693	119.998	13.910	31.824	1.292
LPE(16:0)	1.383	0.931	1.507	1.390	1.052	0.992	1.080
LPE(18:2)	4.829	2.012	5.262	4.589	1.427	1.795	1.128
LPE(18:1)	17.217	4.930	19.548	15.126	2.527	4.363	1.104
LPE(18:0)	0.909	0.770	0.932	0.945	0.794	0.674	0.793

	A	E	G	J	K	M	O
LPE(20:4)	116.967	30.875	151.616	118.176	10.944	27.213	1.084
LPE(22:6)	58.874	21.437	84.739	68.647	6.103	15.939	1.272
LPE(22:5)	40.777	7.233	33.737	30.179	4.154	10.125	0.857
MG(18:3)	1.847	1.042	2.412	1.889	2.602	1.698	1.583
MG(18:2)	1.712	1.853	1.793	1.940	1.300	2.093	1.527
MG(O-17:3)	1.356	1.933	1.859	2.441	2.656	1.452	1.908
MGDG(18:2/18:2)	0.636	0.481	0.300	0.475	0.745	0.618	0.990
MGMG(18:2)	2.668	2.040	3.204	3.395	2.163	2.206	1.294
PA(22:5/18:1)	0.136	0.211	0.036	0.204	1.263	1.046	1.204
PC(P-39:10)	0.693	1.532	0.554	1.082	1.409	1.101	0.000
PC(O-34:2)	1.017	1.302	1.323	1.104	0.958	1.095	1.075
PC(18:1)	2.306	2.126	2.089	2.132	1.744	1.631	1.013
PC(30:0)	0.789	0.975	0.760	0.951	1.112	1.016	1.015
PC(32:3)	1.246	1.381	1.643	1.963	1.980	1.362	1.737
PC(32:2)	0.656	0.725	0.568	0.709	1.011	0.924	0.795
PC(32:1)	0.437	0.994	0.425	0.524	1.177	0.935	1.099
PC(32:0)	0.605	1.000	0.713	0.731	1.170	0.967	1.202
PC(15:2/18:2)	0.646	0.914	0.760	1.060	0.792	0.894	0.851
PC(33:2)	0.521	0.957	0.600	0.698	1.043	0.932	0.926
PC(33:1)	0.352	0.748	0.169	0.324	0.913	0.879	1.067
PC(34:4)	0.488	1.023	0.517	0.636	0.989	0.913	0.948
PC(34:3)	0.707	0.995	0.632	0.836	1.048	0.905	0.928
PC(16:0/18:2)	0.468	0.815	0.434	0.469	0.902	0.756	0.975
PC(34:2)	1.540	3.456	1.328	1.722	0.966	0.650	2.629
PC(34:1)	0.315	0.863	0.266	0.363	0.951	0.898	1.083
PC(16:0/18:1)	0.312	0.811	0.281	0.359	1.051	0.906	1.085
PC(35:2)	0.356	0.951	0.362	0.469	1.013	0.933	1.068
PC(35:1)	0.158	0.723	0.048	0.208	0.841	0.761	1.005
PC(36:6)	1.243	1.507	1.122	1.352	1.372	1.239	1.149
PC(36:5)	0.991	1.298	0.930	1.174	1.247	1.092	0.970
PC(36:5)	1.261	1.121	1.176	1.330	1.244	0.449	0.424

	A	E	G	J	K	M	O
PC(18:2/18:2)	0.733	0.924	0.645	0.864	0.979	0.911	0.891
PC(18:2/18:2)	0.783	0.988	0.701	0.853	0.991	0.901	0.920
PC(36:4)	0.706	0.940	0.661	0.831	0.928	0.814	0.849
PC(18:1/18:2)	0.793	1.089	0.647	0.910	0.860	0.706	0.801
PC(36:2)	0.364	0.910	0.327	0.440	1.011	0.910	1.042
PC(18:0/18:2)	0.328	0.908	0.319	0.366	0.990	0.898	1.096
PC(18:0/18:1)	0.462	0.991	0.342	0.573	1.201	1.152	1.215
PC(36:1)	0.356	0.953	0.326	0.419	0.985	0.932	1.354
PC(38:7)	0.620	0.630	0.288	0.233	0.364	0.605	0.467
PC(16:0/22:6)	0.413	1.342	0.374	0.574	1.362	1.074	1.259
PC(18:0/20:4)	0.389	0.971	0.305	0.480	1.078	1.112	1.124
PC(40:9)	0.215	1.076	0.086	0.058	0.765	0.658	0.757
PC(40:7)	0.000	0.451	0.102	0.037	0.253	0.240	0.610
PC(40:6)	0.395	1.230	0.414	0.574	1.196	1.057	1.171
PC(40:5)	0.329	0.702	0.374	0.691	1.444	1.161	1.326
PE(P-18:0)	18.199	4.915	20.614	15.788	2.591	4.642	1.136
PE(16:0/18:2)	0.292	0.703	0.179	0.309	0.970	0.777	0.960
PE(16:0/18:1)	0.206	0.755	0.146	0.189	0.969	0.790	1.071
PE(18:2/18:2)	0.703	0.945	0.871	1.134	0.942	1.015	0.936
PE(18:2/18:2)	1.362	1.154	1.042	1.274	0.997	0.831	0.918
PE(20:4/16:0)	0.516	0.657	0.588	0.734	0.918	0.566	0.821
PE(36:3)	1.076	1.402	1.087	1.336	1.712	1.287	1.523
PE(18:0/18:2)	0.213	0.785	0.148	0.233	0.943	0.776	1.082
PE(18:0/18:1)	0.141	0.835	0.158	0.225	0.774	0.578	1.272
PE(16:0/22:6)	0.220	0.915	0.100	0.233	0.947	0.808	0.978
PE(20:4/18:1)	0.087	0.185	0.039	0.131	0.855	0.722	0.950
PE(18:0/20:4)	0.438	0.951	0.355	0.495	1.069	0.874	1.153
PEt(10:0/12:1)	1.032	1.023	1.067	0.978	0.950	1.088	1.002
PEt(10:0/18:2)	0.905	0.972	0.987	0.923	1.066	1.097	0.976
PEt(10:0/18:1)	0.977	0.999	0.907	0.973	0.968	1.017	0.981
PEt(10:0/20:2)	0.943	0.995	0.918	0.911	0.990	1.025	0.915

	A	E	G	J	K	M	O
PEt(10:0/22:1)	0.874	1.294	1.205	1.093	1.863	1.136	0.811
PEt(10:0/22:1)	0.987	1.320	0.986	1.000	1.486	0.802	0.457
PEt(10:0/24:1)	1.027	1.222	0.975	1.192	1.274	1.090	1.390
PEt(10:0/24:1)	0.978	1.437	0.978	1.295	1.335	1.047	1.273
PIP3(P-6:0/16:3)	2.146	2.268	2.570	3.222	2.481	2.407	2.385
PIP3(O-4:0/18:4)	2.955	2.645	2.474	3.314	2.873	2.705	2.383
SM(d32:1)	0.971	1.121	1.012	0.997	1.060	1.147	1.100
SM(d34:1)	1.102	0.678	0.990	0.945	0.599	0.893	0.855
SM(d34:0)	0.715	1.461	0.969	1.053	1.016	1.119	1.286
SM(d36:1)	1.075	1.116	1.200	1.166	1.127	1.113	1.076
SM(d40:1)	0.764	0.643	0.352	0.483	0.973	0.475	0.507
SM(d42:1)	0.956	0.911	0.721	0.570	0.614	0.861	0.349
SPH(d14:0)	1.500	1.491	1.733	2.117	1.701	2.162	2.041
SPH(d18:0)	1.169	0.683	1.541	2.521	1.339	1.769	1.563
SPH(d14:1)	1.049	1.464	1.578	1.844	1.640	1.417	1.252
SPH(d16:1)	1.616	1.207	1.464	1.993	1.397	1.622	1.511
SPH(d18:1)	3.875	3.673	3.093	4.568	3.690	3.816	3.189
TG(P-25:6/27:8)	1.730	1.722	1.197	1.603	2.790	2.755	2.705
TG(4:0/6:0/10:0)	1.042	1.389	1.463	1.537	2.093	1.803	2.650
TG(4:0/4:0/14:1)	1.844	2.021	1.598	2.074	2.042	1.855	1.610
TG(4:0/6:0/12:0)	1.289	1.376	1.073	1.509	1.406	1.348	1.306
TG(4:0/6:0/14:1)	0.882	1.063	0.879	0.881	0.883	0.961	1.001
TG(8:0/8:0/8:0)	1.891	1.936	1.681	2.236	2.340	2.172	1.876
TG(2:0/10:0/12:0)	1.046	1.129	1.093	1.124	1.176	1.173	1.098
TG(4:0/4:0/18:2)	1.184	1.365	1.136	1.479	1.535	1.493	0.916
TG(4:0/4:0/18:1)	0.937	1.121	0.717	1.040	1.098	0.934	0.992
TG(8:0/8:0/10:0)	1.875	1.567	1.680	2.042	1.888	2.344	2.397
TG(4:0/6:0/16:0)	1.069	1.085	1.061	1.061	1.121	1.146	1.129
TG(4:0/6:0/17:1)	1.154	1.049	1.053	0.923	1.201	1.107	1.119
TG(4:0/6:0/18:2)	1.057	1.056	1.066	1.112	1.179	1.194	1.055
TG(4:0/6:0/18:1)	1.176	0.745	1.150	0.995	1.312	1.464	1.463

