

# Mathematical modelling of oleic acid epoxidation via a chemo-enzymatic route – From reaction mechanisms to reactor model



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## HIGHLIGHTS

- Oleic acid was successfully epoxidized in the presence of lipase catalyst.
- Experimental data were produced in a multiphase batch reactor.
- Several reaction mechanisms were considered and rate equations were derived.
- The proposed kinetic models are useful for molecular-level understanding and process design.

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## ABSTRACT

The immobilized enzyme lipase acts as an efficient, selective and durable catalyst in the direct transformation of unsaturated carboxylic acids to epoxides, which are used as chemical intermediates and bio-lubricants. Experimental data obtained from the epoxidation of a model molecule, oleic acid in a laboratory-scale isothermal batch reactor were critically evaluated and mathematically modelled in the most precise way. Several rival surface reaction mechanisms were proposed and rate equations based on these mechanisms were derived. The rate equations were implemented in a multiphase model for the laboratory-scale batch reactor and the kinetic and adsorption parameters included in the rate equations were estimated with non-linear regression analysis. Based on the parameter estimation statistics and chemical knowledge, the most plausible kinetic models for the chemo-enzymatic epoxidation of oleic acid on the immobilized lipase catalyst were selected. The best kinetic models gave a good reproduction of the experimental data. The models can be used to predict the performance of enzymatic epoxidation of unsaturated fatty acids.

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## 1. Introduction

Epoxidized fatty acids are exciting green biodegradable components, which are used as chemical intermediates and bio-lubricants. In the Fenno-Scandic area, the main source of fatty acids is the non-edible tall oil, which is a side product of the well-established Kraft pulping process. Tall oil consists of fatty acids, resin acids and neutral components (Aryan and Kraft, 2021). The dominating fatty acids in tall oil are oleic, linoleic, stearic and rosin acids. The classical technology of fatty acid epoxidation relies on the indirect method of Prileschajew (1909), which works well as such and the process can be enhanced by incorporating heterogeneous or homogeneous acid catalysts and exposing microwave

irradiation on the multiphase reaction mixture (Leveneuer et al., 2014; Freites Aguilera et al., 2019). The drawback of the Prilezhaev process is the complex reaction system as such and the risks for temperature runaway in the formation of the percarboxylic acids as reaction intermediates (Pérez-Sena et al., 2020a). Moreover, a part of the epoxides obtained in the Prilezhaev method are destroyed by ring-opening reactions promoted by the presence of acidic species in the reaction mixture (Cai et al., 2018; Freites Aguilera et al., 2019, 2020). Therefore, the scientific community is actively searching for alternative and simpler reaction pathways for fatty acid epoxidation, for instance by using classical heterogeneous catalysts, such as aluminium oxide (Perez-Sena et al., 2020b) or by entering the chemo-enzymatic pathway (Warwel et al., 1995; Orellana-Coca et al., 2005a, 2005b; Vlček and Petrović, 2006; Bhalariao et al., 2017; Zhang et al., 2018; Kirpluks et al., 2019; de Schneider et al., 2009; Hilker et al., 2001; Aouf et al., 2014). In this approach, there is no need for the addition of a carboxylic acid as

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**Nomenclature**

$A$	interfacial area
$c$	concentration
$c$	concentration of vacant sites on the catalyst
$c_0$	total concentration of accessible sites on the catalyst
$K$	equilibrium constant
$k$	rate constant
$k'$	merged rate parameter
$m$	mass
$N$	interfacial flux
$n$	amount of substance
$Q$	objective function
$r$	rate
$s$	observed state variable
$V$	volume
$t$	time
$\alpha$	phase ratio: volume of aqueous phase/ total volume
$\rho$	density

**Subscripts**

a	aqueous phase
c	catalyst

exp	experimental quantity
i, j	component index
o	oil phase
t	experimental time index

**Abbreviations**

A	unsaturated fatty acid (RCOOH)
AH	surface complex of fatty acid and hydrogen peroxide
B	peroxy fatty acid (RCOOOH)
H	hydrogen peroxide
HP	hydrogen peroxide
OA	oleic acid
R	epoxidized fatty acid
RO	ring opening
ROP	ring opening product
ROPR	ring opening product of epoxidized fatty acid (R)
ROPS	ring opening product of epoxidized peroxy fatty acid (S)
S	epoxidized peroxy fatty acid
W	Water
*	active surface site

an oxygen carrier, since the carboxylic group in the fatty acid is performing this role. The use of naturally appearing fatty acid mixtures – e.g. those obtained in tall oil – is a pragmatic view of the technology development, but in order to get a much deeper understanding on the reaction mechanism and kinetics, studies with model molecules are indispensable.

We have investigated the epoxidation of a model molecule for fatty acids, oleic acid, in the presence of immobilized lipase as the heterogenized enzymatic catalyst in a vigorously stirred laboratory-scale batch reactor. Oleic acid has only one double bond which can be epoxidized, which makes the interpretation of the experimental results more straightforward than for multifunctional fatty acids. Kinetic experiments were conducted under isothermal conditions at 50 °C and samples were withdrawn for chemical analysis (Lindroos, 2021; Freites Aguilera et al., 2021). The concentrations of the double bonds and the epoxide groups were determined by separate titrations of the iodine value and the oxirane number (Jay, 1964; Freites Aguilera et al., 2018). The titration method of Greenspan and MacKellar (1948) was used to determine the concentrations of hydrogen peroxide and peroxycarboxylic acids. The catalyst activity, durability and selectivity were confirmed in the experimental work, thus the next step in this series of studies is to take a quantitative approach: detailed consideration of reaction mechanisms, kinetics and mathematical modelling. The reaction stoichiometry and the molecular reaction mechanisms are considered, the rate equations for rival mechanisms are derived and the parameters in the rate equations are estimated with nonlinear regression analysis.

## 2. Stoichiometry, reaction mechanisms and rate equations

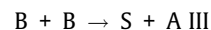
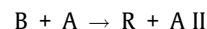
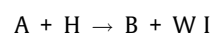
### 2.1. Reaction stoichiometry and overview of mechanisms

Different hypotheses can be proposed for the molecular mechanism for the epoxidation of unsaturated fatty acids with hydrogen peroxide in the presence of lipase as the catalyst (Björkling et al., 1990; Yadav and Maujula Devi, 2001; Rüscher et al., 1999). The fundamental question is, whether the epoxidation takes place directly between hydrogen peroxide and the double bond in the fatty acid or is it initiated by the formation of a peroxycarboxylic acid which

attacks the double bond in the fatty acid forming the epoxide and regenerating the original carboxylic group in the fatty acid. The participating molecules are displayed in Fig. 1.

In 2001 Yadav and Devi proposed the existence of a ternary complex, both the carboxylic acid and hydrogen peroxide being bound to the lipase before the formation of an acyl-enzyme intermediate and elimination of water in the epoxide formation. In 2004, Bugg suggested an alternative mechanism where the catalytic serine stabilizes the carboxylic acid substrate with a hydrogen bond, and the substrates were bound to a lipase enzyme at the same time. In this case, the peroxycarboxylic acid formation takes place without the formation of an acyl enzyme intermediate. Support to this mechanism might originate from specific catalyst analyses, such as an X-ray crystal structure of propionate bound to the active site of a mutated *Pseudomonas fluorescens* esterase which can reveal the perhydrolase activity (Bernhardt et al., 2005; Yin et al., 2010).

