

Matrix Effects on the Kinetics of Lactose Hydrolysis in Fermented and Acidified Milk Products

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Diffusion may limit the homogeneous hydrolysis of the lactose when adding the hydrolyzing enzyme, lactase, to a fermented pasty milk product. The diffusion of lactose in soft cheese (quark) and acid milk gels (obtained by Glucono- δ -lacton) was determined in experiments. By varying amount of dry matter it was possible to study matrix effects on the diffusion rate. The model of one-dimensional infinite media with a constant cross-section based on Fick's second law of diffusion for time-dependent diffusion processes was verified concerning the diffusibility of lactose in viscose milk products. It appeared that the apparent diffusion coefficient " D_{app} " showed a linear decline as the dry matter of the product increased. Concerning the kinetics of lactose hydrolysis, no significant limitation was caused by the diffusion resistance of the matrix of fermented or acidified milk products.

Key words:

Milk products, lactose, lactase, diffusion, hydrolysis

Introduction

In the late 70s and 80s, many studies were published which demonstrated the enzymatic hydrolysis of lactose at a neutral pH level in whole milk, skim milk and whey, allowing lactose-intolerant consumers to consume milk and milk products. The hydrolysis took place in the liquid phase so it was possible to prevent the restriction of hydrolysis through diffusion effects.

In a new approach, a fungal lactase was added directly to the viscose product matrix of fermented milk products before packaging¹, and the distribution period was intended mainly to be used to hydrolyze the lactose. The idea was to reduce the lactose content and to produce sweet natural milk products without any or with a lower addition of sugars by hydrolyzing the remaining lactose in the fermented milk product. In order to minimize enzyme costs, only a minimal amount of the enzyme may be added, although a high degree of homogeneous hydrolysis must nevertheless be ensured during the distribution period of about 3 to 5 days at 6 to 10 °C. An advantage is that the enzyme is directly and continuously injected into the fermented product prior to the filler, which means that the fermentation process and equipment remain unchanged and milk products with lactose and with a reduced amount of it may be easily produced on the same processing line.

For the introduction of this technology, the kinetic of the lactose hydrolysis of a fungal lactase in fermented milk products was studied and described in detail². However, the efficiency of the process may be restricted by the matrix of the fermented milk product, hindering the diffusion of the lactase or the lactose and enabling homogeneous hydrolysis.

This paper is concerned with the effects of a possible restriction of diffusion on the kinetics of lactose hydrolysis caused by the product matrix. Based on Fick's second law of diffusion for time-dependent diffusion processes, several experiments have already been carried out in different food matrices such as e.g. in cheese^{3,4} or in rice starch / water-solutions⁵, but not in fermented milk products. As a measure of the mobility, the diffusion coefficient can mainly be described as being dependent on the process temperature, the viscosity of the flow media, and the radius of the diffusing molecule in these experiments. Further researchers discuss resistance and prolongation distance effects caused by the texture of product matrix⁶⁻⁸.

The lactose can be expected to diffuse faster than the enzyme because of its molecular radius. After mixing the enzyme into the viscose matrix, contact between lactose and enzyme molecules is the first step before the hydrolysis of the lactose can start. Thus, the diffusion of lactose molecules is to be assumed as being an important step for hydrolysis.

Experiment

Materials

A skimmed quark (Deller, Munich, Germany) and acid gels (GdL-gels) were used as products, the latter being produced through the reconstitution of milk protein powder (Alaplex 4850, protein content 81.5 %, New Zealand Milk Products, Rellingen, Germany). Subsequently glucono- δ -lacton, GdL (purity 99 %, Fluka, Neu-Ulm, Germany) was added directly as an acidification step (cf. *Hammelehle* et al., 1997 for method)⁹.

The enzyme used for lactose hydrolysis was derived from *Asp. oryzae* (Fungal Lactase 30 000, DMS Food Specialities, Delft, Netherlands). The added lactose was purchased from Meggle, Wasserburg, Germany (quality edible <G>; lactose monohydrate > 99 %).

