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Non-enzymatic browning in hydrolysed concentrated cheese whey permeate

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The influence of the degree of hydrolysis (DH) on the rate of brown colour development in a hydrolysed concentrated Grana whey permeate (HCCWP), to be used as osmotic agent, was studied.

The colour (L^*, a^*, b^*) and the optical density (420 nm) were measured for 7 days at approximately 1-day intervals, on three concentrates at pH 6 at 65, 75 and 85% degree of hydrolysis, and heated at 35, 45 and 55°C.

The activation energy of the browning process was correlated with the DH values of HCCWP: increasing the DH decreased the activation energy and increased the rate of colour change.

The choice of degree of lactose hydrolysis of concentrated permeate seems to be an effective way of controlling the rate of brown colour development and of improving the shelf life of the product. Copyright © 1996 Elsevier Science Ltd.

INTRODUCTION

Whey has always been regarded as a large mass, low value by-product of cheesemaking, difficult to dispose of, and highly polluting. It retains approximately 50% of the milk nutrients and its volume is 80–85% of the whole milk.

The high lactose content of the ultrafiltration (UF) permeate, which remains after whey protein concentrate has been produced, limits its use even for animal feeding.

The enzymatic hydrolysis of lactose of UF permeate, that increases its sweetness, solubility, fermentability and digestibility (Marwaha & Kennedy, 1988), opens new possibilities for a better utilisation of this valuable by-product.

Recently, hydrolysed concentrated cheese whey permeate has been suggested as a sweetener in ice cream, biscuits, drinks and other products (Moore, 1978; Rothwell, 1984; Chiu & Kosikowski, 1985; Prendergast, 1985; Rexroat & Bradley, 1986; Ogunrinola *et al.*, 1988; Arndt & Wehling, 1989*a,b*).

Rondo Brovetto *et al.* (1991*a,b*) and Torreggiani *et al.* (1992, 1994) characterised and proposed the use of hydrolysed syrup from cheese whey permeate in the osmodehydration of fruits and vegetables. These syrups are just as effective as osmotic solutions, as sweet corn syrups or glucose syrups and, owing to the low sweetness of D-glucose and D-galactose (hydrolysed lactose), could be used for both fruit and vegetable processing. To obtain a shelf-stable syrup it has to be taken into account that the presence of relatively high concentrations of sugars, and low amounts of proteins at low molecular weight (about 0.5% in a concentrate at 50% total solids), could favour non-enzymatic browning which may affect the appearance and possibly the nutritional quality of the product.

As a matter of fact, non-enzymatic browning, via the Maillard reaction, has already been reported as one important mode of deterioration in whey (Saltmarch *et al.*, 1981). Concentrated whey contains a high concentration of lactose and proteins high in lysine and these components participate in the Maillard reaction (Kanterewicz & Chirife, 1986) albeit slowly as compared to systems containing sugars more reactive than lactose. However the lactose hydrolysis, which increases the concentration of non-enzymatic browning reactive groups, could greatly influence the rate of colour change (Buera *et al.*, 1990).

As already reported for model systems (Petriella *et al.*, 1985; Buera *et al.*, 1987) other parameters, such as pH and temperature, are relevant in non-enzymatic browning of non-hydrolysed and hydrolysed concentrated cheese whey (Kanterewicz & Chirife, 1986; Buera *et al.*, 1990) and of hydrolysed cheese whey permeate (Torreggiani *et al.*, 1993).

The purpose of this study was to determine the effects of the degree of lactose hydrolysis on colour changes of

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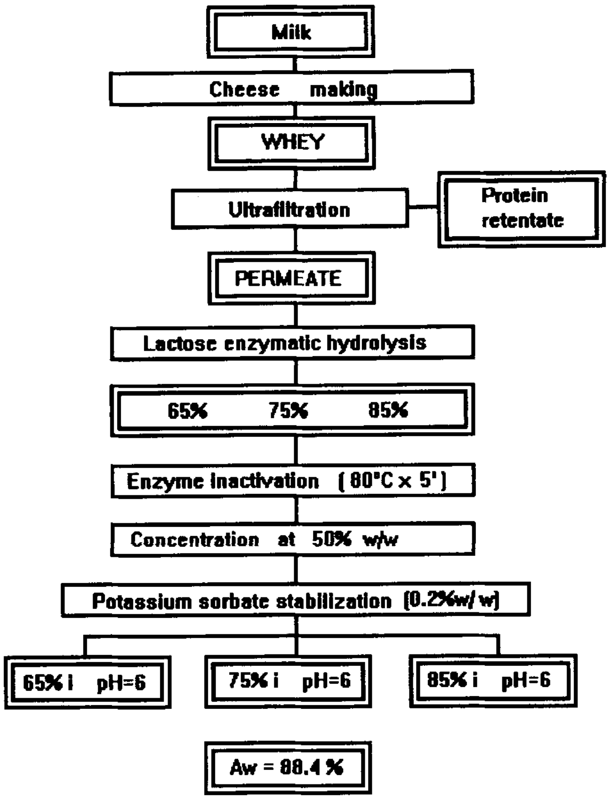