	A	E	G	J	K	M	O
TG(8:0/10:0/10:0)	0.957	0.932	0.897	23.742	23.365	1.378	1.808
TG(4:0/10:0/14:0)	1.145	1.150	1.134	1.178	0.977	1.086	1.174
TG(4:0/8:0/17:1)	1.073	1.086	1.138	1.130	1.306	1.225	1.140
TG(4:0/8:0/18:2)	2.056	2.704	2.386	2.374	2.306	2.689	2.984
TG(4:0/8:0/18:1)	1.056	1.040	1.040	1.052	1.119	1.125	1.106
TG(4:0/6:0/20:0)	0.860	0.760	0.867	1.079	1.122	1.468	1.892
TG(4:0/10:0/16:0)	1.104	1.129	1.092	0.987	1.113	1.184	1.036
TG(4:0/10:0/16:0)	1.044	1.376	0.951	0.860	0.918	0.945	1.025
TG(6:0/8:0/17:1)	1.165	1.492	1.164	1.344	1.647	0.829	1.153
TG(6:0/8:0/18:2)	1.095	0.878	1.052	1.041	0.921	0.925	1.002
TG(4:0/14:0/14:1)	0.914	1.005	1.013	0.950	0.985	1.017	1.008
TG(4:0/8:0/20:0)	0.761	0.822	0.803	0.967	1.050	1.305	1.846
TG(4:0/12:0/16:0)	1.159	1.015	1.163	0.987	1.417	1.197	1.431
TG(4:0/12:0/16:0)	0.987	0.942	1.101	1.110	1.236	1.067	1.530
TG(4:0/15:0/14:1)	0.966	0.801	0.888	0.885	1.062	0.861	0.900
TG(6:0/10:0/17:1)	1.033	1.143	1.753	1.221	1.386	1.192	1.010
TG(4:0/12:0/18:2)	1.206	1.127	1.166	0.717	0.963	1.212	1.071
TG(4:0/12:1/18:1)	1.214	1.119	1.105	0.950	0.970	1.221	1.079
TG(4:0/12:0/18:1)	1.020	0.869	0.952	0.935	0.853	0.905	0.948
TG(4:0/12:0/18:1)	1.127	1.024	0.844	1.161	1.030	0.627	1.086
TG(4:0/10:0/20:0)	0.804	0.786	0.809	1.011	1.073	1.235	1.531
TG(4:0/14:0/16:0)	1.094	0.964	1.218	1.111	1.079	0.963	1.287
TG(4:0/14:0/16:0)	1.134	0.889	0.913	0.974	0.744	1.019	0.685
TG(8:0/10:0/17:1)	0.922	0.911	0.864	0.864	0.963	0.818	1.175
TG(8:0/10:0/17:1)	0.965	0.911	0.891	0.792	0.842	0.818	1.175
TG(4:0/14:0/18:3)	0.585	0.564	0.854	0.659	0.957	0.882	0.839
TG(4:0/14:0/18:3)	0.884	0.621	0.773	0.684	0.946	0.958	0.931
TG(4:0/14:0/18:2)	0.909	1.053	1.013	1.187	0.701	1.127	1.627
TG(4:0/14:0/18:1)	0.899	0.901	0.841	1.047	1.115	1.235	1.770
TG(4:0/14:0/18:1)	0.926	1.231	0.939	0.912	1.114	0.988	1.021
TG(4:0/14:0/18:1)	1.347	1.252	1.506	1.359	1.114	1.601	1.021

	A	E	G	J	K	M	O
TG(4:0/14:0/18:1)	0.944	0.996	0.754	1.048	1.157	0.890	0.873
TG(4:0/16:0/16:0)	1.043	1.029	1.012	1.069	1.162	1.196	1.703
TG(6:0/14:0/16:0)	1.010	0.955	1.085	0.895	1.085	1.037	1.078
TG(4:0/16:0/16:0)	1.349	1.158	1.403	1.114	0.806	1.135	1.124
TG(4:0/16:0/16:0)	0.814	1.075	1.183	1.066	1.034	1.079	1.071
TG(4:0/16:0/17:0)	1.145	1.212	1.137	0.847	1.374	1.098	0.942
TG(6:0/14:0/17:0)	1.058	1.155	1.122	0.718	1.185	1.033	0.886
TG(6:0/14:0/17:0)	0.838	1.086	1.078	0.697	1.164	1.128	0.676
TG(30:2/4:0/4:0)	1.121	1.011	0.726	0.974	1.218	1.308	1.594
TG(4:0/16:1/18:1)	0.750	0.763	0.513	0.663	0.702	0.680	0.837
TG(4:0/16:0/18:2)	0.842	0.934	1.051	1.103	0.762	1.140	1.001
TG(4:0/16:0/18:1)	0.890	1.310	0.853	1.438	1.613	1.104	0.938
TG(4:0/16:0/18:1)	0.983	1.005	0.911	0.951	0.989	1.293	1.208
TG(4:0/16:0/18:1)	1.015	1.049	1.073	1.068	1.107	1.145	0.844
TG(4:0/16:0/18:0)	0.885	0.763	0.621	0.885	0.926	1.130	1.317
TG(4:0/16:0/18:0)	1.110	1.101	1.004	0.945	1.101	1.227	1.433
TG(10:0/12:0/16:0)	1.173	1.189	1.139	1.005	1.049	1.055	1.198
TG(4:0/16:0/18:0)	1.327	1.350	0.738	1.150	1.093	1.462	1.185
TG(4:0/17:1/18:1)	0.575	1.099	1.154	0.628	0.617	1.194	0.943
TG(4:0/17:0/18:1)	0.787	1.196	1.286	0.683	1.282	0.978	0.990
TG(4:0/17:0/18:1)	0.595	0.957	1.049	0.796	1.050	0.723	0.741
TG(4:0/18:1/18:2)	3.157	0.928	2.448	2.770	2.725	2.648	2.778
TG(4:0/18:1/18:1)	0.710	0.698	0.719	0.781	0.849	1.151	0.970
TG(4:0/18:1/18:1)	1.008	1.058	1.093	1.127	1.124	1.114	1.112
TG(10:0/12:0/18:2)	1.008	1.058	1.183	1.127	1.124	1.114	1.112
TG(4:0/18:0/18:1)	1.051	0.744	0.765	1.157	1.247	0.779	1.679
TG(4:0/18:0/18:1)	1.133	0.906	0.775	0.858	0.887	1.046	1.129
TG(6:0/16:0/18:1)	0.976	1.078	1.022	1.024	1.119	1.037	0.863
TG(4:0/16:0/20:0)	0.795	0.908	0.800	0.936	0.901	1.150	1.272
TG(6:0/16:0/18:0)	0.684	0.682	0.556	0.986	0.723	0.959	0.829
TG(10:0/14:0/16:0)	0.973	0.858	0.842	0.782	0.950	1.014	0.966

	A	E	G	J	K	M	O
TG(4:0/18:1/19:1)	1.445	1.321	1.043	1.187	1.016	1.317	1.347
TG(6:0/17:1/18:1)	4.073	0.847	4.018	3.447	1.779	3.763	4.283
TG(6:0/17:1/18:1)	1.530	1.413	1.130	1.276	0.887	1.283	1.544
TG(18:0/4:0/19:1)	0.608	0.929	0.705	1.071	0.996	0.851	0.917
TG(4:0/20:0/18:2)	0.827	0.653	0.621	0.693	0.936	0.968	0.758
TG(4:0/20:0/18:2)	1.011	0.943	1.253	5.548	1.687	1.926	1.056
TG(6:0/18:1/18:1)	0.396	0.382	0.420	0.600	0.439	0.431	0.482
TG(6:0/18:1/18:1)	1.220	1.203	1.201	1.200	1.306	1.340	1.347
TG(4:0/20:0/18:1)	1.219	1.468	1.020	1.143	1.569	0.987	1.282
TG(10:0/14:0/18:1)	1.264	1.045	1.672	1.185	1.319	1.421	1.107
TG(8:0/16:0/18:1)	1.269	1.078	1.699	1.222	1.340	1.421	1.117
TG(8:0/16:0/18:1)	1.034	1.031	0.974	1.107	0.963	1.051	0.996
TG(6:0/16:0/20:0)	0.892	0.765	0.895	0.890	0.969	0.917	1.111
TG(8:0/16:0/18:0)	0.608	0.674	0.571	0.608	1.270	1.390	0.724
TG(12:0/14:0/16:0)	0.924	0.986	0.902	0.797	0.877	0.953	0.808
TG(8:0/17:1/18:1)	0.854	0.926	0.896	0.912	0.826	0.829	0.934
TG(10:0/16:0/17:1)	0.919	0.765	2.370	0.790	0.825	1.000	2.116
TG(10:0/16:0/18:2)	0.784	1.143	0.579	0.830	1.028	1.001	2.803
TG(10:0/16:0/18:2)	1.199	1.200	1.172	1.201	1.125	1.189	1.122
TG(10:0/16:0/18:1)	1.392	0.915	0.901	0.857	0.592	0.994	1.140
TG(10:0/16:0/18:1)	0.974	1.035	0.962	0.983	0.962	0.971	0.965
TG(8:0/16:0/20:0)	0.822	0.980	0.943	0.908	0.052	0.058	0.946
TG(10:0/16:0/18:0)	1.037	1.034	0.988	0.979	0.751	1.068	0.995
TG(8:0/18:1/19:1)	0.334	1.020	1.027	0.482	1.291	0.416	0.274
TG(8:0/18:1/19:1)	0.375	1.317	1.270	1.313	1.278	0.184	1.286
TG(12:0/17:0/16:1)	0.893	0.983	1.019	0.965	1.059	0.817	0.928
TG(10:0/18:0/18:3)	0.499	1.119	1.194	1.385	1.477	1.541	1.225
TG(14:0/14:0/18:3)	1.217	1.115	1.075	1.038	1.333	1.307	0.435
TG(14:0/14:1/18:1)	0.973	0.932	0.919	0.897	0.926	0.966	0.998
TG(14:0/14:1/18:1)	0.949	0.958	0.927	0.834	0.961	0.994	0.965
TG(12:0/16:0/18:1)	0.977	0.831	1.151	1.144	1.174	0.840	0.873