Experimental set-up

A two-chamber diffusion tube consisting of two symmetrical steel cylinders (inner diameter: 100 mm) was used to determine the apparent coefficients of lactose diffusion. The product mass was transported in millimeters over the opening with a rotating linkage provided with a thread (Fig. 1). The maximum distance for the diffusion was 40 mm. The diffusion chamber with the sample was placed in a tempering box to ensure a constant temperature of (4.0 ± 0.1) °C for the experiment.

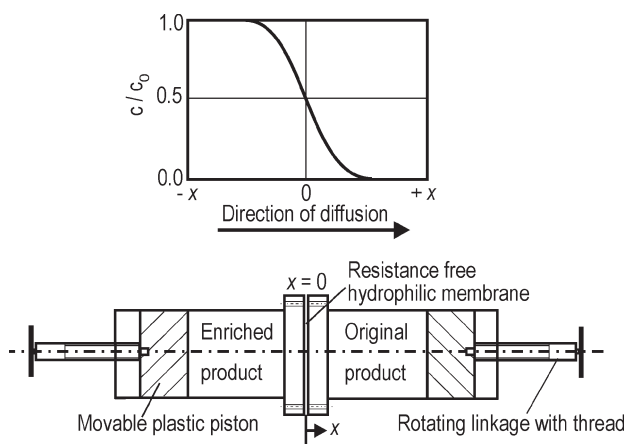


Fig. 1 – Two-chamber diffusion tube to determine the diffusion coefficient and the resulting concentration profile

Details of the experiment

The product enriched with lactose was filled into one of the two cylinders and the product with the original lactose content into the other to ensure the concentration difference required for diffusion. (Fig. 1). A hydrophilic membrane of cellulose acetate (pore diameter $1.2 \mu\text{m}$) (Schleicher & Schuell,

Dassel, Germany) was placed between the 2 cylinders, after screwing down the cylinders, filled to the top with the sample. The membrane prevented the adhesion of the samples and the carry-over of material when opening the chamber. Connecting both cylinders started the diffusion in the two-chamber diffusion tube. After diffusion, the samples were scraped off in layers of 1 mm thickness. The concentration of the diffusing lactose was determined by means of a HPLC analysis¹ and/or enzymatic test kits (Lactose/Galactose, Boehringer, Mannheim, Germany). The result was a concentration profile similar to that shown in Fig. 1. All analyses were carried out twice.

A statistical function of Excel 5.0 enabled data to be obtained on the Gauss Error Function which is necessary to calculate the concentration profile. Through iterating and setting the time of diffusion (t), via equation 2, it was possible to calculate the relative concentrations (c/c_0) for every distance of diffusion (x).

Results and discussion

Model development and characterization

Fick's second law of diffusion for time-dependent diffusion processes was used to calculate the diffusion process. This law includes the dependency of the concentration (c) on time (t) and on the distance (x):

$$\frac{\partial c}{\partial t} = D \cdot \frac{\partial^2 c}{\partial x^2} \quad (1)$$

The statement made by this important basic equation is that the mass of substance, which flows more into the element with the thickness (∂x) during the time (∂t) than it flows out of it, serves to increase the contained substrate concentration (∂c). The diffusion coefficient (D) is regarded as constant and therefore assumed as being independent of the concentration. Furthermore, Fick's second law is based on the assumption that diffusion processes run one-dimensionally and not in all directions as in reality. In the relevant literature many solutions for the time-dependent diffusion equation are given^{10,11}. A special solution for practical use can be developed using the model of the one-dimensional infinite media with a constant cross-section. This model is valid since significant changes in the concentration do not reach the boundary of real finite systems¹⁰. To determine the diffusion coefficient between a media, with and without a diffusing substance in practice, the following concentration profile (see also Fig. 1) must be assumed. For $t = 0$, the result is $c = c_0$ for $x < 0$ and $c = 0$ for $x > 0$ in which

c_0 is the enriched initial concentration of the diffusing substance.