Fig. 1. Flow-sheet of the process.

hydrolysed, concentrated shelf-stable cheese whey permeate at 50% total solids.

MATERIALS AND METHODS

Whey

Grana Padano cheese whey supplied by the Institute of Dairy Science ILC of The Ministry of Agriculture (Lodi, Italy) was ultrafiltered using an ILC pilot plant provided with 2.2 m^2 PVDF (polyvinylenedifluoride) flat membranes (cut-off 40,000 Da) (IRIS 3065, Rhone-Poulenc, Miribel, France).

The working conditions were: pressure in 4×10^5 Pa, pressure out 2.2×10^5 Pa, recirculation rate 12 000 litres/h.

The chemical characteristics of permeate were: total solids 5.16%, lactose 4.14%, ash 0.6%, proteins 0.04%, and pH 6.

The hydrolysis and concentration were performed following the flow sheet shown in Fig. 1.

Hydrolysis

Hydrolysis was carried out at 37°C with 0.2 g lactase (MAXILAT LX5000, Gist-brocades, Delft, The Netherlands)/100 g concentrated permeate.

Lactase inactivation was carried out after the required degree of hydrolysis (DH = 65–75–85%) was reached by heating the concentrate at 80°C for 5 min. To evaluate the degree of hydrolysis, lactose, glucose and galactose were quantified by HPLC according to the method of Forni *et al.* (1992); because of the poor resolution between glucose and galactose the method was modified by using, under the same condition, an Aminex HPX-87P (BioRad, Richmond, California, USA) column (0.70x30 cm) (85°C and deionised water as eluent) and a Jasco (Tokyo, Japan) 830-RI refractometer as detector.

Concentration

The permeates at different DH were concentrated under vacuum at 50°C by Rotavapor R100 (Buchi, Flawil, Switzerland) to 50% solids content.

The concentrates were stabilised by the addition of potassium sorbate (Merck, Darmstad, Germany) (0.2% w/w).

Colour measurement

Glass cells, 5 cm in diameter and 4 cm in depth, with black walls, were used to contain 35 ml of each syrup, stoppered and stored at selected temperatures in constant temperature ovens. Concentrated cheese whey permeates at 65, 75 and 85% DH were heated at 55°C and the colour measurements were made at approximately 1-day intervals for 300 h. Successively the same concentrates were heated at 35, 45 and 55°C for 180 h. The colour measurements were repeated daily.

A Minolta Chroma Meter CR 2000 (Minolta Camera Co. Ltd, Osaka, Japan) was used to follow the progress of colour changes in the heated, concentrated cheese whey permeate. The CIE tristimulus L^*, a^*, b^* were obtained directly from the instrument. Concentrates in the above described cells were measured by being placed on a white ($L^*=97.79$, $a^*=-0.21$, $b^*=2.38$) standard plaque (Buera *et al.*, 1990) and the measuring device just touched the surface of the concentrates.

Using L^*, a^*, b^*, the following were calculated:

$$\Delta\text{Lightness}(\Delta L^*) = L_0^* - L^*,$$

where $L_0^* = L^*$ value of the concentrate at zero time;

$$\text{Saturation } a^*, b^*(s_{ab}) = (a^{*2} + b^{*2})^{1/2}$$

$$\text{Saturation } u^*, v^*(s_{uv}) = 13[(u' - u_0)^2 + (v' - v_0)^2]^{1/2}.$$

All the colour parameters are CIE 1976.

Optical density measurement

Optical density measurements at 420 nm were carried out using a UV–Visible Spectrophotometer PU 8800 (PYE UNICAM Ltd, Cambrige, UK) according to Lerici *et al.* (1990)

Solutions were diluted with distilled water in relation to the degree of browning in order to have optical density signals on the scale.