	A	E	G	J	K	M	O
TG(14:0/16:0/16:0)	0.951	0.787	0.762	0.887	0.928	0.913	0.921
TG(14:0/14:1/19:1)	0.952	0.990	1.153	1.095	1.161	1.063	1.189
TG(14:0/16:0/17:0)	0.227	0.898	0.766	0.735	0.810	0.741	0.889
TG(14:1/16:1/18:2)	1.090	0.884	1.076	0.938	0.938	1.159	1.165
TG(12:0/18:1/18:2)	0.848	1.226	0.933	0.898	1.208	1.186	1.120
TG(12:0/18:1/18:2)	0.850	1.227	0.936	0.899	1.212	1.188	1.121
TG(12:0/18:1/18:1)	0.818	1.190	0.558	1.136	1.162	1.028	0.315
TG(16:0/14:1/18:1)	0.785	1.206	0.431	1.008	0.963	0.152	0.115
TG(14:0/16:0/18:1)	0.951	0.937	0.926	0.959	0.902	0.971	1.008
TG(14:0/16:0/18:0)	1.077	1.138	1.129	1.159	1.241	0.659	0.728
TG(14:1/18:1/18:2)	0.570	1.209	0.890	0.969	0.982	0.807	1.012
TG(14:0/18:1/18:3)	0.520	1.211	0.333	0.978	0.920	0.809	1.103
TG(14:0/18:1/18:2)	1.003	0.384	1.628	2.133	1.510	1.825	1.645
TG(16:0/16:1/18:1)	1.083	1.012	0.957	1.024	0.999	0.947	0.918
TG(16:0/16:0/18:2)	6.319	6.396	5.932	6.523	6.323	5.060	5.445
TG(16:0/16:0/18:1)	0.901	0.934	0.895	0.975	0.964	1.020	1.039
TG(16:0/16:0/18:0)	0.922	0.890	0.731	0.642	0.570	0.614	0.672
TG(16:0/17:1/18:1)	0.631	0.830	0.935	0.827	0.785	1.008	0.949
TG(16:0/17:1/18:1)	0.693	0.862	0.965	0.957	0.831	0.857	0.745
TG(16:0/17:0/18:1)	1.129	1.086	1.115	1.171	1.052	0.889	1.104
TG(16:0/17:0/18:1)	0.931	1.194	1.328	1.134	0.762	1.028	1.133
TG(17:0/17:0/17:0)	0.985	1.057	1.012	1.120	0.947	0.876	0.880
TG(16:0/17:0/18:0)	0.950	1.221	1.086	1.284	1.198	0.853	0.901
TG(16:0/18:2/18:3)	1.164	1.331	1.251	1.280	1.175	1.071	1.207
TG(16:1/18:1/18:2)	1.258	0.701	0.893	0.855	1.018	0.601	1.103
TG(16:1/18:1/18:2)	1.255	0.541	0.891	0.853	1.016	0.677	1.084
TG(16:0/18:2/18:2)	9.968	8.164	7.025	10.184	7.982	5.916	4.724
TG(16:0/18:2/18:2)	0.736	0.827	0.771	1.113	0.915	0.835	0.735
TG(16:0/18:1/18:2)	0.726	0.547	0.882	1.114	0.800	1.298	0.983
TG(16:0/18:1/18:1)	0.947	0.882	0.803	0.795	0.529	0.843	0.883
TG(16:0/18:1/18:1)	4.345	3.988	3.850	4.429	3.933	3.869	3.597

	A	E	G	J	K	M	O
TG(16:0/18:1/18:1)	0.817	0.961	0.881	0.907	1.003	0.908	0.944
TG(16:0/18:1/18:1)	0.866	0.992	0.914	0.889	1.007	0.964	0.913
TG(16:0/18:0/18:1)	1.436	1.224	1.340	0.779	0.887	0.855	0.935
TG(16:0/18:0/18:0)	1.152	1.088	0.943	0.783	0.955	1.045	0.780
TG(17:0/18:1/18:1)	0.962	0.754	0.999	0.908	1.017	0.752	0.840
TG(18:0/17:1/18:1)	0.878	0.926	0.910	0.952	0.998	0.672	0.571
TG(17:0/18:0/18:1)	0.648	0.858	0.790	0.762	0.870	0.844	0.621
TG(18:2/18:2/18:3)	2.233	1.986	1.735	2.209	1.771	1.839	1.413
TG(17:0/17:0/19:0)	0.885	0.899	0.767	0.794	0.610	0.712	0.688
TG(18:1/18:2/18:3)	1.718	1.284	0.736	1.718	1.792	1.578	1.264
TG(18:1/18:2/18:2)	1.261	1.557	1.399	1.306	1.557	1.298	1.025
TG(18:1/18:1/18:2)	0.936	1.148	1.031	1.036	1.133	0.971	0.982
TG(18:1/18:1/18:2)	1.097	0.885	0.914	0.684	0.955	0.874	0.762
TG(18:0/18:2/18:2)	1.024	0.988	0.891	1.095	0.972	0.858	0.816
TG(18:0/18:1/18:2)	1.036	1.106	1.087	1.056	0.822	1.046	1.052
TG(18:0/18:1/18:2)	0.875	1.011	1.087	0.853	0.822	0.734	0.720
TG(18:0/18:1/18:1)	1.160	1.347	1.279	1.060	1.275	1.191	1.191
TG(18:0/18:0/18:1)	0.787	0.767	0.572	0.444	0.606	0.690	0.588
TG(16:0/18:0/20:0)	0.521	0.804	0.466	0.357	0.328	0.460	0.426
TG(18:1/18:1/19:1)	0.829	1.217	0.887	1.167	1.034	1.024	1.063
TG(18:0/18:1/19:1)	0.814	1.029	0.980	0.741	0.775	0.796	0.833
TG(16:0/21:0/18:1)	0.851	0.703	0.835	0.675	0.927	0.700	0.513
TG(16:0/16:0/23:0)	1.135	1.245	1.122	1.117	0.971	0.869	1.133
TG(16:0/18:1/22:5)	1.122	1.106	1.142	1.154	1.152	1.116	1.159
TG(18:1/18:1/20:1)	0.861	1.144	0.847	1.088	0.889	0.969	1.032
TG(18:0/18:1/20:1)	1.205	1.270	1.127	1.020	1.189	0.835	1.101
TG(16:0/22:0/18:1)	0.846	0.793	0.807	0.932	0.943	1.003	0.722
TG(16:0/18:0/22:0)	1.032	1.206	1.151	0.956	1.065	1.164	1.074
TG(16:1/18:1/23:1)	0.898	0.898	0.923	0.961	0.899	0.919	0.887
TG(16:0/18:1/23:1)	1.174	1.605	1.256	1.471	1.257	1.524	1.063
TG(16:0/23:0/18:1)	1.000	0.924	0.905	0.857	0.995	0.937	0.857

	A	E	G	J	K	M	O
TG(16:0/18:0/23:0)	1.111	1.034	0.976	1.087	1.089	1.087	0.988
TG(18:0/18:1/22:5)	0.938	0.849	0.939	0.877	0.791	0.746	0.886
TG(18:0/18:1/22:4)	1.133	0.881	1.204	1.238	1.215	1.172	1.126
TG(22:0/18:1/18:3)	1.332	1.384	1.226	1.395	1.356	1.268	1.150
TG(22:0/18:1/18:2)	1.015	1.115	0.756	1.253	1.183	1.179	1.101
TG(16:0/18:1/24:1)	0.951	1.101	0.962	1.165	0.860	0.953	1.163
TG(16:0/24:0/18:1)	1.070	0.926	1.185	1.106	0.996	1.019	1.019
TG(16:0/18:0/24:0)	1.077	1.081	1.030	0.994	1.024	1.031	1.003
TG(18:1/18:1/23:1)	1.135	1.247	1.166	1.292	1.055	1.130	1.083
TG(18:0/18:1/23:1)	1.159	1.090	0.837	1.044	1.139	1.066	0.996
TG(16:0/25:0/18:1)	0.941	1.087	1.142	1.166	1.129	1.074	1.001
TG(16:0/18:0/25:0)	1.170	1.200	1.140	1.158	1.175	0.904	1.058
TG(24:0/18:1/18:2)	1.416	1.446	1.187	1.340	1.276	1.190	1.276
TG(16:0/26:0/18:2)	1.027	1.230	0.775	1.351	1.005	1.091	1.024
TG(16:0/26:0/18:1)	1.075	1.107	1.017	0.876	1.109	0.895	0.667
TG(16:0/18:0/26:0)	1.113	1.079	0.870	0.978	1.136	0.946	0.955
TG(18:0/26:0/17:1)	1.192	1.047	1.128	1.119	1.066	1.151	1.095
TG(26:0/18:1/18:1)	1.385	1.338	1.340	1.400	1.282	1.214	1.249
TG(18:0/26:0/18:1)	1.163	1.249	1.179	1.185	1.144	1.119	1.112

Table 34: Substrate specificity ratios for the lipases A, E, G, J, K, M and O towards different lipids in brioche dough (n = 3). Data already prepared for publication ([162]).