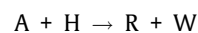
If the reaction proceeds via the formation of the peroxycarboxylated species (B), the overall stoichiometry is (see the molecules in Fig. 1)



This reaction scheme is illustrated in Fig. 1.1.

It should be noticed that the molecule A on the right-hand side of step II is generated from the molecule B on the left-hand side, i.e. the peroxycarboxylic acid formed in step I.

The alternative mechanistic hypotheses suggest that the peroxycarboxylic acid (B) does not exist, and consequently, the epoxidized peroxycarboxylic acid (S) does not exist. The reaction stoichiometry is thus very straightforward,



and this reaction scheme is illustrated below in Fig. 1.2.

To progress further towards operational rate equations, it is necessary to assume a detailed molecular reaction mechanism of

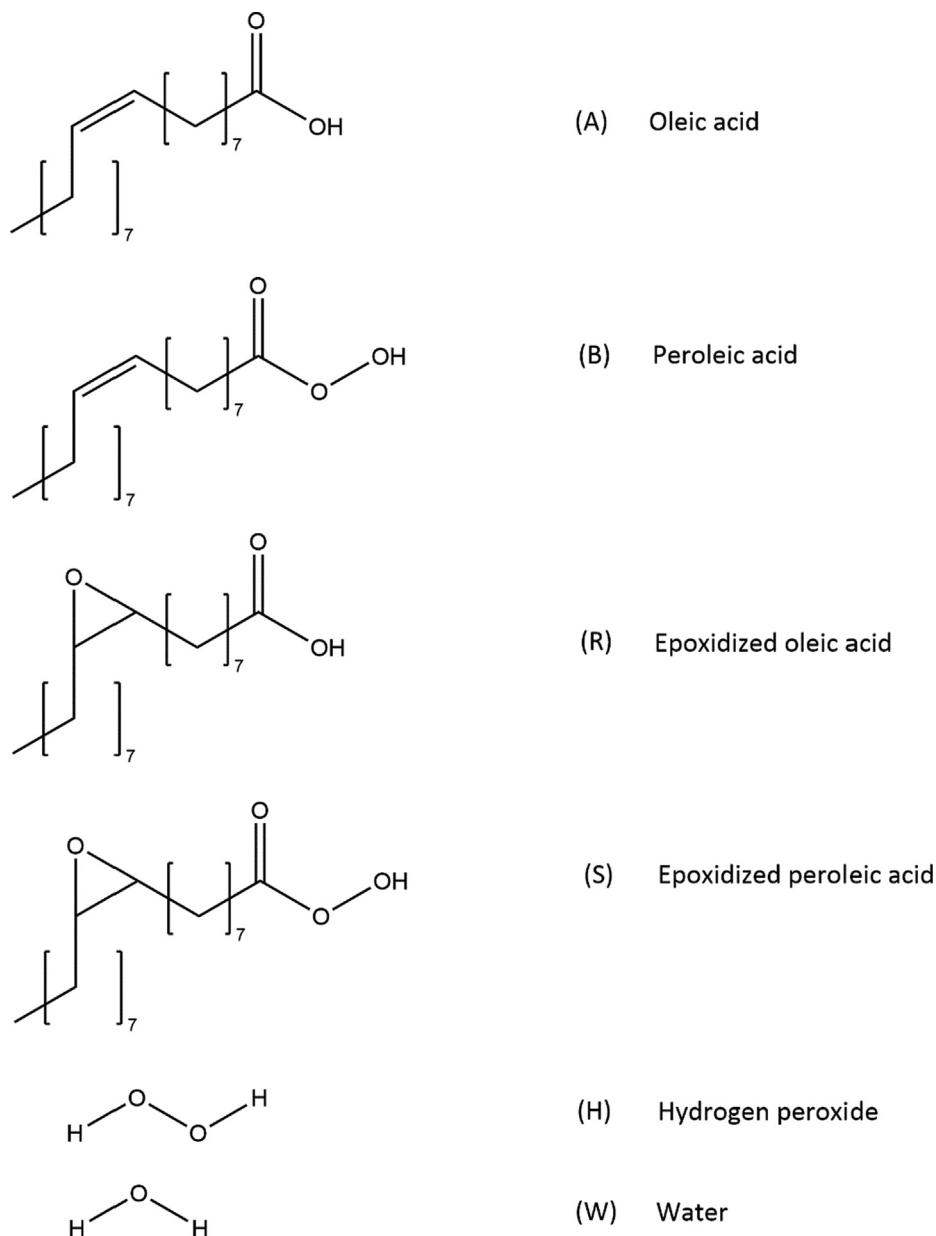


Fig. 1. Molecules participating in the epoxidation of unsaturated carboxylic acid.

the fatty acid epoxidation on the catalyst surface. The accessible surface sites are denoted by an asterisk (\*) in the sequel.

Starting from the assumption that a percarboxylic acid (B) exists as a reaction intermediate and presuming that all the components are able to adsorb on the catalyst surface, the overall reactions (I-III) are split to surface reaction and adsorption/ desorption steps. The adsorption and desorption steps are taken as rapid reversible steps, whereas the surface reaction steps are assumed to be irreversible, because hydrogen peroxide cannot be rebuilt after the peroxide bond has been broken, either by donating an oxygen atom to form an epoxide or by decomposing to molecular oxygen and water.

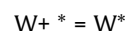
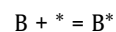
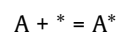
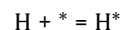
## 2.2. Derivation of rate equations for rival epoxidation mechanisms

It is not a priori clear, how the epoxidation of a double bond of a carboxylic acid proceeds in the presence of a lipase catalyst. There-

fore, alternative mechanistic hypotheses are considered and the corresponding rate equations are derived.

### 2.2.1. Mechanism 1A

The elementary steps for reactions I-III are listed below. For the sake of simplicity, it is assumed that hydrogen peroxide, water, the double bonds and epoxides occupy only one basic site on the catalyst surface. The mechanistic sequence is given below (rds = rate determining step).



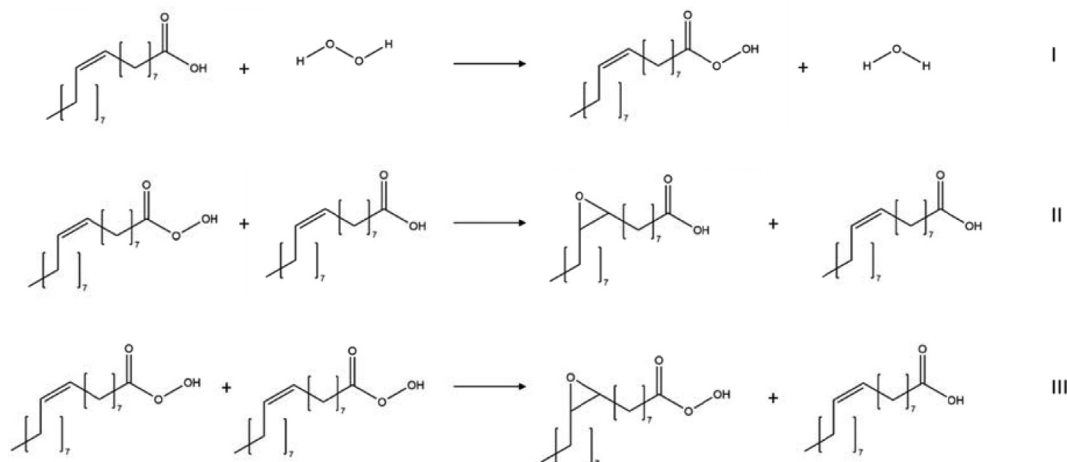


Fig. 1.1. Reaction scheme via the formation of the percarboxylated species.