Distilled water was used as reference.

By optical density measurement, the following change was calculated:

$$\text{Optical density } (A) = A - A_0$$

where $A_0 = A$ value of the concentrate at zero time.

The data reported both for optical and L^*, a^*, b^* measurements are the average of five replications.

Water activity measurement

The a_w, as Equilibrium Vapour Pressure/100 was measured by a Thermoconstanter Hygrometer (Novasina, Zurich, Switzerland) at 25°C. The value reported in Fig. 1 is the average of three replications.

The experiment conducted at 55°C for 300 h was repeated twice and the experiment conducted on the same concentrates at 35, 45 and 55°C for 180 h, was repeated three times. The analysis of variance and Duncan's multiple range test were used to determine statistically significant differences ($P \leqslant 0.05$)

RESULTS AND DISCUSSION

Among the examined colour functions (ΔL^*, s_{ab}, s_{uv}), L^* was the most suitable for studying the rate of colour development during non-enzymatic browning in hydrolysed concentrated cheese whey permeate (HCCWP).

In Fig. 2 the changes of ΔL^* values of the concentrates at pH 6 and at 65, 75 and 85% of DH, heated at 55°C, are shown.

The colour development of the concentrate at 65% DH shows a linear trend ($R^2 = 95.08\%$), while the colour developments of concentrates at 75 and 85% DH show a curvilinear trend [$y = a + b \cdot \ln(x)$; $R^2 = 98.16\%$ and $R_2 = 97.12\%$, respectively].

Table 1. Zero-order rate constant k_0 ($\Delta L^* \cdot h^{-1}$) values obtained by lightness measurement over 180 and 300 h

% DH	180 h		300 h	
	R_2	k_0	R_2	k_0
65	91.10	0.050	95.08	0.038
75	92.47	0.074	89.57	0.043
85	92.36	0.087	84.33	0.054

$\Delta L^* = k_0 \cdot t$; $T = 55°C$.

As already reported (Torreggiani *et al.*, 1993), over the first 180 h, which could be considered a period of practical interest, browning of HCCWP may be described by a zero-order reaction model. Buera *et al.*, 1990 observed the same behaviour in a hydrolysed concentrated cheese whey (HCCW). So the experimental data were fitted by:

$$\Delta L^* = k_0 \times t$$

where k_0 = rate coefficent and t = time.

The k_0 values obtained by L^* measurements over 180 and 300 h are reported in Table 1.

On the basis of these results, the same concentrates at 65, 75 and 85% of DH were heated at 35°C, 45°C and 55°C for 180 h and the changes of ΔL^* values are shown in Figs 3–5. The k_0 values reported in Table 2 are significantly different ($P \leqslant 0.05$) at all tested DH and temperatures except for k_0 of 75 and 85% DH at 45°C, so showing that the browning rate increases as the DH increases. The concentrate at 65% DH is the most stable at all selected temperatures. The influence of the temperature on colour change is also confirmed.

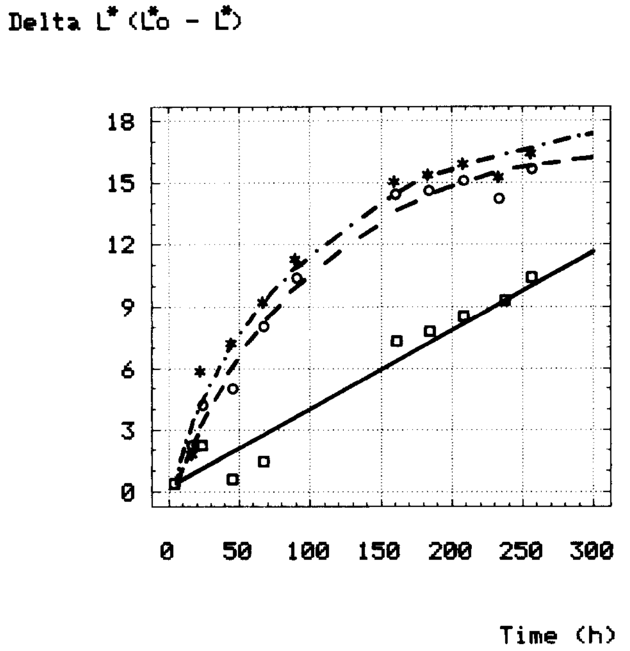

Fig. 2. Effect of lactose hydrolysis on colour development (ΔL^*) in concentrated cheese whey permeate, heated at 55°C for 300 h. DH = 65% —□—; DH = 75% —O—; DH = 85% ---*---.