	A	E	G	J	K	M	O
BisMePA(34:3)	0.989	1.037	0.984	0.780	1.161	1.125	0.958
BisMePA(34:2)	2.307	1.075	1.155	0.808	0.891	1.177	2.097
BisMePA(36:5)	1.109	0.966	1.110	0.781	1.218	1.201	1.034
BisMePA(36:3)	2.235	1.242	1.420	0.977	1.093	1.273	1.664
DG(P-24:1)	1.026	1.002	1.099	1.071	0.666	0.936	0.833
DG(P-46:5)	0.994	0.432	0.272	0.891	0.365	1.048	0.438
DG(P-25:8/23:6)	1.505	1.158	1.305	0.998	1.338	1.232	1.119
DG(P-30:4/18:2)	1.075	1.352	1.124	1.201	0.960	1.037	1.276

	A	E	G	J	K	M	O
DG(O-30:6)	0.927	0.887	0.766	0.959	1.254	1.096	1.419
DG(O-30:6/19:0)	0.991	1.050	0.884	1.012	0.773	0.816	1.003
DG(6:0/12:1)	2.088	1.196	1.468	0.743	1.689	2.240	2.345
DGDG(16:0/18:2)	0.725	0.953	1.133	0.768	0.971	1.118	1.198
DGDG(18:2/18:3)	1.690	1.097	1.183	0.734	0.924	1.139	1.270
DGDG(18:2/18:2)	1.822	1.499	1.589	1.257	1.098	1.598	2.140
DGMG(18:2)	0.630	0.565	0.922	0.664	0.933	0.865	0.615
LPC(16:1)	1.287	0.773	1.135	0.583	1.018	1.224	1.234
LPC(18:1)	0.679	0.637	0.970	0.671	0.945	1.045	0.624
LPC(18:0)	1.430	0.816	1.040	0.557	0.940	1.041	1.496
LPC(20:4)	0.149	0.504	0.672	0.307	0.778	1.443	0.188
LPC(22:6)	0.028	0.449	0.711	0.173	0.729	1.322	0.129
LPC(22:5)	0.000	0.367	0.721	0.167	0.677	1.373	0.059
LPC(O-12:1)	0.854	0.606	0.916	0.470	1.774	0.950	0.422
LPE(16:0)	1.461	0.660	0.860	0.588	0.707	1.029	0.988
LPE(18:2)	1.042	1.011	1.004	0.861	1.494	2.230	0.981
LPE(18:2)	0.887	0.572	0.797	0.563	0.694	1.032	0.625
LPE(18:1)	0.289	0.257	0.698	0.473	0.613	1.043	0.334
LPE(20:4)	0.000	0.574	0.770	0.167	0.785	1.367	0.065
MG(18:2)	0.708	0.692	1.150	0.550	0.821	0.872	0.775
MGDG(18:2/18:2)	2.322	1.248	1.415	1.062	1.045	1.152	1.625
MGMG(18:2)	0.550	0.470	0.795	0.508	0.753	0.633	0.532
PC(O-28:5/20:5)	1.452	0.900	1.109	0.852	1.398	1.069	1.237
PC(30:0)	1.085	1.240	0.934	1.235	0.786	1.073	1.241
PC(32:2)	1.416	1.094	1.318	0.825	1.047	0.652	0.951
PC(32:1)	1.629	1.059	1.102	0.752	0.791	1.094	1.463
PC(32:0)	1.820	1.323	1.194	0.955	0.793	1.219	1.610
PC(33:4)	1.135	0.814	1.068	0.562	1.028	0.740	0.838
PC(34:3)	1.522	0.890	1.075	0.746	1.074	1.017	1.132
PC(16:0/18:2)	1.560	0.985	1.262	0.842	1.226	1.098	1.329
PC(16:0/18:1)	1.735	0.770	1.155	0.545	0.977	0.999	1.449

	A	E	G	J	K	M	O
PC(34:1)	2.010	1.007	1.116	0.196	0.974	0.841	1.473
PC(18:2/18:2)	1.385	1.009	1.170	0.815	1.260	1.104	1.204
PC(18:0/18:2)	2.327	1.022	1.144	0.729	0.995	1.021	1.507
PC(36:1)	1.972	0.923	1.057	0.524	0.814	0.928	1.569
PC(38:6)	2.129	0.802	1.271	0.565	0.955	0.908	1.504
PC(38:4)	2.019	0.968	0.970	0.518	0.848	0.770	1.345
PE(18:2/18:2)	1.071	0.766	0.711	0.653	0.952	0.873	0.902
PEt(O-26:3/18:4)	1.228	0.887	1.025	0.813	1.008	0.814	1.108
PEt(10:0/18:1)	1.029	0.978	0.908	1.055	0.909	1.080	1.066
PEt(24:6/18:2)	1.349	1.290	1.249	0.860	0.856	0.943	1.379
PIP2(16:3/6:0)	1.400	0.968	1.184	1.055	1.362	1.322	1.326
PIP2(28:6)	1.391	1.010	1.096	0.850	1.279	1.037	1.202
PIP3(P-6:0/16:3)	1.267	11.838	1.466	0.970	2.164	2.150	1.098
SM(d34:1)	1.605	1.072	1.063	0.787	0.993	0.770	1.100
SPH(d14:0)	1.599	1.216	1.556	0.887	1.787	2.165	1.476
SPH(d18:2)	1.624	0.724	1.125	0.486	0.902	0.961	1.026
SPH(d18:0)	2.258	1.626	2.307	1.180	3.083	2.317	1.547
SPH(d14:1)	1.345	1.080	1.592	1.164	1.484	1.062	1.487
SPH(d16:1)	1.859	1.604	2.391	1.655	2.425	1.876	1.904
SPH(d18:1)	0.843	0.440	1.211	1.042	1.423	0.975	0.986
TG(P-32:8/24:6)	1.280	1.105	1.625	0.884	1.330	1.248	0.964
TG(P-20:6/21:6/21:6)	2.200	2.021	2.228	1.656	1.952	1.818	1.733
TG(P-38:10/25:7)	1.348	0.702	1.249	1.341	2.079	1.042	1.677
TG(P-25:8/22:6/22:6)	1.660	1.682	1.892	1.301	1.795	1.734	1.585
TG(O-4:0/14:1)	2.039	1.150	1.503	0.786	1.930	1.747	2.328
TG(O-25:7/27:8)	1.430	1.054	1.172	0.907	1.467	1.169	1.428
TG(O-28:8/27:7)	1.011	0.232	0.737	0.686	1.090	1.293	0.604
TG(4:0/6:0/12:0)	0.742	1.042	0.897	0.901	0.664	0.913	2.004
TG(4:0/8:0/12:0)	0.743	0.660	0.942	0.669	0.594	0.789	1.025
TG(4:0/4:0/18:1)	0.934	1.064	1.080	1.184	0.884	1.124	0.936
TG(4:0/6:0/16:0)	0.244	0.958	0.772	1.110	0.699	0.903	1.048