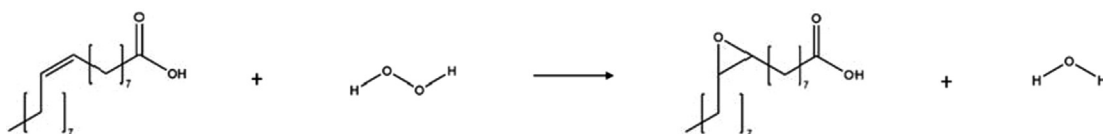
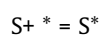
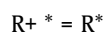
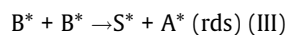
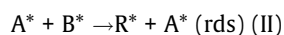
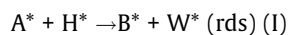
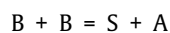
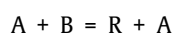
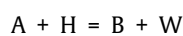


Fig. 1.2. Reaction scheme in the absence of the formation of the percarboxylated species.



Overall reactions



The rate determining surface reaction steps are denoted by Roman numerals (I-III) above. The rates of the steps are given by

$$r_I = k_I c_A^* c_{H^*} \quad (1)$$

$$r_{II} = k_{II} c_A^* c_{B^*} \quad (2)$$

$$r_{III} = k_{III} c_{B^*}^2 \quad (3)$$

where  $c_A^*$ ,  $c_{B^*}$  and  $c_{H^*}$  denote the surface concentrations of the adsorbed species.

Application of the quasi-equilibrium hypothesis on the adsorption and desorption steps implies that the expression

$$K_i = \frac{c_i^*}{c_i c^*} \quad (4)$$

is valid for all the adsorbed components molecules. The total balance of the surface species is valid, which implies that the total concentration of the surface species ( $c_0$ ) is equal to the sum of adsorbed species ( $c_j^*$ ) and vacant surface sites ( $c^*$ ),

$$c^* + \sum c_j^* = c_0 \quad (5)$$

Taking into account the quasi-equilibrium equilibria, the concentration of vacant sites is easily solved, from the total site balance,

$$c^* = \frac{c_0}{1 + \sum K_j c_j} \quad (6)$$

Analogously, for each adsorbed molecule is obtained

$$c_i^* = \frac{K_i c_i c_0}{1 + \sum K_j c_j} \quad (7)$$

The expressions for  $c^*$  and  $c_j^*$  are inserted in rate equations (1)-(3) which become

$$r_I = \frac{k_I K_A K_H c_A c_H c_0^2}{(1 + \sum K_j c_j)^2} \quad (8)$$

$$r_{II} = \frac{k_{II} K_A K_B c_A c_B c_0^2}{(1 + \sum K_j c_j)^2} \quad (9)$$

$$r_{III} = \frac{k_{III} K_B^2 c_B^2 c_0^2}{(1 + \sum K_j c_j)^2} \quad (10)$$

If the adsorption of a component is weak, the corresponding adsorption equilibrium parameter ( $K_j$ ) is set to zero. In case of catalyst deactivation, the total concentration of active sites ( $c_0$ ) decreases with time and from batch to batch. The products of rate and equilibrium constants can be merged to single parameters. The operative forms of the rate equations are

$$r_I = \frac{k'_1 c_A c_H c_0^2}{(1 + \sum K_j c_j)^2} \quad (11)$$

$$r_{II} = \frac{k'_2 c_A c_B c_0^2}{(1 + \sum K_j c_j)^2} \quad (12)$$

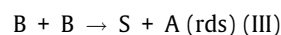
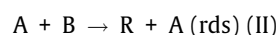
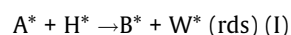
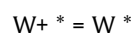
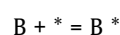
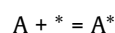
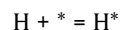
$$r_{III} = \frac{k'_3 c_B^2 c_0^2}{(1 + \sum K_j c_j)^2} \quad (13)$$

where

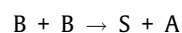
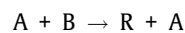
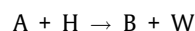
$$\sum K_j c_j = K_A c_A + K_B c_B + K_R c_R + K_S c_S + K_H c_H + K_W c_W \quad (14)$$

### 2.2.2. Mechanism 1B

A modification of Mechanism 1A is proposed. This time the formation of the peracid is presumed to take place on the lipase surface, whereas the epoxidation itself proceeds in the bulk phase of the oil, according to the Prilezhaev mechanism. The mechanism is given below,



Overall reactions



The rate determining surface reaction steps are denoted by Roman numerals (I-III) above. The rates of the elementary steps are written as

$$r_I = k_I c_A^* c_H^* \quad (15)$$

$$r_{II} = k_{II} c_A c_B \quad (16)$$

$$r_{III} = k_{III} c_B^2 \quad (17)$$

where  $c_A^*$ ,  $c_B^*$  and  $c_H^*$  denote the surface concentrations of the adsorbed species.

Application of the quasi-equilibrium hypothesis on the adsorption and desorption steps implies that the expression

$$K_i = \frac{c_i^*}{c_i c^*} \quad (18)$$

is valid for all the adsorbed components molecules. The total balance of the surface species implies that the total concentration of the surface species ( $c_0$ ) is equal to the sum of adsorbed species ( $c_j^*$ ) and vacant surface sites ( $c^*$ ),

$$c^* + \sum c_j^* = c_0 \quad (19)$$

The concentration of vacant sites is solved from the total site balance,

$$c^* = \frac{c_0}{1 + \sum K_j c_j} \quad (20)$$

For the adsorbed species is obtained

$$c_i^* = \frac{K_i c_i c_0}{1 + \sum K_j c_j} \quad (21)$$

The expressions for  $c^*$  and  $c_j^*$  are inserted in rate equations (1)-(3) which become

$$r_I = \frac{k_I K_A K_H c_A c_H c_0^2}{(1 + \sum K_j c_j)^2} \quad (22)$$

$$r_{II} = k_{II} c_A c_B \quad (23)$$

$$r_{III} = k_{III} c_B^2 \quad (24)$$

The products of rate and equilibrium constants are merged, giving the rate equations

$$r_I = \frac{k'_1 c_A c_H c_0^2}{(1 + \sum K_j c_j)^2} \quad (25)$$

$$r_{II} = k'_2 c_A c_B \quad (26)$$

$$r_{III} = k'_3 c_B^2 \quad (27)$$

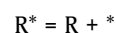
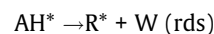
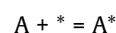
where

$$\sum K_j c_j = K_A c_A + K_B c_B + K_H c_H + K_W c_W \quad (28)$$

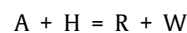
From a formal viewpoint, model 1B (equations (25)–(27)) is a special case of model 1A (equations (11)–(13)).