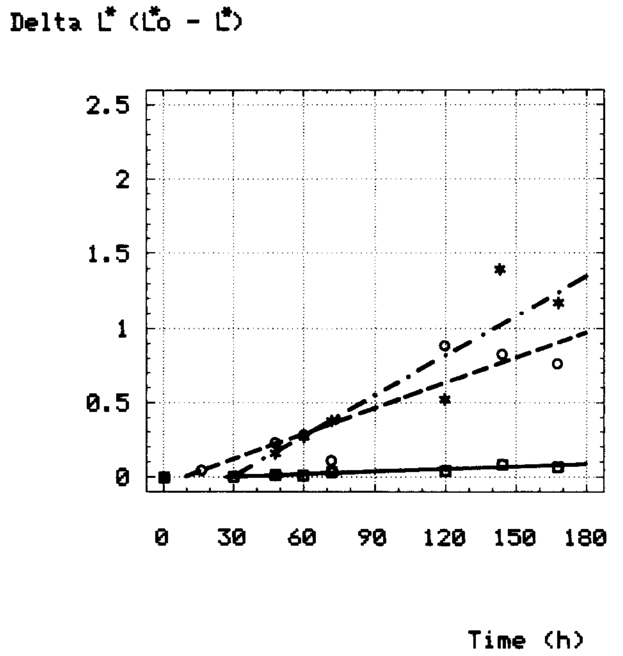

Fig. 3. Effect of lactose hydrolysis on colour development (ΔL^*) in concentrated cheese whey permeate, heated at 35°C for 180 h. DH = 65% —□—; DH = 75% —O—; DH = 85% ---*---.

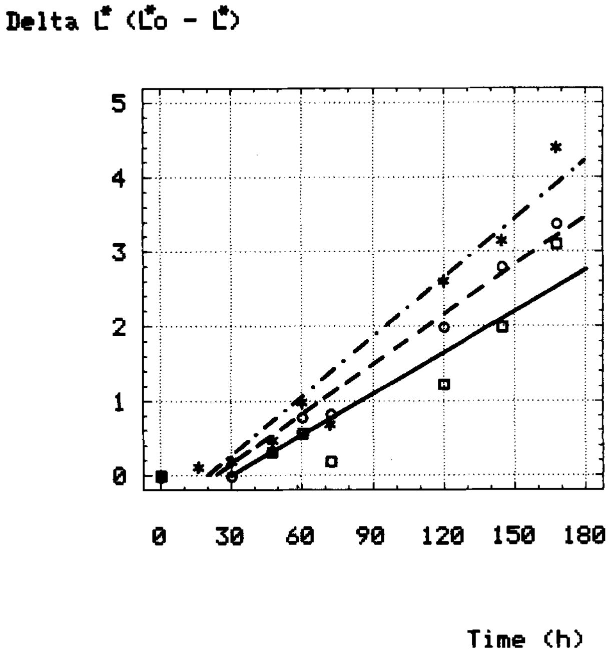

Fig. 4. Effect of lactose hydrolysis on colour development (ΔL^*) in concentrated cheese whey permeate, heated at 45°C for 180 h. DH = 65% —□—; DH = 75% —○—; DH = 85% ---*---.