	A	E	G	J	K	M	O
TG(4:0/6:0/18:1)	0.817	1.139	0.732	0.969	0.714	0.945	0.804
TG(8:0/10:0/10:0)	1.025	0.895	0.702	1.389	1.256	1.761	1.283
TG(4:0/10:0/14:0)	1.079	1.117	0.878	0.818	0.850	0.766	1.021
TG(4:0/8:0/18:2)	0.990	0.752	0.874	0.778	0.799	1.024	1.050
TG(4:0/8:0/18:1)	0.870	0.634	0.839	0.673	0.663	0.911	0.882
TG(6:0/10:0/14:0)	1.032	0.858	0.743	1.767	1.219	1.767	1.485
TG(4:0/10:0/16:0)	0.989	1.028	0.912	1.170	0.843	1.152	1.117
TG(4:0/8:0/18:0)	0.974	1.084	0.890	1.099	0.827	1.067	1.024
TG(4:0/10:0/17:1)	0.998	1.165	0.961	1.244	0.837	1.125	1.179
TG(4:0/10:0/18:2)	1.172	1.255	1.479	1.315	1.010	1.612	1.682
TG(4:0/10:0/18:1)	1.128	1.232	1.121	1.089	0.911	0.927	1.415
TG(6:0/8:0/18:1)	1.010	1.103	1.004	0.975	0.815	1.261	1.080
TG(4:0/12:0/16:0)	0.917	0.956	0.695	1.200	0.657	1.165	0.917
TG(6:0/8:0/18:0)	0.746	1.205	1.088	0.765	0.514	0.827	0.950
TG(6:0/10:0/17:0)	0.569	1.089	0.978	1.371	0.521	1.065	0.982
TG(4:0/12:0/18:3)	1.002	0.922	1.051	1.209	0.864	0.846	1.020
TG(4:0/12:0/18:2)	1.217	1.411	0.939	1.481	0.420	1.202	1.307
TG(12:0/4:0/18:1)	1.117	1.009	0.926	1.907	1.348	1.810	1.608
TG(4:0/12:0/18:1)	0.869	1.099	1.097	1.450	0.717	1.083	1.271
TG(6:0/10:0/18:1)	0.954	1.033	1.052	1.092	0.792	0.776	0.980
TG(4:0/14:0/17:1)	1.112	0.795	1.047	1.715	1.014	1.245	1.195
TG(6:0/10:0/20:3)	1.112	1.023	0.960	0.774	0.902	1.203	1.050
TG(4:0/14:0/18:2)	1.211	2.299	2.613	3.278	0.771	3.100	3.239
TG(4:0/14:0/18:1)	0.889	1.297	0.896	1.430	0.819	1.130	1.106
TG(6:0/12:0/18:1)	0.975	1.014	1.072	0.968	0.803	1.090	1.053
TG(4:0/16:0/16:0)	1.610	2.443	0.844	2.467	0.829	2.055	1.800
TG(4:0/16:0/17:0)	0.577	0.847	0.837	0.275	0.338	1.077	0.957
TG(4:0/16:0/18:3)	1.180	1.043	0.918	1.309	0.919	1.167	0.974
TG(4:0/16:0/18:2)	0.440	0.984	1.160	1.135	0.519	1.298	1.008
TG(4:0/16:1/18:1)	1.147	1.454	1.380	1.638	1.401	1.924	0.555
TG(4:0/16:0/18:1)	1.049	0.858	0.413	0.781	0.544	1.282	89.088

	A	E	G	J	K	M	O
TG(6:0/14:0/18:0)	1.043	1.014	0.784	1.801	1.239	1.648	1.623
TG(4:0/16:0/18:0)	0.387	1.312	0.346	1.381	0.336	0.408	0.363
TG(4:0/17:1/18:1)	0.854	1.724	0.478	1.429	0.619	1.248	1.031
TG(4:0/17:0/18:1)	0.363	0.890	0.829	0.487	0.594	1.016	1.102
TG(4:0/18:1/18:2)	0.864	1.132	0.912	1.108	0.792	0.702	1.040
TG(4:0/18:1/18:1)	0.933	1.388	1.307	2.094	0.847	1.496	1.915
TG(6:0/16:0/18:1)	0.279	0.650	0.244	0.411	0.231	0.814	0.317
TG(4:0/16:0/20:0)	1.284	0.972	0.956	1.493	1.358	1.829	1.401
TG(10:0/14:0/16:0)	0.981	0.654	0.462	0.771	1.189	1.151	1.025
TG(6:0/16:0/18:0)	0.750	0.856	0.759	1.059	0.689	0.983	0.874
TG(10:0/14:0/16:0)	0.962	1.000	0.895	0.980	0.885	1.073	1.050
TG(6:0/17:0/18:1)	0.915	1.091	0.898	1.094	0.823	1.082	1.072
TG(6:0/18:1/18:3)	0.833	1.013	0.802	0.751	0.618	0.598	0.647
TG(6:0/18:0/18:2)	1.915	1.712	1.271	1.069	1.616	1.045	0.823
TG(6:0/18:1/18:1)	1.036	1.021	1.032	0.634	0.862	1.164	1.089
TG(4:0/20:0/18:1)	1.797	1.092	1.177	1.162	0.958	1.093	1.092
TG(12:0/14:0/16:1)	0.610	0.615	1.380	0.505	1.004	1.056	0.977
TG(8:0/16:0/18:1)	0.840	0.943	1.097	0.962	0.731	0.840	1.030
TG(10:0/12:0/20:0)	1.231	0.787	0.906	1.650	1.273	1.445	1.716
TG(12:0/14:0/16:0)	1.364	1.963	1.720	1.819	1.195	1.321	1.698
TG(8:0/17:1/18:1)	0.934	0.952	0.997	0.941	0.917	1.125	1.022
TG(10:0/16:0/17:1)	1.279	0.819	0.982	1.207	0.910	1.406	0.985
TG(8:0/18:1/18:3)	0.959	1.093	0.897	0.844	0.855	1.152	0.980
TG(10:0/16:0/18:3)	0.653	0.988	1.070	0.289	0.664	0.767	0.938
TG(8:0/18:1/18:2)	0.729	1.307	1.299	0.921	0.685	1.215	1.109
TG(10:0/16:1/18:1)	0.807	0.888	0.870	1.512	0.965	1.178	1.018
TG(10:0/16:1/18:1)	0.885	1.505	1.015	1.443	1.043	1.319	1.099
TG(10:0/16:0/18:1)	0.913	1.101	0.978	1.066	0.459	0.934	1.169
TG(10:0/14:0/20:0)	0.955	0.955	0.810	0.964	0.838	0.778	0.012
TG(10:0/16:0/18:0)	0.663	0.933	0.977	0.997	0.820	1.082	1.091
TG(10:0/17:1/18:1)	1.103	1.284	0.681	1.042	0.883	1.101	1.236

	A	E	G	J	K	M	O
TG(10:0/17:1/18:1)	1.110	0.825	0.609	1.045	0.868	1.101	1.619
TG(10:0/17:0/18:2)	0.962	0.996	1.152	1.130	0.730	1.194	1.402
TG(10:0/18:1/18:3)	1.063	0.980	1.051	1.137	0.883	1.222	1.122
TG(10:0/18:1/18:3)	1.101	1.276	1.089	1.179	0.915	1.270	1.163
TG(10:0/18:1/18:2)	1.074	0.712	1.156	0.987	0.841	0.917	1.097
TG(12:0/16:0/18:3)	1.075	1.011	1.156	1.119	1.082	0.844	1.384
TG(14:0/14:1/18:1)	1.348	1.198	1.247	1.659	0.587	1.043	1.510
TG(14:0/14:1/18:1)	1.070	1.076	0.985	1.285	0.927	1.287	1.276
TG(12:0/16:0/18:1)	0.931	0.822	0.883	0.784	0.765	0.830	0.757
TG(12:0/16:0/18:1)	0.916	0.917	0.934	1.024	0.797	1.087	0.880
TG(10:0/18:0/18:1)	0.916	0.917	0.934	1.024	0.797	1.087	0.852
TG(14:0/16:0/16:0)	2.869	4.528	0.985	1.199	0.864	1.156	3.286
TG(14:0/16:0/16:0)	0.801	0.717	0.922	1.081	0.858	1.098	1.116
TG(14:0/16:0/17:0)	0.761	0.152	0.654	0.423	0.775	0.989	1.138
TG(12:0/18:1/18:3)	0.881	1.048	1.014	1.121	0.901	1.031	1.124
TG(16:0/14:1/18:2)	0.996	0.822	1.087	1.059	0.955	1.242	1.133
TG(16:0/14:1/18:1)	1.360	1.351	0.770	1.362	1.133	0.920	0.964
TG(14:0/16:0/18:1)	1.253	0.951	1.175	1.477	1.135	1.555	1.322
TG(14:0/16:0/18:0)	0.130	1.051	1.044	1.176	0.775	1.333	1.433
TG(14:0/18:1/18:3)	0.565	0.865	0.685	1.032	0.864	1.101	1.103
TG(16:0/16:1/18:2)	1.046	0.698	0.617	0.805	0.646	0.954	1.310
TG(16:1/16:1/18:1)	1.049	0.976	0.750	0.981	0.589	1.033	1.097
TG(20:2/14:0/16:0)	0.938	1.232	1.199	1.329	1.211	0.883	0.884
TG(16:0/16:1/18:1)	0.837	1.078	1.037	1.148	1.049	0.842	1.064
TG(16:0/16:1/18:1)	0.965	1.078	1.038	1.151	1.050	0.842	0.974
TG(16:0/16:0/18:1)	1.143	0.874	1.133	1.275	1.330	1.062	1.317
TG(16:0/16:0/18:1)	0.985	1.004	0.734	0.715	0.872	0.851	0.974
TG(14:0/18:0/18:1)	1.326	1.270	0.712	1.302	1.104	0.944	0.766
TG(16:0/16:0/18:0)	0.480	0.979	0.859	0.800	0.822	1.104	0.917
TG(16:0/17:1/18:1)	0.973	1.321	1.412	1.300	1.505	1.854	1.021
TG(16:0/17:0/18:1)	1.024	0.869	1.036	0.966	0.654	1.203	0.785