### 2.2.3. Mechanism 2

The mechanism is based on direct epoxidation of the carboxylic acid with hydrogen peroxide. An active complex is formed with the enzyme catalyst. A set of steps can be written in the spirit of [Yadav and Maujula Devi \(2001\)](#) as follows,



Overall reaction



It should be noticed that this mechanism excludes the existence of adsorbed water and hydrogen peroxide on the active sites of the catalyst. If the reaction of the active complex ( $AH^*$ ) is assumed to be the rate determining step, the reaction kinetics is given by

$$r = k c_{AH^*} \quad (29)$$

After assuming that the adsorption equilibria are rapid compared to the surface reaction step, the concentrations of the adsorbed species are obtained from

$$K_A = \frac{c_A^*}{c_A c^*} \quad (30)$$

$$K_R = \frac{c_R^*}{c_R c^*} \quad (31)$$

$$K_{AH} = \frac{c_{AH^*}}{c_A^* c_H} \quad (32)$$

A combination of these expressions with the total balance

$$c_A^* + c_{AH^*} + c_R^* + c^* = c_0 \quad (33)$$

gives the rate equation

$$r = \frac{k K_A K_{AH} c_A c_H c_0}{1 + K_A c_A + K_A K_{AH} c_A c_H + K_R c_R} \quad (34)$$

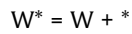
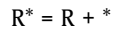
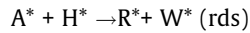
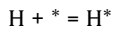
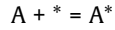
which can be written in a compressed form

$$r = \frac{k' c_A c_H c_0}{1 + K_A c_A + K_A K'_{AH} c_A c_H + K_R c_R} \quad (35)$$

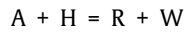
If it is assumed that both water and hydrogen peroxide adsorb on the surface but act as spectators, the adsorption terms are added to the denominator of equation (35).

#### 2.2.4. Mechanism 3

Provided that the direct epoxidation proceeds via a surface reaction between adsorbed fatty acid and hydrogen peroxide and the reaction product on the surface is the activated complex, a plausible reaction mechanism can be sketched as follows



Overall reaction



As step 3 is presumed to be the rate determining one, the rate expression becomes

$$r = k c_A^* c_H^* \quad (36)$$

After applying the adsorption equilibria on rapid steps and the total balance for the for the surface species,

$$c_A^* + c_H^* + c_R^* + c_W^* + c^* = c_0 \quad (37)$$

the rate equation becomes

$$r = \frac{k K_A K_H c_A c_H c_0^2}{(1 + \sum K_j c_j)^2} \quad (38)$$

The operative form of the rate equation is

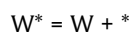
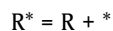
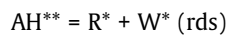
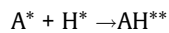
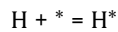
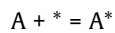
$$r = \frac{k' c_A c_H c_0^2}{(1 + \sum K_j c_j)^2} \quad (39)$$

where

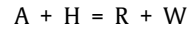
$$\sum K_j c_j = K_A c_A + K_H c_H + K_R c_R + K_W c_W \quad (40)$$

#### 2.2.5. Mechanism 4

Continuing the consideration of direct epoxidation mechanisms, the hypothesis behind the previous mechanisms can be extended so that the adsorbed fatty acid and hydrogen peroxide form a surface complex (AH\*\*) which gives the epoxidized species. The mechanism is displayed below,



Overall reaction



The rate of the rate-determining step is

$$r = k c_{AH}^{**} \quad (41)$$

The quasi-equilibrium hypothesis is applied on the adsorption equilibria

$$K_A = \frac{c_A^*}{c_A c^*} \quad (42)$$

$$K_H = \frac{c_H^*}{c_H c^*} \quad (43)$$

$$K_R = \frac{c_R^*}{c_R c^*} \quad (44)$$

$$K_W = \frac{c_W^*}{c_W c^*} \quad (45)$$

$$K_{AH} = \frac{c_{AH}^{**}}{c_A^* c_H^*} \quad (46)$$

The total balance of the surface species becomes

$$c_A^* + c_H^* + c_{AH}^{**} + c_R^* + c_W^* + c^* = c_0 \quad (47)$$

which in fact is a second degree equation with respect to the concentration of vacant sites ( $c^*$ ). After a straightforward algebraic treatment, it can be shown that the solution of total balance equation (47) can be written in a comfortable form,

$$c^* = \frac{2c_0}{1 + \sum K_j c_j + \sqrt{(1 + \sum K_j c_j)^2 + 4K c_A c_H}} \quad (48)$$

where  $K = K_A K_H K_{AH}$ .

Consequently, the rate equation obtains an operative form

$$r = \frac{k' c_A c_H c_0^2}{((1 + \sum K_j c_j)/2 + \sqrt{(1 + \sum K_j c_j)^2/4 + K c_A c_H})^2} \quad (49)$$

where

$$\sum K_j c_j = K_A c_A + K_H c_H + K_R c_R + K_W c_W \quad (50)$$

and  $k' = k K_A K_H K_{AH}$ .

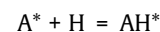
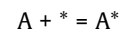
The details of the derivation equations (48)–(49) are given in Appendix.

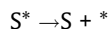
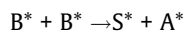
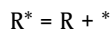
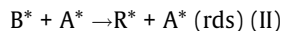
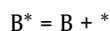
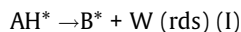
A special case is worth of attention. If the concentration of the adsorbed activated complex is low (i.e.  $K_{AH}$  has a low value,  $K \rightarrow 0$ ), the rate equation (49) is simplified to

$$r = \frac{k' c_A c_H c_0^2}{(1 + \sum K_j c_j)^2} \quad (51)$$

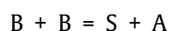
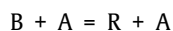
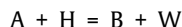
#### 2.2.6. Mechanism 5

According to this mechanism an activated complex is formed in a reaction between adsorbed fatty acid and hydrogen peroxide from the bulk liquid. The activated complex forms the percarboxylic acid (B) which reacts with adsorbed fatty acid (A) giving an epoxidized fatty acid (R). Analogously, two molecules of adsorbed percarboxylic acid could form an epoxidized product (S). The mechanism is summarized below,





Overall reactions



By considering the slow steps, the rate equations are easily written as

$$r_I = k_I c_{AH^*} \quad (52)$$

$$r_{II} = k_{II} c_{A^*} c_{B^*} \quad (53)$$

$$r_{III} = k_{III} c_{B^*}^2 \quad (54)$$

The adsorption quasi-equilibria are applied on the surface species, giving

$$K_i = \frac{c_i^*}{c_i c^*} \quad (55)$$

for  $i = A, B, R, S$  and

$$K_{AH} = \frac{c_{AH^*}}{c_H c_{A^*}} \quad (56)$$

for the surface species  $AH^*$ . The total balance of the surface species is

$$c_A^* + c_{AH^*} + c_B^* + c_R^* + c_S^* + c^* = c_0 \quad (57)$$

After inserting the adsorption equilibrium expressions in the site balance (57), the concentration of vacant sites is solved,

$$c^* = \frac{c_0}{1 + \sum K_j c_j + K_A K_{AH} c_A c_H} \quad (58)$$

The operative forms of the rate equations are finally obtained as

$$r_1 = \frac{k'_1 c_A c_H c_0}{1 + \sum K_j c_j + K_{CA} c_H} \quad (59)$$

$$r_2 = \frac{k'_2 c_A c_B c_0^2}{(1 + \sum K_j c_j + K_{CA} c_H)^2} \quad (60)$$

$$r_3 = \frac{k'_3 c_B^2 c_0^2}{(1 + \sum K_j c_j + K_{CA} c_H)^2} \quad (61)$$

where

$$\sum K_j c_j = K_A c_A + K_B c_B + K_R c_R \quad (62)$$

and  $K = K_A K_{AH}$ .