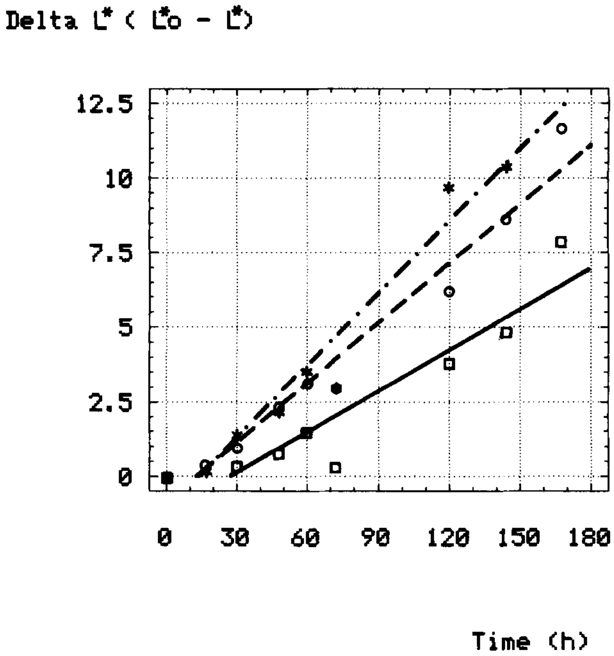

Fig. 5. Effect of lactose hydrolysis on colour development (ΔL^*) in concentrated cheese whey permeate, heated at 55°C for 180 h. DH = 65% —□—; DH = 75% —○—; DH = 85% ---*---.

The activation energy values of the browning process (obtained from k_0 data) were calculated to be 46.3, 24.8 and 22.1 kcal/mol for DH 65, 75 and 85%, respectively. They are significantly different ($P \leqslant 0.05$) and are correlated with the DH values of HCCWP: increasing the DH decreased the activation energy value.

These activation energy values are higher than those reported by Kanterewicz & Chirife (1986) and Buera *et al.* (1990) for browning in non-hydrolysed and hydrolysed concentrated cheese whey, respectively. The HCCWP could be a less reactive system than HCCW because of the lower content of whey proteins: in a 50% solid HCCW the average amount of proteins is 7.5 g/100 g compared with 0.4 g/100 g in HCCWP at the same solids and lactose concentration.

The influence of the DH on the rate of browning of HCCWP has also been followed by spectrophotometric measurement of the optical density at 420 nm (Lerici *et al.*, 1990).

Table 2. Zero-order rate constant k_0 ($\Delta L^* \cdot h^{-1}$) values obtained by lightness measurement over 180 h

% DH	Temperature (°C)	R^2	k_0 ($\Delta L^* \cdot h^{-1}$)
65	35	84.65	4.58513×10^{-4}
75	35	85.81	5.63729×10^{-3}
85	35	87.95	8.88636×10^{-3}
65	45	90.06	1.87668×10^{-2}
75	45	96.97	2.23769×10^{-2}
85	45	95.06	2.63023×10^{-2}
65	55	90.05	4.50620×10^{-2}
75	55	97.03	6.65525×10^{-2}
85	55	97.19	8.06782×10^{-2}

$\Delta L^* = k_0 \cdot t$.

The effect of DH on colour development (ΔA at 420 nm) of HCCWP heated at 35, 45 and 55°C is shown in Figs 6, 7 and 8, respectively.

The reported data confirm that browning of HCCWP may be described by a zero-order reaction model. The k_0 values (Table 3) at all selected DH and temperatures are significantly different ($P \leqslant 0.05$) as such are the activation energy values of the browning process (48.2,

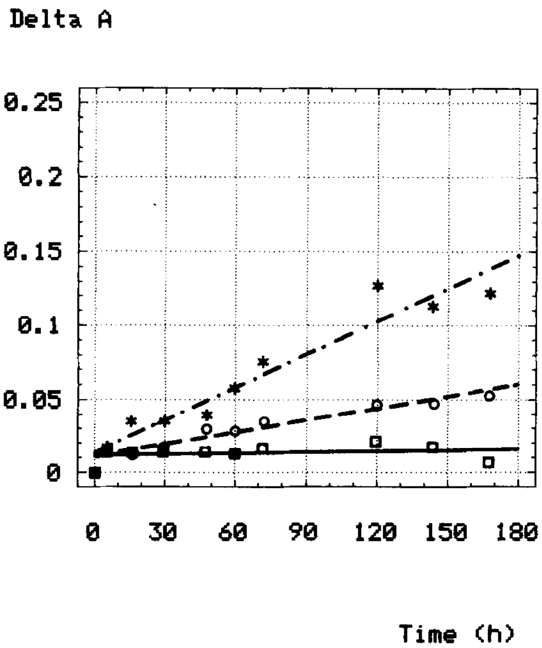

Fig. 6. Effect of lactose hydrolysis on colour development (ΔA) in concentrated cheese whey permeate, heated at 35°C for 180 h. DH = 65% —□—; DH = 75% —○—; DH = 85% ---*---.