	A	E	G	J	K	M	O
TG(16:0/18:2/18:3)	1.293	0.999	1.134	0.886	1.207	1.002	1.322
TG(16:0/18:2/18:2)	1.715	1.556	1.537	1.127	1.525	1.138	1.815
TG(16:0/18:1/18:2)	1.386	1.082	1.116	0.939	1.214	1.226	1.215
TG(16:0/18:1/18:1)	1.106	1.040	1.043	0.999	0.968	1.090	1.068
TG(16:0/18:0/18:1)	0.923	0.840	1.038	0.913	0.713	1.262	1.060
TG(16:0/18:0/18:0)	1.080	1.176	1.030	0.815	0.719	0.831	0.973
TG(17:0/18:1/18:1)	1.064	1.081	1.029	1.032	0.859	1.001	1.090
TG(17:0/18:0/18:1)	1.008	0.857	1.084	0.969	0.724	0.932	1.056
TG(18:2/18:2/18:3)	1.182	1.007	0.869	0.745	1.074	1.079	1.294
TG(18:1/18:2/18:3)	1.288	0.877	1.110	1.055	1.504	1.155	1.355
TG(18:1/18:2/18:3)	1.362	0.692	1.466	1.074	1.591	1.526	1.433
TG(18:1/18:2/18:2)	1.270	0.941	1.090	0.885	1.311	1.119	1.123
TG(18:1/18:1/18:2)	1.020	0.911	1.064	0.875	1.077	1.103	1.177
TG(18:1/18:1/18:2)	1.072	0.871	1.113	0.846	1.034	0.983	1.099
TG(18:0/18:1/18:2)	1.149	1.025	1.111	1.038	1.002	0.983	1.105
TG(18:0/18:1/18:1)	1.080	0.910	1.029	1.104	0.865	1.136	1.090
TG(18:0/18:0/18:1)	0.889	0.808	0.769	0.859	0.901	0.725	0.736
TG(16:0/18:0/20:0)	1.227	1.022	0.698	0.659	0.771	0.958	1.008
TG(18:1/18:1/19:1)	1.175	0.999	1.154	1.013	1.027	1.049	1.099
TG(18:0/19:0/18:2)	1.104	0.787	1.061	0.990	0.956	0.931	0.881
TG(18:0/19:0/18:1)	0.876	1.061	0.696	1.013	0.770	1.089	0.573
TG(16:0/18:0/21:0)	0.987	1.045	1.070	0.906	0.949	1.109	1.062
TG(16:0/18:1/22:5)	1.164	1.017	1.185	1.019	1.181	1.259	1.128
TG(20:1/18:2/18:2)	0.960	0.668	0.926	1.072	1.420	1.369	1.004
TG(18:0/18:1/20:3)	1.179	0.572	1.150	0.784	1.134	1.156	1.214
TG(18:1/18:1/20:1)	1.340	1.188	1.121	1.147	1.223	0.998	1.313
TG(18:0/18:1/20:1)	0.993	0.989	0.832	0.747	0.842	0.924	0.375
TG(16:0/22:0/18:1)	1.092	1.242	1.556	1.288	1.533	1.700	1.378
TG(16:0/18:0/22:0)	0.999	1.045	0.874	0.858	0.748	0.750	1.047
TG(16:0/18:1/23:1)	0.992	1.001	1.055	0.902	0.958	1.114	0.961
TG(16:0/18:0/23:1)	0.890	1.069	1.036	0.948	0.960	1.080	1.095

	A	E	G	J	K	M	O
TG(16:0/17:0/24:0)	1.185	0.965	1.107	0.889	1.147	1.047	0.891
TG(22:1/18:2/18:2)	1.235	0.963	1.081	0.843	1.038	1.041	1.141
TG(16:0/24:1/18:2)	0.981	1.015	1.178	0.979	1.075	1.008	1.045
TG(16:0/24:0/18:2)	1.501	1.139	0.968	1.501	1.218	0.893	1.196
TG(16:0/24:0/18:1)	1.367	0.953	0.968	1.413	1.250	0.989	1.193
TG(16:0/18:0/24:0)	1.083	0.973	0.936	0.942	0.952	0.958	1.100
TG(24:0/18:1/18:1)	1.109	0.954	1.065	0.968	1.014	1.059	1.145
TG(16:0/26:0/18:1)	1.034	0.831	1.027	1.030	0.928	1.105	0.810

Table 35: Substrate specificity ratios for the lipases A, E, G, J, K, M and O towards different lipids in baked brioche (n = 3). – not detected. Data already prepared for publication ([162]).

	A	E	G	J	K	M	O
BisMePA(20:1/27:5)	0.911	1.049	0.906	0.921	1.076	1.062	1.082
DG(O-30:6)	1.205	1.296	1.049	1.319	0.716	1.362	1.316
DG(O-42:6)	0.000	0.852	0.000	0.000	1.141	0.529	1.713
DG(6:0/12:1)	0.697	0.662	0.896	0.823	1.188	1.066	0.662
DGDG(16:0/18:2)	1.335	1.321	1.193	1.248	1.542	1.527	1.489
DGDG(18:2/18:3)	1.226	1.342	1.040	0.957	1.280	1.070	1.381
DGDG(18:2/18:2)	1.176	1.249	1.184	0.879	1.126	1.140	1.473
DGMG(18:2)	0.449	0.829	1.063	0.922	0.740	0.596	0.593
LPC(16:1)	0.927	1.145	1.063	1.079	1.176	0.966	1.138
LPC(18:3)	1.290	1.446	1.313	1.354	1.415	1.232	1.190
LPC(18:1)	0.761	1.031	1.038	0.964	0.559	0.992	0.814
LPC(18:0)	0.696	0.933	0.866	0.685	0.672	0.875	0.777
LPC(22:6)	0.000	1.612	1.425	1.195	1.003	0.702	–
LPE(16:0)	1.096	1.210	1.109	0.983	1.190	1.137	1.369
LPE(18:2)	0.990	1.269	1.093	1.061	1.174	1.149	1.326
MG(18:3)	0.899	0.628	1.260	1.264	0.421	1.328	1.144
MG(18:2)	1.049	0.931	1.122	1.002	1.031	0.737	0.872
MGDG(18:3/18:2)	1.312	1.147	0.841	0.785	1.168	1.141	1.397

	A	E	G	J	K	M	O
MGDG(18:2/18:2)	1.381	1.147	0.979	0.882	1.184	1.126	1.589
MGMG(18:2)	0.601	1.029	1.267	1.330	0.974	0.819	0.815
PC(O-26:4/22:6)	1.026	0.985	0.932	1.109	1.334	1.069	1.050
PC(30:0)	1.358	1.170	0.975	1.036	1.043	0.954	1.554
PC(32:2)	1.062	1.064	1.159	1.127	1.126	1.154	1.130
PC(32:1)	1.404	1.292	1.075	1.086	1.138	1.182	1.634
PC(32:0)	1.253	1.176	1.028	0.930	1.136	1.010	1.361
PC(34:3)	0.836	1.029	0.999	0.835	1.032	0.771	0.979
PC(34:2)	0.997	1.115	1.049	1.025	1.040	1.048	1.194
PC(16:0/18:2)	1.086	1.119	1.107	1.152	1.103	1.127	1.150
PC(16:0/18:1)	1.876	1.183	1.150	1.070	1.408	1.226	1.909
PC(36:4)	0.850	0.942	0.753	1.173	0.989	1.075	0.686
PC(18:2/18:2)	0.956	1.021	1.005	0.999	1.019	1.033	0.999
PC(36:4)	0.986	1.477	1.249	1.458	2.421	1.160	1.302
PC(18:1/18:2)	0.772	0.823	0.910	0.728	0.764	0.856	0.562
PC(18:0/18:2)	1.324	1.303	0.906	1.004	1.052	1.161	1.453
PC(36:2)	1.234	1.266	1.041	0.925	1.097	1.083	1.385
PC(36:1)	1.553	1.254	1.079	0.982	1.293	1.205	1.774
PC(38:6)	1.437	1.459	1.247	1.151	1.223	1.120	1.510
PC(38:4)	1.120	1.654	1.242	1.106	1.523	1.318	1.683
PE(18:2/18:2)	0.938	1.459	1.237	1.179	1.339	1.184	1.353
PE(18:2/18:2)	0.822	1.535	1.256	1.078	1.162	0.978	1.086
PEt(O-26:3/18:4)	1.077	1.188	1.316	1.073	1.277	1.085	1.166
PEt(16:1/16:1)	1.222	1.219	1.020	0.950	1.204	1.314	1.085
PEt(10:0/22:1)	0.947	0.781	1.047	0.697	0.876	0.892	0.726
PEt(24:6/18:2)	1.628	1.519	0.807	1.195	1.432	1.314	2.054
PG(O-21:0/20:4)	0.741	1.043	0.826	0.831	0.494	1.055	0.349
PG(O-28:5/20:3)	1.072	1.144	1.842	1.449	1.359	1.864	0.953
PG(O-28:3/20:4)	1.051	1.099	1.169	1.030	1.012	1.016	0.907
PG(28:0/16:3)	0.933	1.076	1.042	1.216	0.852	0.723	0.982
PG(28:1/22:5)	1.180	1.393	0.959	1.401	1.082	1.435	1.351