If adsorbed water and hydrogen peroxide are included as adsorbed species, but spectators on the catalyst surface, terms  $K_H$  and  $K_W$  are added to the denominators of the rate equations. The very interesting feature of this kinetic model is that the rate equa-

tions for the formation of the percarboxylic acid (B) and the final products (R and S) are fundamentally different.

### 2.3. Ring opening reactions

The existence of some ring opening reactions was confirmed in kinetic experiments and in NMR analysis of the reaction products (Lindroos, 2021; Freites Aguilera et al., 2021). Therefore, it was necessary to add the ring opening kinetics in the model. The rate of ring opening was assumed to follow the kinetics

$$r_{ROPI} = -k_{CA} c_i \quad (63)$$

where  $i = R$  or  $i = S$ .

### 2.4. Generation rates of components

The generation rates (production and consumption rates) of the components are determined by the stoichiometry. The rates of perhydrolysis, epoxidation and ring-opening reactions contribute to the generation rates.

For mechanisms 1 and 5, the component generation rates are given by

$$r_A = -r_I + r_{III} - r_{ROPR} - r_{ROPS} \quad (64)$$

$$r_B = r_I - r_{II} - 2r_{III} \quad (65)$$

$$r_R = r_{II} - r_{ROPR} \quad (66)$$

$$r_S = r_{III} - r_{ROPS} \quad (67)$$

$$r_H = -r_I \quad (68)$$

$$r_W = r_I \quad (69)$$

In case that the formation of the epoxidized carboxylic acid is negligible, the rate  $r_{III}$  and the generation rate of the component S eliminated from the above equations.

For mechanisms 2, 3 and 4, the generation rates are obtained from

$$r_A = -r - r_{ROPR} \quad (70)$$

$$r_R = r - r_{ROPR} \quad (71)$$

$$r_H = -r \quad (72)$$

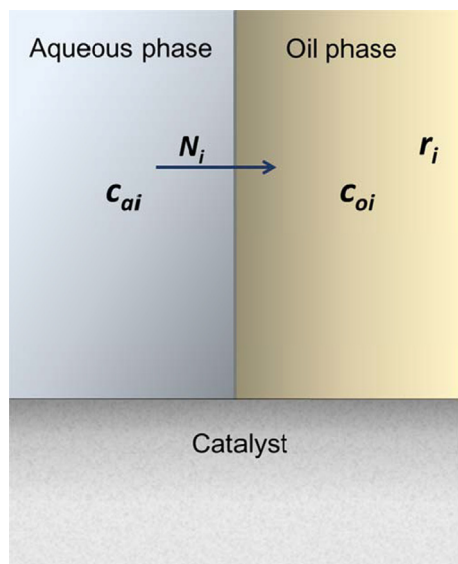
$$r_W = r \quad (73)$$

## 3. Reactor model

### 3.1. Reactor model – Component mass balances

A vigorously stirred three-phase batch reactor is considered here. Oil droplets are dispersed in the aqueous phase, which is the continuous phase. It is assumed that the chemical reactions proceed in the oil phase adjacent to the catalyst particles. The solubility of the fatty acid and the epoxides in the aqueous phase are negligible, whereas hydrogen peroxide is distributed between the aqueous and the oil phase (Freites Aguilera et al., 2019). Interfacial mass transfer from hydrogen peroxide and water takes place as illustrated in Fig. 2.

In our experimental work (Freites Aguilera et al., 2021) we have studied the effect of different stirring rates on the epoxidation kinetics of oleic acid in the presence of lipase. The stirring rate 1000 rpm was chosen because it resulted in kinetic control. In addition, very small catalyst particles (approx. 0.34 mm diameter)



**Fig. 2.** Schematic view of the phases and the interfacial mass transfer in the epoxidation of unsaturated acid with hydrogen peroxide in the presence of an immobilized enzyme catalyst.

were used. The reaction rate was kept slow by using small amounts of catalyst in the liquid phase (catalyst load 7%) to guarantee the operation in the regime of intrinsic kinetics.

For a component (hydrogen peroxide and water) which co-exists in the aqueous and oil phases, the mass balance for the aqueous phase (a) can be written as

$$0 = N_i A + \frac{dn_{ai}}{dt} \quad (74)$$

where A denotes the aqueous phase-oil phase interfacial area. For the oil-catalyst phase (o) the mass balance is

$$N_i A + r_i m_{cat} = \frac{dn_{oi}}{dt} \quad (75)$$

Because the chemical reactions in the current case were slow compared to the interfacial mass transfer, the mass balance equations (74) and (75) can be added giving the expression

$$r_i m_{cat} = \frac{dn_{ai}}{dt} + \frac{dn_{oi}}{dt} \quad (76)$$

The amounts of substance are expressed with the concentrations and the phase volumes. Provided that the volumes of the aqueous and oil phases remain constant during the progress of the reaction, we can write

$$\frac{dn_{ai}}{dt} = V_a \frac{dc_{ai}}{dt} \quad (77)$$

$$\frac{dn_{oi}}{dt} = V_o \frac{dc_{oi}}{dt} \quad (78)$$

The distribution coefficient ( $K_i$ ) of a molecule (i) between the aqueous and oil phases is defined by

$$K_i = \frac{c_{ai}}{c_{oi}} \quad (79)$$

After taking this relation into account, the mass balance equation becomes

$$\frac{dc_{ai}}{dt} V_a + \frac{dc_{oi}}{K_i dt} V_o = r_i m_{cat} \quad (80)$$

The catalyst bulk density is defined as the mass of catalyst-to-volume of oil phase,  $\rho_c = m_{cat}/V_o$ . The bulk density is introduced into equation (80) which is rearranged to

$$\frac{dc_{ai}}{dt} = \frac{r_i \rho_c}{V_a/V_o + 1/K_i} \quad (81)$$

The phase ratio ( $\alpha$ ) is defined as

$$\alpha = \frac{V_a}{V_a + V_o} \quad (82)$$

and it is inserted in equation (81), which becomes

$$\frac{dc_{ai}}{dt} = \frac{(1 - \alpha)r_i \rho_c}{\alpha + (1 - \alpha)/K_i} \quad (83)$$

The time derivative of the oil-phase concentration is expressed in an analogous manner by taking into account the relation (82),

$$\frac{dc_{oi}}{dt} = \frac{(1 - \alpha)r_i \rho_c}{\alpha K_i + 1 - \alpha} \quad (84)$$

Equations (83) and (84) are the general ones for components which co-exist in both phases. For the components which do not dissolve in the aqueous phase,  $K_i = 0$ , equation (84) shrinks to

$$\frac{dc_{oi}}{dt} = r_i \rho_c \quad (85)$$

Summa summarum: for the oil-phase components (A, B, R, S) the mass balance equation (85) is used ( $c_{oi}$  is denoted by  $c_i$  for the sake of brevity) and for the aqueous-phase components (H =  $H_2O_2$  and W =  $H_2O$ ) the balance equation (83) is used in the reactor modelling and parameter estimation.