Equation 2 shows the resulting solution of equation 1:

$$c(x,t) = \frac{c_0}{2} \cdot \left[1 - \operatorname{erf}\left(\frac{x}{2 \cdot \sqrt{D \cdot t}}\right) \right] \quad (2)$$

The term

$$\operatorname{erf}\left(\frac{x}{2 \cdot \sqrt{D \cdot t}}\right) = \operatorname{erf}(z) \quad (3)$$

is known as the error function. The solution is given in extensive tables¹¹. Assuming $x = 0$ leads to

$$\operatorname{erf}(z) = 0 \quad (4)$$

Thus

$$c(0,t) = \frac{c_0}{2} \quad (5)$$

can be written. A concentration profile according to Fig. 1 is obtained by determining each concentration at different distances (x) and at a constant time (t) on the basis of the above mentioned assumptions. If it is unexpectedly ascertained that the value of “ c ” at $x = 0$ is not $c_0/2$, another process must have occurred. Flows must always be considered if volume changes of the media have arisen. For fermented milk products, the syneresis may be taken into account.

In fermented milk products diffusion may be influenced by diffusion resistances due to the kind of matrix. In porous solids it is to be expected that the whole cross-section of the pore will be smaller than the total area (\rightarrow cross-section reducing factor, μ_A) and that, due to windings, the diffusion distance will be longer than the shortest distance (\rightarrow distance prolongation factor, μ_l)^{1,8}. For homogeneous pore systems the factor “ μ_A ” approximately corresponds to the reciprocal value of the porosity (ϵ). From these qualities a resistance factor (μ) results, which can be examined experimentally.

$$\mu = \mu_A \cdot \mu_l = \frac{\mu_l}{\epsilon} \quad (6)$$

The factor μ , as the product of the indicated resistances, reduces the diffusion coefficient of pure water to the apparent diffusion coefficient (D_{app}) in the product. In accordance with *Mersmann* (1986)⁶, “ μ ” can be obtained from the relation between the molecular and the effective diffusion coefficient in the product. The following result is obtained when modified for this particular case:

$$\mu = \frac{D}{D_{app}} > 1 \quad (7)$$

Since the existing resistances restrict the mobility of the molecules with the consequence that the dominating rate of diffusion becomes slower, the factor “ μ ” must be > 1 .

Verification of the diffusion model

The following data of the ascertained diffusion coefficients for lactose were worked out using curves, the courses, and dependencies, of which followed the model of the one-dimensional infinite media with a constant cross-section. Fig. 2 shows the concentration profile of lactose diffusion in skimmed quark. The curve was obtained using a value of $D \pm s = (1.37 \cdot 10^{-10} \pm 0.13) \text{ m}^2 \text{ s}^{-1}$ for the diffusing coefficient from 5 independent experiments (s : standard deviation). The concentrations were made dimensionless by relating them to the adjusted difference of the lactose concentration (Δw).

Since the product matrix offers resistance to the diffusion of the molecules, it has an effect on an apparent reduction of the diffusion coefficient (D). Thus, the diffusion coefficient must be dependent on the dry matter of the product and is to be regarded as being the apparent diffusion coefficient (D_{app}).

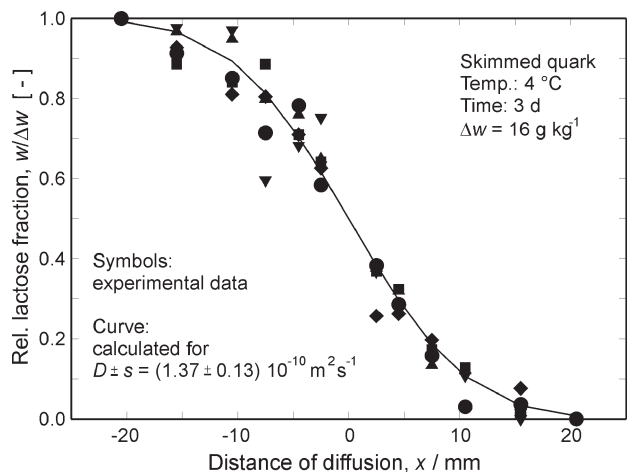


Fig. 2 – Concentration profile for the diffusion of lactose in skimmed quark (s : standard deviation)