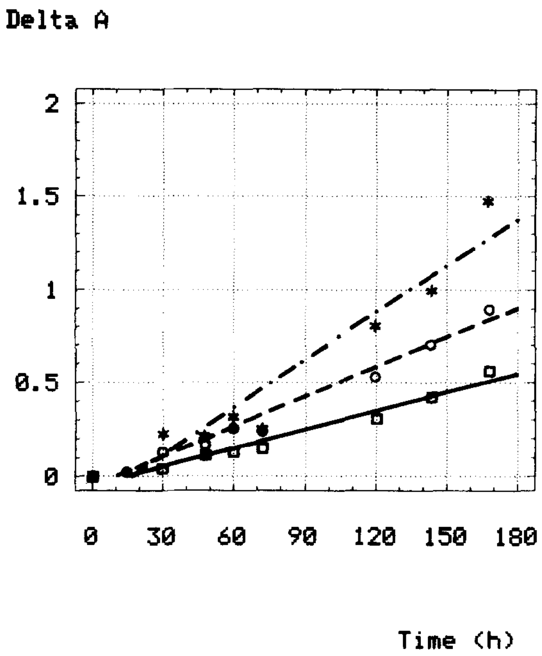

Fig. 7. Effect of lactose hydrolysis on colour development (ΔA) in concentrated cheese whey permeate, heated at 45°C for 180 h. DH = 65% —□—; DH = 75% —○—; DH = 85% ---*---.

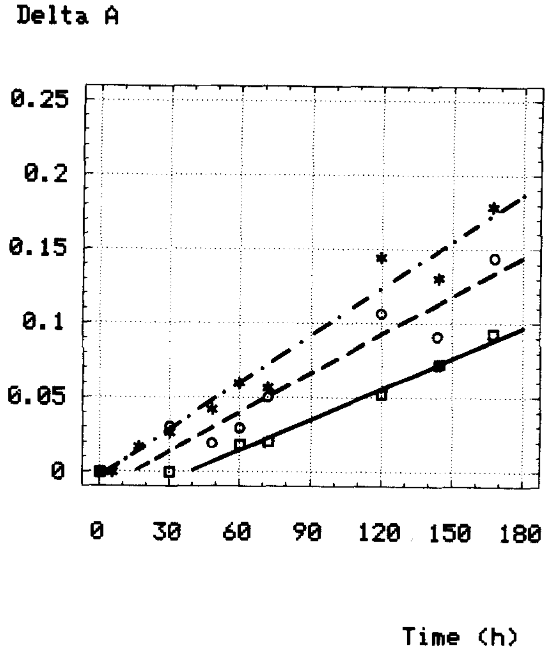

Fig. 8. Effect of lactose hydrolysis on colour development (ΔA) in concentrated cheese whey permeate, heated at 55°C for 180 h. DH = 65% —□—; DH = 75% —○—; DH = 85% ---*---.

29.7 and 24.3 kcal/mol for DH 65, 75 and 85%, respectively). These data confirm the correlation between the DH of HCCWP and the rate of browning. Further studies should be made in order to understand if it is only the DH which influences the browning reaction rate or if other mechanisms could be considered.

The activation energies obtained considering both the absorbance changes at 420 nm and the L^* changes values, are very close, as these colour parameters are both related to the presence of melanoidin compounds.

As the production of hydrolysed lactose syrup is simple, requiring no special plant and utilising material which is often pumped to waste or low value outlets, there is high economic potential for these products.

However, the data indicate that, as reported for HCCW, HCCWP is also very susceptible to browning. By choosing the degree of hydrolysis, the development of the browning could be effectively controlled and the shelf life improved. To widen its practical use, further research is being carried out on the crystallisation and on the microbiological stabilisation of the product.

Table 3. Zero-order rate constant k_0 ($\Delta[L^*]$A·h^{-1}) values obtained by optical density measurement at 420 nm over 180 h

% DH	Temperature (°C)	R^2	$k_0(\Delta A \cdot h^{-1})$
65	35	8.14	2.78680×10^{-5}
75	35	91.61	2.73268×10^{-4}
85	35	92.44	7.37284×10^{-4}
65	45	91.85	6.28290×10^{-4}
75	45	93.61	8.69589×10^{-4}
85	45	96.77	1.05574×10^{-3}
65	55	97.29	3.36661×10^{-3}
75	55	98.29	5.33198×10^{-3}
85	55	94.32	8.44481×10^{-3}

$\Delta A = k_0 \cdot t$.

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