	A	E	G	J	K	M	O
PIP2(16:3/6:0)	0.915	0.803	1.270	0.868	0.904	1.222	0.861
PIP3(P-6:0/16:3)	0.901	1.074	1.330	1.216	1.352	1.209	1.068
SM(d34:1)	0.786	1.207	0.786	0.839	1.200	1.005	1.235
SM(d40:1)	0.316	0.483	1.255	1.090	0.000	1.636	1.642
SPH(d18:0)	1.380	0.829	0.982	0.933	0.722	1.069	0.959
SPH(d14:1)	3.242	0.773	1.378	1.058	0.792	1.654	1.275
SPH(d16:1)	2.539	1.060	1.197	1.636	0.878	1.390	0.964
SPH(d20:1)	0.971	1.648	1.515	1.344	1.147	1.789	1.435
TG(P-32:8/24:6)	0.923	0.903	0.654	0.871	0.495	0.649	0.429
TG(P-25:8/36:9)	1.165	1.396	1.015	1.245	1.208	1.469	1.450
TG(P-62:18)	1.142	1.148	1.271	1.023	1.195	0.954	0.922
TG(P-38:10/24:7)	1.173	1.160	0.874	1.224	0.592	1.038	0.881
TG(P-38:10/25:7)	0.991	1.003	1.184	1.194	0.776	0.876	0.689
TG(P-22:7/23:7/23:7)	0.852	1.190	1.134	1.121	1.210	0.959	1.108
TG(P-50:16/18:4)	0.864	1.225	0.986	0.862	0.725	1.188	1.077
TG(P-43:13/26:7)	0.906	1.021	0.985	0.893	1.096	1.062	0.937
TG(O-4:0/12:1/2:0)	0.819	0.934	1.171	1.158	3.259	1.212	0.631
TG(O-28:8/24:7)	1.030	0.993	0.950	1.040	1.362	1.244	0.972
TG(O-28:8/28:7)	0.937	1.018	0.849	0.676	0.889	0.662	0.546
TG(O-47:15/22:6)	1.045	1.102	1.056	0.828	1.195	1.138	1.001
TG(4:0/8:0/12:0)	0.846	0.733	0.411	0.357	0.637	0.608	0.837
TG(4:0/4:0/18:1)	1.122	1.085	1.071	0.721	1.060	1.100	1.230
TG(4:0/8:0/14:0)	1.410	1.413	1.839	1.317	1.461	1.063	0.934
TG(4:0/6:0/18:1)	1.496	1.464	1.533	1.525	1.290	0.765	1.765
TG(8:0/10:0/10:0)	0.900	0.962	1.310	1.510	1.420	1.047	1.409
TG(4:0/10:0/14:0)	1.014	0.923	0.874	0.829	0.667	0.758	1.001
TG(6:0/8:0/16:2)	1.023	0.980	0.908	0.889	0.877	0.939	1.030
TG(4:0/8:0/18:1)	1.033	0.984	1.165	0.780	0.880	0.749	1.287
TG(10:0/10:0/10:0)	0.884	0.935	1.434	1.927	1.674	1.343	1.417
TG(4:0/12:0/14:0)	1.258	1.146	1.180	1.196	0.652	1.024	1.004
TG(6:0/8:0/16:0)	1.338	1.178	1.180	1.196	0.652	1.024	1.258

	A	E	G	J	K	M	O
TG(4:0/10:0/18:2)	1.031	1.059	0.730	0.947	0.900	1.022	1.090
TG(4:0/10:0/18:2)	1.031	0.973	0.730	0.947	0.900	1.021	1.115
TG(4:0/10:0/18:1)	0.980	0.957	0.861	0.770	0.824	0.852	0.961
TG(4:0/12:0/16:0)	1.301	1.083	1.035	0.819	0.948	0.832	1.293
TG(4:0/12:0/16:0)	0.894	0.984	1.055	1.010	1.187	1.081	1.115
TG(4:0/12:0/17:1)	1.809	1.373	1.847	1.323	1.427	1.016	1.992
TG(4:0/12:0/18:3)	1.201	0.979	1.170	1.175	1.169	1.134	0.997
TG(4:0/12:0/18:2)	1.025	0.778	0.864	0.871	0.831	0.950	1.010
TG(6:0/10:0/18:1)	0.943	1.098	1.060	0.995	0.841	0.710	1.138
TG(4:0/14:0/16:0)	0.779	0.881	0.858	0.699	0.747	0.797	0.857
TG(4:0/14:0/17:1)	1.292	0.429	0.448	0.526	0.709	0.797	1.481
TG(4:0/14:0/18:3)	1.142	0.912	1.084	1.098	0.768	0.935	0.951
TG(4:0/14:0/18:2)	0.885	0.795	1.059	0.529	0.687	0.816	0.907
TG(4:0/14:0/18:1)	0.979	1.115	1.141	0.843	0.905	0.830	1.039
TG(6:0/14:0/16:0)	0.983	1.041	1.609	2.472	2.513	1.515	1.884
TG(4:0/14:0/18:0)	0.926	0.750	1.687	2.191	2.613	1.570	1.971
TG(4:0/16:0/16:0)	1.072	1.174	1.105	1.087	2.382	0.976	2.840
TG(4:0/16:0/17:0)	0.309	0.354	0.335	0.254	0.097	0.336	0.378
TG(4:0/16:0/18:3)	0.827	0.979	0.947	0.766	0.868	0.899	0.992
TG(4:0/16:0/18:2)	0.779	0.834	1.105	0.896	0.509	0.877	0.694
TG(4:0/16:0/18:1)	0.463	0.545	0.497	0.642	1.003	0.866	0.510
TG(4:0/16:0/18:0)	0.901	0.639	1.591	2.314	2.233	1.263	1.871
TG(6:0/16:0/16:0)	1.030	1.398	1.023	1.200	1.044	0.974	0.939
TG(4:0/16:0/18:0)	0.665	1.129	0.741	0.865	1.046	0.863	0.954
TG(8:0/14:0/16:0)	0.929	1.282	0.817	1.271	1.422	0.894	0.734
TG(4:0/17:1/18:1)	1.875	1.884	1.980	1.391	1.789	1.475	2.598
TG(4:0/17:0/18:1)	1.927	0.818	1.159	0.710	11.920	1.373	0.655
TG(4:0/18:1/18:3)	0.919	1.058	0.840	0.746	0.814	0.814	0.900
TG(4:0/18:1/18:2)	0.909	0.992	1.061	0.852	0.900	1.012	0.941
TG(30:2/4:0/6:0)	0.724	1.062	1.011	1.049	0.072	0.966	1.005
TG(4:0/18:1/18:1)	0.865	1.045	1.339	1.155	1.217	1.723	1.059

	A	E	G	J	K	M	O
TG(6:0/16:1/18:1)	0.940	1.053	1.076	1.008	1.034	1.083	1.061
TG(6:0/16:0/18:1)	0.895	0.934	1.186	0.905	1.224	0.910	1.034
TG(6:0/16:0/18:0)	1.038	0.912	1.613	2.572	2.033	1.271	1.840
TG(6:0/16:0/18:0)	1.394	1.075	1.556	1.483	1.393	1.009	1.216
TG(10:0/14:0/16:0)	0.955	0.758	0.790	0.833	0.882	0.643	0.827
TG(6:0/17:0/18:1)	0.958	0.981	0.973	1.012	0.940	0.970	1.049
TG(6:0/18:1/18:2)	1.699	0.882	1.994	2.127	2.669	1.256	1.462
TG(18:2/12:0/12:0)	0.916	1.262	1.070	2.206	1.373	0.957	1.298
TG(4:0/20:0/18:1)	2.285	2.092	3.927	4.535	3.726	2.106	3.747
TG(8:0/16:0/18:1)	1.035	0.357	0.791	0.271	0.021	0.999	0.310
TG(8:0/16:0/18:1)	0.540	0.745	0.975	0.810	0.693	0.821	0.848
TG(10:0/12:0/20:0)	0.939	0.871	0.959	2.014	1.781	1.066	1.583
TG(12:0/14:0/16:0)	1.024	1.164	0.951	1.290	1.133	1.206	1.170
TG(8:0/18:0/17:1)	0.255	0.267	0.351	1.186	0.335	0.800	0.445
TG(8:0/18:1/18:2)	0.693	0.704	0.934	0.938	0.500	0.634	0.926
TG(28:2/6:0/10:0)	0.158	1.108	0.847	0.249	1.226	1.057	1.016
TG(10:0/16:0/18:2)	0.869	0.553	1.142	1.297	1.167	0.960	0.569
TG(10:0/16:1/18:1)	0.953	0.934	0.897	0.991	1.024	0.851	0.962
TG(28:1/6:0/10:0)	0.076	0.099	0.146	0.189	0.118	0.098	0.129
TG(12:0/14:0/18:1)	1.620	1.120	1.353	1.018	1.598	1.119	1.048
TG(10:0/16:0/18:1)	0.971	0.972	0.970	0.900	0.347	0.921	1.209
TG(10:0/16:0/18:0)	0.779	1.103	1.032	0.941	0.898	0.845	0.600
TG(10:0/17:1/18:1)	1.157	0.738	0.901	1.265	0.813	1.127	0.435
TG(10:0/18:1/18:3)	0.995	1.019	0.986	1.008	0.956	0.982	1.018
TG(10:0/18:1/18:2)	0.807	0.856	0.961	0.348	0.141	0.733	1.140
TG(10:0/18:1/18:1)	1.074	0.865	0.829	1.177	1.010	0.743	0.853
TG(10:0/18:1/18:1)	1.052	0.843	0.691	1.024	0.961	0.751	0.856
TG(10:0/18:0/18:1)	0.936	0.780	1.005	1.053	0.958	0.913	1.262
TG(12:0/16:0/18:1)	0.942	0.929	1.033	1.087	0.833	0.966	1.162
TG(14:0/16:0/16:0)	1.078	1.302	2.172	1.311	0.944	1.039	3.387
TG(12:0/16:0/18:0)	1.020	0.426	0.965	1.080	0.697	0.911	1.295