Influence of the product matrix. – Variation of the dry matter

General considerations

Fig. 3 illustrates the effects of different dry matters (wDM) in the GdL-gel on the mobility of the lactose in the product matrix. The diffusion rate characterized by the apparent diffusion coefficient decreased as the dry matter increased. The resistance of the product matrix had an increasing influ-

ence on the diffusion of lactose. The steeper the straight line, the greater is the effect of the diffusion resistance of the matrix on the diffusion coefficient¹². The intersection of the straight line with the ordinate at a fraction of dry matter of 0 % represents the diffusion coefficient “D” in pure water, assumed as independent of any influencing ingredients^{13,14}. The regression ($r^2 = 0.966$) results in

$$D_{app} = D - a \cdot w_{DM} \quad (8)$$

with a diffusion coefficient having a value of $D = 3.18 \cdot 10^{-10} \text{ m}^2 \text{ s}^{-1}$ and a rate coefficient of $a = 1.36 \cdot 10^{-10} \text{ m}^2 \text{ kg s}^{-1} \text{ g}^{-1}$. The value corresponds to data of lactose diffusion in pure water^{15,16}.

However, the apparent diffusion rate (D_{app}) of the skimmed quark is higher than the value achieved in the GdL-gel. As a result of processing, quark is a suspension of coagulated casein particles dispersed in the milk serum phase¹⁷. After being filled into the test chamber, diffusion mainly takes place in the serum phase, but the dispersed particles obstruct diffusion. In comparison, the structure of GdL-gels was built up directly in the chamber resulting in a homogeneous protein network with a high diffusion resistance.

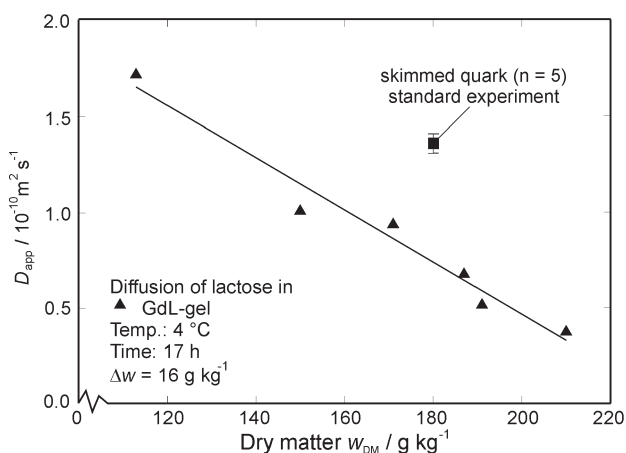


Fig. 3 – Apparent diffusion coefficient D_{app} as a function of the mass fraction dry matter in the GdL-gel; skimmed quark: experimental data ($n = 5$)

In experiments Kessler¹⁸ showed that the flow through the matrix of spheres indicated that, within diffusion processes, the factor “ μ_1 ” is almost independent of spheres order and the diameter of the spheres. Knowing the structure of the matrix and the form of the molecules it is possible to estimate an apparent porosity with equation 6 and equation 7 for each dry matter. Thus, porosity can be classified in comparison to other matrices (equation 9).

$$\varepsilon = \mu_l \cdot \frac{D_{app}}{D} \quad (9)$$

Assuming that the protein aggregates connected in the gel structure are spherically shaped, the dry matter, varying between 8 % and 15 %, depending on the increase of the protein concentration, results in a greater density of the structure elements in the matrix of the GdL-gel. The porosity decreases or the cross-section reducing factor “ μ_A ” increases, so that the mobility of the diffusing molecules is limited.