## 4. Parameter estimation

### 4.1. Parameter estimation procedure

Three different and independent chemical analyses were carried out to catch the progress of the reaction kinetics: determination of the double bonds and epoxide groups as well as the aqueous-phase concentration of hydrogen peroxide. Double bonds appear in the fatty acids A and B, while epoxide groups are found in the products R and S. In fact, the states (s) are observed with the aid of chemical analysis

$$s_1 = c_A + c_B \quad (\text{oil phase}) \quad (86)$$

$$s_2 = c_R + c_S \quad (\text{oil phase}) \quad (87)$$

$$s_3 = c_H \quad (\text{aqueous phase}) \quad (88)$$

The rate parameters are estimated in such a way that the objective function (Q), the sum of squares is minimized,

$$Q = \sum_i \sum_t (s_{i,t,exp} - s_{i,t})^2 \quad (89)$$

where the index 'exp' refers to the experimental observation and  $s_{i,t}$  is the state value obtained from the model.

The ordinary differential equations (83) and (85) were solved repeatedly with different values of the rate and adsorption equilibrium parameters until the minimum of the objective function is found with a Levenberg-Marquardt algorithm. The libraries LsqFit (J. M. White et al., 2020) and NLOpt (S. G. Johnson, 2020) in Julia were used for the computations.

### 4.2. Parameter estimation results

Separate calculations were performed for the five different mechanisms described on the previous section. Tables 1–5 depict



**Table 1A**

Estimated parameters for chemo-enzymatic epoxidation of oleic acid according to Mechanism 1A.

Parameter	Value	Standard error (%)
$k'_1$ ( $L^2mol^{-1}min^{-1}g^{-1}$ )	3.0674e-4	16.1
$k'_2$ ( $L^2mol^{-1}min^{-1}g^{-1}$ )	2.382e-2	40.2
$k_{ROPR}$ ( $L^2mol^{-1}min^{-1}g^{-1}$ )	1.414e-5	29.8
$K_A$ (-)	0.870	58.7
$K_H$ (-)	0.357	19.3
Sum of squares (Q)	4.4893	

**Table 1B**

Estimated parameters for chemo-enzymatic epoxidation of oleic acid according to Mechanism 1B.

Parameter	Value	Standard error (%)
$k'_1$ ( $L^2mol^{-1}min^{-1}g^{-1}$ )	2.9672e-4	15.1
$k'_2$ ( $L^2mol^{-1}min^{-1}g^{-1}$ )	0.1079	134.0
$k_{ROPR}$ ( $L^2mol^{-1}min^{-1}g^{-1}$ )	1.4643e-5	26.4
$K_A$ (-)	0.994	9.2
$K_H$ (-)	0.356	14.2
Sum of squares (Q)	4.4970	

**Table 2**

Estimated parameters for chemo-enzymatic epoxidation of oleic acid according to Mechanism 2.

Parameter	Value	Standard error (%)
$k'$ ( $L^2mol^{-1}min^{-1}g^{-1}$ )	6.94e-5	20.3
$k_{ROPR}$ ( $L^2mol^{-1}min^{-1}g^{-1}$ )	1.38e-5	31.3
$K_A$ (-)	8.27	263.8
Sum of squares (Q)	6.2113	

**Table 3**

Estimated parameters for chemo-enzymatic epoxidation of oleic acid according to Mechanism 3.

Parameter	Value	Standard Error (%)
$k'$	0.3888	14.9
$k_{ROPR}$	1.418e-5	26.7
$K_A$	45.318	7.36
$K_R$	16.742	1.78
$K_H$	0.3542	11.4
Sum of squares (Q)	4.5956	

the results from the parameter estimation for the models based on Mechanisms 1–5. A summary and comparison of the between the different models is shown in Table 6.

#### 4.2.1. Mechanism 1A (rate equations (11)–(13))

In general lines, the model developed based on Mechanism 1 gives a good representation of the experimental data, since the sum of squares is among the lowest between all the mechanisms investigated and the parameters became estimated with moderate standard errors. Besides, no significant correlations were found between the estimated parameters.

After some preliminary calculations, it was confirmed that the formation of the per-epoxyoleic acid ( $r_{III}$ ) was negligible, as well as the ring opening by peroleic acid ( $r_{ROPS}$ ), therefore the rate constants  $k'_3$  and  $k_{ROPS}$  were set to zero. The adsorption constants of hydrogen peroxide ( $K_H$ ), epoxyoleic acid ( $K_R$ ), per-epoxyoleic acid ( $K_S$ ), peroleic acid ( $K_B$ ) and water ( $K_W$ ) were also neglected for simplification purposes, since the adsorption parameter of oleic acid ( $K_A$ ) turned out to be to be the most dominant one in the preliminary modelling results.

**Table 4**

Estimated parameters for chemo-enzymatic epoxidation of oleic acid according to Mechanism 4.

Parameter	Value	Standard Error (%)
$k'$	1.318	5.55
$k_{ROPR}$	1.414e-5	26.6
$K_A$	83.9	4.50
$K_R$	31.0	6.39
$K_H$	0.354	18.0
Sum of squares (Q)	4.6048	

**Table 5**

Estimated parameters for chemo-enzymatic epoxidation of oleic acid according to Mechanism 5.

Parameter	Value	Standard Error (%)
$k'_1$	7.08e-5	9.4
$k'_2$	20.614	276.5
$k_{ROPR}$	1.409e-5	24.7
$K_A$	19.307	213.1
$K_H$	0.3612	19.2
Sum of squares (Q)	4.52268	

**Table 6**

Estimation statistics for the models based on mechanisms 1–5.

Mechanism	Sum of squares	Standard errors	Correlation
1A	4.489	Moderate	Low
1B	4.497	High	Low
2	6.211	High	Low
3	4.596	Moderate	Low
4	4.605	Moderate	Low
5	4.523	High	Low

Overall, mechanism 1A seems to give a good representation of the system, where the formation of the peroleic acid is assumed to be the rate-determining step of the process, and the impact of the ring opening reactions is rather small.

#### 4.2.2. Mechanism 1B (rate equations (25)–(27))

In case of this simplified version of Mechanism 1A, the differences between the model fit for this mechanism and Mechanism 1A is almost imperceptible, since the rate equations are based on the same principles but the epoxidation reaction is assumed to take place in the bulk of the oil instead of the catalyst surface. The simplifications of the previous model were used here, too. The estimated parameters are virtually within the same confidence intervals, except for the rate constant of epoxidation ( $k'_2$ ) which falls in a wide confidence interval. This could be explained by a lack of specification of this parameter and this problem could be solved by providing the model data about the peroleic acid concentration over time. In this sense, the model developed for Mechanism 1A is a better choice to represent our experimental data, even though the assumptions made for Mechanism 1B might very well describe the chemical reality of the process.