	A	E	G	J	K	M	O
TG(12:0/17:1/18:1)	1.150	1.052	1.191	1.187	0.759	1.013	1.010
TG(14:0/16:0/17:0)	1.058	0.878	0.853	0.866	0.835	0.959	1.108
TG(12:0/18:1/18:3)	0.965	0.999	1.008	1.007	0.976	1.019	1.072
TG(12:0/18:1/18:2)	0.979	0.843	1.223	0.855	0.957	0.844	1.053
TG(16:0/14:1/18:1)	2.962	3.288	2.436	3.376	2.666	3.209	2.147
TG(14:0/16:0/18:1)	1.077	9.178	2.893	1.230	1.309	9.676	0.886
TG(14:0/16:0/18:0)	0.949	1.346	0.935	0.936	0.253	0.769	1.511
TG(14:0/18:1/18:3)	1.009	1.123	1.228	1.285	1.108	1.141	7.186
TG(14:0/18:1/18:2)	0.829	1.022	0.869	0.931	0.970	0.808	0.215
TG(16:0/16:1/18:1)	0.963	0.992	0.812	1.037	0.777	0.984	0.190
TG(16:0/16:0/18:1)	1.248	0.330	1.096	0.143	1.142	0.658	1.121
TG(16:0/16:0/18:0)	1.016	0.905	1.054	1.049	0.863	0.829	4.022
TG(16:0/17:1/18:1)	1.406	1.124	1.131	1.157	1.133	0.805	0.804
TG(16:0/17:0/18:1)	1.180	1.215	1.132	1.027	0.902	1.065	1.004
TG(16:0/18:2/18:3)	0.944	1.121	1.008	1.039	0.999	1.034	2.406
TG(16:0/18:2/18:2)	0.988	0.984	1.056	0.991	1.780	1.097	0.185
TG(16:0/18:1/18:2)	0.880	1.033	0.954	0.969	1.026	0.966	0.242
TG(16:0/18:1/18:1)	1.228	1.083	1.195	1.064	1.265	1.275	0.715
TG(16:0/18:1/18:1)	1.773	1.354	1.567	1.698	1.090	1.785	1.761
TG(16:0/18:0/18:1)	0.842	0.979	1.204	1.216	0.817	0.829	1.984
TG(16:0/18:0/18:0)	0.776	0.951	0.717	0.730	0.672	0.586	9.787
TG(17:0/18:1/18:1)	1.143	1.296	1.190	0.470	1.254	1.129	0.255
TG(17:0/18:0/18:1)	0.742	1.171	1.130	1.076	1.189	1.159	1.201
TG(18:2/18:2/18:3)	0.973	0.902	1.135	1.173	1.133	1.164	1.633
TG(18:2/18:2/18:3)	0.999	1.299	1.172	1.392	1.128	1.162	1.315
TG(18:1/18:2/18:3)	0.845	0.661	0.718	0.736	0.622	0.737	0.168
TG(18:1/18:2/18:3)	0.907	0.885	0.874	0.846	1.206	0.741	4.321
TG(18:1/18:2/18:2)	1.027	1.063	0.827	1.346	1.139	1.031	0.242
TG(18:1/18:1/18:2)	0.801	1.054	0.979	0.989	1.090	1.084	0.692
TG(18:0/18:2/18:2)	0.801	1.054	0.979	0.989	1.068	1.084	1.086
TG(18:1/18:1/18:2)	0.731	1.076	1.165	1.141	1.127	1.136	1.360

	A	E	G	J	K	M	O
TG(18:0/18:1/18:2)	0.996	0.928	0.612	0.759	1.038	0.895	0.523
TG(18:1/17:2/20:4)	1.296	1.051	0.883	0.511	15.901	1.532	43.377
TG(16:0/18:0/20:0)	0.798	0.940	0.908	1.185	0.997	0.615	1.291
TG(18:1/18:1/19:1)	0.946	1.221	1.209	1.157	1.165	1.014	0.381
TG(18:0/18:1/19:1)	0.939	1.041	0.960	0.731	0.988	1.009	0.661
TG(16:0/21:0/18:1)	1.008	1.159	1.027	1.147	0.774	1.039	1.233
TG(18:1/18:2/20:2)	0.726	0.925	0.777	0.854	0.976	0.865	0.463
TG(18:1/20:1/18:2)	1.041	0.976	1.306	1.169	1.244	1.260	1.129
TG(18:1/18:1/20:1)	1.601	1.354	1.779	1.158	1.502	1.580	1.584
TG(18:0/18:1/20:1)	0.856	1.055	1.032	1.022	0.848	0.857	0.816
TG(16:0/22:0/18:1)	0.296	1.242	1.189	1.259	1.054	1.247	1.699
TG(16:0/18:0/22:0)	0.906	0.994	0.946	0.800	0.953	0.851	1.914
TG(16:0/18:1/23:1)	0.728	0.952	1.043	1.062	0.995	1.010	0.472
TG(16:0/18:0/23:1)	0.915	0.976	1.015	0.982	0.979	0.987	1.051
TG(16:0/17:0/24:0)	0.935	1.100	1.052	0.991	0.976	1.084	2.260
TG(22:0/18:1/18:2)	0.946	0.961	1.002	1.104	1.038	0.965	0.917
TG(16:0/24:0/18:2)	0.899	0.847	1.195	0.622	1.095	1.147	0.726
TG(16:0/24:0/18:1)	2.612	1.811	2.787	2.692	5.840	2.794	4.126
TG(16:0/18:0/24:0)	0.912	1.010	0.857	0.859	0.702	0.835	0.953

A.4. Establishment of a new lipase activity assay for the use of baking lipases in fine bakery goods

Table 36: Lipase activities as measured using three commercially available lipase activity assay kits. Values are given as mean (n = 3) ± standard deviation.

Lipase	Assay I	Assay II	Assay III
	Lipase activity [µmol $min^{-1}mg^{-1}$]	Lipase activity [nmol $min^{-1}mg^{-1}$]	Lipase activity [nmol $min^{-1}mg^{-1}$]
A	1.779 ± 0.119	63.865 ± 1.129	1427.38 ± 21.95
E	0.574 ± 0.038	23.301 ± 0.317	1306.23 ± 8.55
G	2.045 ± 0.077	121.990 ± 2.565	2003.26 ± 158.15
J	1.820 ± 0.044	201.963 ± 3.214	2244.74 ± 81.07
K	0.640 ± 0.043	6.684 ± 0.143	162.78 ± 12.42
M	1.055 ± 0.078	60.956 ± 0.725	1490.79 ± 69.54
O	0.267 ± 0.023	68.841 ± 2.298	861.91 ± 70.54

Table 37: Lipase activities towards different substrates measured using the newly developed lipase activity assay. Values are given as mean (n = 6) ± standard deviation. DOPC - 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine

Substrate	Olive oil	Wheat germ oil	Flaxseed oil	Coconut oil	Butter	Triolein	DOPC
Lipase	[mmol $min^{-1}mg^{-1}$]						
A	39.610 ± 4.857	121.212 ± 5.517	90.990 ± 1.994	51.505 ± 0.670	59.092 ± 5.688	31.469 ± 3.233	4.119 ± 0.458
E	149.336 ± 11.628	19.564 ± 1.723	131.911 ± 4.991	45.714 ± 2.418	38.313 ± 5.850	9.287 ± 0.472	5.334 ± 0.612
G	59.452 ± 4.367	93.303 ± 10.303	96.200 ± 6.707	42.729 ± 1.673	90.176 ± 10.651	28.069 ± 0.206	4.833 ± 0.397
J	83.704 ± 2.846	124.643 ± 15.111	158.030 ± 18.337	88.888 ± 6.548	75.170 ± 0.351	104.475 ± 9.583	16.773 ± 1.700
K	10.875 ± 1.510	10.418 ± 1.287	63.434 ± 1.807	26.100 ± 2.842	42.626 ± 3.795	1.244 ± 0.176	5.315 ± 0.451
M	100.287 ± 11.190	9.227 ± 0.257	129.097 ± 7.274	40.738 ± 6.816	30.393 ± 0.428	5.282 ± 0.346	5.397 ± 0.637
O	107.298 ± 12.064	34.653 ± 2.300	108.492 ± 9.314	196.512 ± 9.851	93.833 ± 1.091	61.452 ± 5.048	7.671 ± 0.708