Effects of diffusion resistances of the matrix on the lactose hydrolysis during distribution

The next question was: does the diffusion rate influence the degree of hydrolysis obtainable after 3 days of distribution? Fig. 4 demonstrates that after 3 days the degrees of hydrolysis do not differ in spite of the separation of almost the entire product matrix consisting mainly of proteins (rhombic symbol). Resistance in the matrix is negligible. Any delays in time, caused by diffusion resistances, do not need to be taken into account considering the long period of distribution of about 3 to 5 days. Furthermore, in overlapping experiments it was ascertained that the enzyme also diffuses. The distance of the reacting molecules are overcome more quickly. Thus, in spite of existing diffusion resistances of the protein matrix, the desired rate of hydrolysis is not reduced during distribution, owing to the sufficient mobility of both the lactose and the enzyme. The long period of distribution is adequate for the participating molecules to come into contact for hydrolysis.

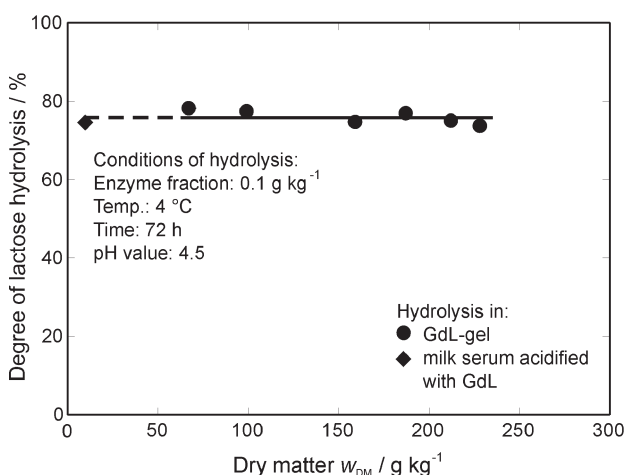


Fig. 4 – Degree of hydrolysis as a function of the mass fraction dry matter in GdL-gels (lactose mass fraction varied between 29 – 32 g kg^{-1} depending on the mass fraction dry matter of the sample)

Conclusion

Experiments were carried out in order to examine the influence of a limiting diffusion process of lactose on the kinetics of lactose hydrolysis caused

by the product matrix. Both, quark and model gels with different fraction of dry matters, the latter produced through the reconstitution of a protein powder and direct acidification with glucono- δ -lacton (GdL), were used as diffusion media.

i) The model of one-dimensional infinite media with a constant cross-section, deduced from Fick's second law of diffusion for time-dependent diffusion processes, was verified for the mobility of lactose in pasty fermented and acidified milk product matrices.

ii) There was a linear reduction of the ascertained apparent diffusion coefficients D_{app} of the lactose as the dry matter increased due to the prolongation of the distance and the narrowing cross-section in the product matrix. The slope of the straight line was characteristic for the resistance of the product matrix to lactose diffusion. Further experiments served to prove how the destruction of the gel structure into small suspended gel particles influences the diffusion rate.

iii) Experiments showed that the diffusion resistance of the matrix did not significantly limit the kinetics of lactose hydrolysis. Delays in hydrolysis caused by diffusion resistances do not need to be taken into consideration when considering that a distribution period of 3 to 5 days is available for the hydrolysis of the lactose. In fermented and acidified milk products, the enzyme kinetic mainly restricts enzymatic hydrolysis, and diffusion resistance may be negligible.

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Nomenclature

a	– rate coefficient, $m^2 \text{ kg s}^{-1} \text{ g}^{-1}$
c	– concentration, mol dm^{-3}
w	– substrate mass fraction, g kg^{-1}
w_0	– initial mass fraction, g kg^{-1}
Δw	– difference of lactose mass fraction, g kg^{-1}
D	– diffusion coefficient of pure water, $\text{m}^2 \text{ s}^{-1}$
D_{app}	– apparent diffusion coefficient, $\text{m}^2 \text{ s}^{-1}$
w_{DM}	– mass fraction of dry matter
erf	– error function

GdL	– Glucono- δ -lacton
r^2	– regression
s	– standard deviation
t	– time, s, d
x	– distance of diffusion, mm
ε	– porosity
μ	– resistance factor
μ_A	– cross-section reducing factor
μ_l	– distance prolongation factor

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