#### 4.2.3. Mechanism 2 (rate equation (35))

This mechanism is based on the direct epoxidation between oleic acid and hydrogen peroxide through the formation of an active complex in the surface of the catalyst. After some evaluations of the behaviour of the model, it was concluded that the determination of the rate and adsorption parameters could be simplified to three main parameters: the rate constants for epoxidation and ring opening, and the adsorption constant of oleic acid on the lipase. It was observed that the distribution coefficient of the hydrogen peroxide between the aqueous and oil phase,  $K_{H,}$

tended to big values and its determination did no longer affect the modelling results, so it was fixed as a high value (10,000) which that hydrogen peroxide was assumed to exist only in the aqueous phase. Likewise, the adsorption constants of epoxyoleic acid ( $K_R$ ) and the complex AH ( $K_{AH}$ ) were found to be negligible and the constants were fixed to zero.

Even though no strong correlations were found between the parameters, the determination of the adsorption constant of oleic acid was rather cumbersome and the resulting confidence intervals were quite large. It was noticed that at high concentrations of oleic acid, the values of the adsorption constant of oleic acid were very high, and the influence of  $K_A$  was no longer relevant in the process, which might indicate that the catalyst surface becomes saturated by oleic acid. In this case, the rate equation can be simplified to  $r = k_{CH_2O}$ . However, at low concentrations of oleic acid this is no longer valid. In order gain more identification for the determination of  $K_A$  more experiments would be needed with a wider range of oleic acid concentrations.

In this model, the estimated values for the rate constants of the overall reactions are close to the perhydrolysis rate constant in Mechanisms 1A and 1B, and the influence of the ring opening reactions was observed to be minimal. Mechanism 2 is a reasonable representation of the system; however, the fit of this model to the experimental data is rather poor compared to the models based on the other mechanisms.

#### 4.2.4. Mechanism 3 (rate equation (39))

From the results collected in Table 3, it can be observed that the estimated parameters are well specified, since the standard errors are moderate and no significant correlations were found. The model represents adequately the experimental results, and the sum of squares results in a low value, compared to the rest of the models. The influence of the adsorption of water and hydrogen peroxide in the catalyst surface ( $K_W$  and  $K_H$ ) was found to be negligible and the adsorption terms for oleic acid and epoxyoleic acid showed to be the most dominant ones in the preliminary model effort.

#### 4.2.5. Mechanism 4 (rate equation (49))

Table 4 depicts the estimated parameters for the model based on Mechanism 4. This model shows similar results to those of the previous model, since they are basically based on similar principles and in some cases, they can be reduced to the same expression (Eq. (51)). The simplifications made for the previous model were applied also for this case. Together with the models based on Mechanisms 1A and 3, this model represents the best alternative to describe the system according to the regression analysis.

#### 4.2.6. Mechanism 5 (rate equations (59)–(61))

In terms of the fit of the model, mechanism 5 is a good representation of the system because the total sum of squares is as low as for the other models (with the exception of 2). Even though no serious correlations were found between the parameters, a large confidence interval was determined for the epoxidation rate constant ( $k'_2$ ). Similarly to Mechanism 1B, this problem could be solved by providing experimental data about the peroleic acid concentration as a function of the reaction time. Since some parameters did not show an effect in the fit of the model, simplifications were made, such as neglecting the adsorption constants epoxyoleic acid ( $K_R$ ), peroleic acid ( $K_B$ ) and the AH complex ( $K_{AH}$ ).

#### 4.3. Discussion of modelling results and reaction mechanisms

Table 6 summarizes the parameter estimation results for the different models. Virtually an equally good fit was achieved for all the models with the exception of Mechanism 2 - inspired by the work of Yadav and Maujula Devi, 2001 - which was the least representative one. For the viewpoint of parameter estimation, mechanisms 1, 3 and 4 showed the best performances. The experimental error in the data varied in the experiments; a typical error caused by the analytics was 4–5%. A representative model fit is shown in Fig. 3. The model fit is based on mechanism 1A. Concerning the complex chemical system and the complicated chemical analysis, the fit can be regarded as satisfactory: all the main trends of the experimental data are reproduced by the model.

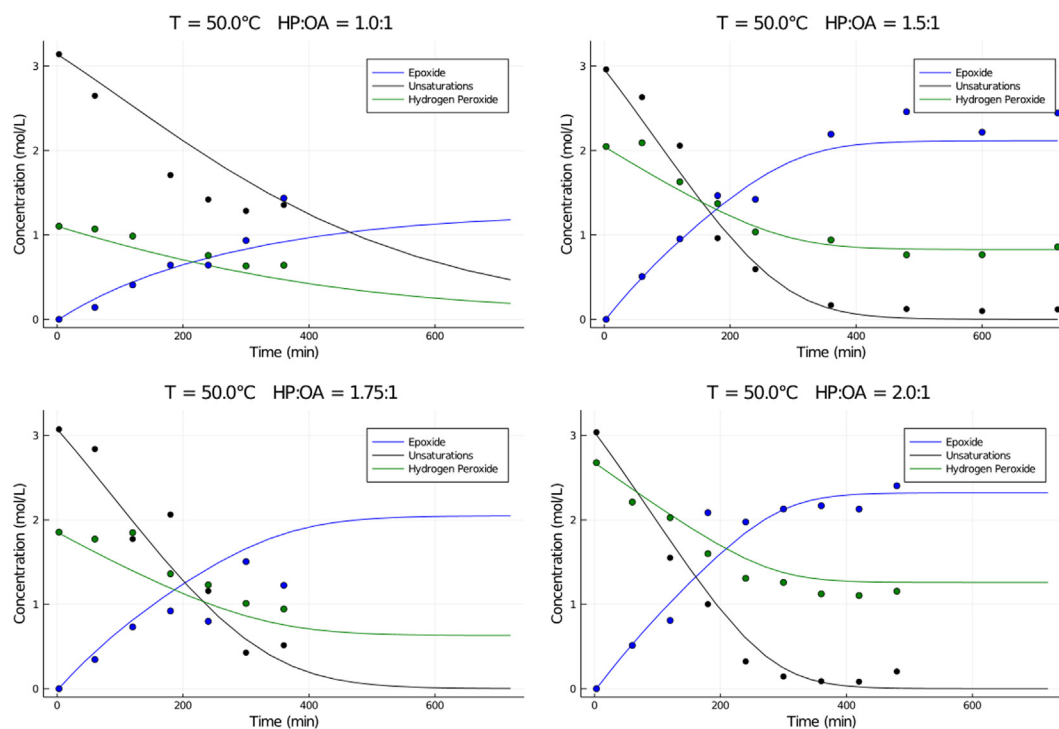


Fig. 3. Experimental vs. predicted concentrations at four different hydrogen peroxide-to-oleic acid ratios (HP:OA). Continuous lines represent the model fit (1A).

Even though the five mechanisms are based on different chemical assumptions, some commonalities were found. From the parameter estimation of all the mechanisms, it was determined without any doubt that the adsorption of oleic acid on the enzyme is predominant: the adsorption parameter of oleic acid was very dominant compared to other adsorption parameters and the effective reaction order with respect to oleic acid was less than one. This is an interesting discovery and it is important to examine in more detail the character of the active site to judge whether this binding occurs at the double bond of the carboxylic acid molecule or at the carboxylic group. Adsorption of hydrogen peroxide, water, peroleic acid, per-epoxyoleic acid and epoxyoleic acid turned out to be negligible in all the models considered. This could point to a catalytic mechanism based on the Eley-Rideal principle or the perhydrolysis proceeding on the catalyst surface and the epoxidation occurring in the bulk of the oil phase as in Mechanism 1B.

A discussion of the chemical character of the lipase catalyst might be of help. Lipases are a subgroup of enzymes called serine hydrolases. For serine hydrolases, a catalytic triad, a combination of three amino acid residues (serine, histidine, and aspartic or glutamic acid), is in the active site of the hydrolase, where the histidine residue works as a general acid-base catalyst. The active site is claimed to consist of two binding sites, one for a carbonyl group containing acyl donor substrate and the other for a nucleophilic substrate (Uppenberg et al., 1994, 1995). The reaction starts by the addition of serine hydroxyl to the carbonyl group of the acid acyl donor, forming an acyl enzyme intermediate while the first product (water in case of peroxyacid formation) is eliminated from the active site. In the second step, an added nucleophile (here: hydrogen peroxide) attacks the carbonyl carbon of the acyl enzyme intermediate, completing the reaction with the elimination of the second product (here: the percarboxylic acid) into the bulk of the system and the regeneration of the lipase for a new catalytic cycle. The epoxidation with the percarboxylic acid evidently takes place without enzymatic assistance in the solvent. This description fits very well with the proposed mechanism 1B in the current work.

The rival kinetic models that assume the direct epoxidation between oleic acid and hydrogen peroxide gave a good identification of the parameters. However, this does not overrule the formation of the peroleic acid, since the estimated values for the rate constants of the direct reaction are within the same orders of magnitude as the perhydrolysis rate constant. This might suggest that the rate constant for the direct reaction englobes the perhydrolysis and the epoxidation via peroleic acid. If this is the case, the numerical value for the overall reaction rate constant is in fact determined by the value of the slow reaction step, which is perhydrolysis.

Sustaita-Rodriguez et al. (2018) concluded that lipase catalysed the epoxidation of fatty acid methyl esters in the absence of added carboxylic acids, but they proposed that lipase catalyses the hydrolysis of the ester to the corresponding fatty acid which is oxidized by the enzyme itself to form a peracid. In any case, the most effective ways to determine whether the epoxidation proceeds through the percarboxylic acid route or not are to attempt to detect the peroleic acid by chemical analysis during the epoxidation experiments or to perform an epoxidation experiment with an olefin that does not have a carboxylic group, such as octene, dodecene or octadecene. Consequently, three additional experiments were conducted by us to elucidate the reaction mechanism.

An experiment with oleic acid, hydrogen peroxide and immobilized lipase was performed under identical conditions with the previous experiments in an attempt to detect the presence of peroleic acid by titration analyses (Greenspan and Mackellar, 1948), using 2-propanol as the solvent. Concentrations of around 0.5–1.0 wt-% of peroleic acid were observed by the titrimetric analysis.

Because of the low concentrations, the determination was rather cumbersome and the results were semi-quantitative.

Subsequently, two experiments were conducted using octadecene as an olefin similar to oleic acid, but lacking the carboxylic group. The experiments were performed at 50 °C, under vigorous stirring and, after a few hours, the oxirane groups were measured by potentiometric titration (Jay, 1964). The first experiment was done with octadecene, hydrogen peroxide and immobilized lipase and no oxirane groups were found after three hours of reaction. In the second experiment, the same components co-existed in the same amounts, but acetic acid in excess was added as well. This second experiment resulted in a content of around 14 wt-% of oxirane after three hours. This result reveals that the epoxidation of octadecene proceeded via the formation of a percarboxylic compound, in this case peracetic acid.

The experimental observation is in accordance with the Prilezhaev principle: a carboxylic acid (here: acetic acid) reacts with hydrogen peroxide in the presence of a catalyst (here: lipase) forming a percarboxylic acid (here: peracetic acid), which epoxidizes the double bond in the hydrocarbon. Taking into account the reflections concerning the character of the active sites on the lipase catalyst and the supplementary experiments performed with octadecene, there is no doubt that the epoxidation of oleic acid assisted by immobilized lipase occurs via the formation of peroleic acid.

## 5. Conclusions

The chemo-enzymatic epoxidation of oleic acid with hydrogen peroxide in the presence of immobilized lipase catalyst was investigated and five alternative reaction mechanisms were studied by kinetic modelling. The mechanisms that showed to best represent the experimental data were based on the assumption of the formation of peroleic acid as the epoxidation agent, the direct epoxidation via surface reaction between adsorbed oleic acid and hydrogen peroxide and the formation of peroleic acid through an active complex. The rate equations based on these mechanisms were able to describe the experimental trends.

Even though we obtained experimental evidence that indicates the formation of peroleic acid as the reaction carrier, it was observed that mathematical modelling of the reaction through direct epoxidation (mechanisms 3 and 4), described the data as well as the ones obtained for the mechanisms including the formation of peroleic acid (mechanisms 1A, 1B and 5).

Upon statistical analysis of the models, it was confirmed that the adsorption of oleic acid to the enzyme is predominant while the adsorption of hydrogen peroxide, water, peroleic acid and epoxyoleic acid are negligible from a practical point of view. After additional experimental work with octadecene and examination the character of the lipase catalyst, a final conclusion is that mechanism 1B was the one that came nearest to a description of the chemical reality of the reaction system.

## CRedit authorship contribution statement

**Tapio Salmi:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Resources, Project administration, Funding acquisition, Supervision. **Adriana Freites Aguilera:** Conceptualization, Methodology, Formal analysis, Software, Writing – original draft, Writing – review & editing, Supervision. **Pontus Lindroos:** Methodology, Formal analysis, Software, Writing – original draft. **Liisa Kanerva:** Conceptualization, Methodology, Writing – original draft, Supervision.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A

The following parameters are defined

$$\alpha = K_A K_H K_{AH} C_{AC_H} \quad (A1)$$

$$\beta = 1 + \sum K_j C_j \quad (A2)$$

$$K = K_A K_H K_{AH} \quad (A3)$$

These parameters are inserted in the site balance

$$C_A^* + C_H^* + C_{AH}^* + C_R^* + C_W^* + C^* = C_0 \quad (A4)$$

which is developed to a second-degree equation with respect to the vacant sites,

$$C^* = \frac{-\beta + \sqrt{\beta^2 + 4\alpha C_0}}{2\alpha} \quad (A5)$$

The nominator and denominator are multiplied by

$$\beta + \sqrt{\beta^2 + 4\alpha C_0}$$

Equation (A5) becomes after multiplication

$$C^* = \frac{2C_0}{\beta + \sqrt{\beta^2 + 4\alpha C_0}} \quad (A6)$$

Back-substitution of  $\alpha$  and  $\beta$  gives

$$C^* = \frac{2C_0}{1 + \sum K_j C_j + \sqrt{(1 + \sum K_j C_j)^2 + 4K C_A C_H}} \quad (A7)$$

where  $K = K_A K_H K_{AH}$ .

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