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Short communication

Lipolysis of pork fat by the meat starter culture *Debaryomyces hansenii* at various environmental conditions

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Abstract

Lipolysis of pork fat by the meat starter culture *Debaryomyces hansenii* added at a level of 3.5×10^6 cells/ml was investigated at different temperatures (10–30°C), pH values (4.7–6.0), NaCl concentrations (2.5–7.5% w/v), and times of incubation (5–15 days). Pronounced growth was obtained amounting to 10^7–10^9 cells/ml even at conditions combining the lowest temperature, the lowest pH and the highest NaCl concentration. Pork fat was hydrolysed to an extent depending on the environmental conditions. A quadratic polynomial model was developed describing the combined effects of environmental conditions on lipolysis. Regression analysis of data indicated that temperature, pH and time of incubation at conditions of meat fermentation were all significant factors in controlling lipolysis whereas NaCl concentration at the levels studied had no significant effect. Lipolysis increased when temperature increased. At 10°C, lipolysis was very restricted even though growth was observed. An increase in pH resulted in higher lipolysis, the effect being most pronounced at high temperatures.

Keywords: Lipolysis; *Debaryomyces hansenii*; Fermented meats; Pork fat; Environmental conditions

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1. Introduction

Strains of *Debaryomyces hansenii* are used as starter culture in meat fermentations (Jessen, 1995). The desired properties of yeast starter cultures in relation to meat fermentation include their NaCl tolerance, their ability to grow at low water activities and at low pH values (Leistner and Bem, 1970; Jessen, 1995) in combination with their consumption of oxygen improving and accelerating color formation (Leistner and Bem, 1970; Gehlen et al., 1991; Jessen, 1995). Furthermore, several studies have found that the addition of *D. hansenii* improves flavor development (Gökalp and Ockerman, 1985; Grazia et al., 1989; Gehlen et al., 1991).

Studies of the lipolysis caused by various yeasts isolated from fermented meat products have been carried out (Molina et al., 1991; Boissonnet et al., 1994; Buzzini and Haznedari, 1995). However, only few studies have used pork fat as a substrate to examine lipolysis by yeasts (Molina et al., 1991) and the investigations reported have not considered the combined effects of the environmental factors controlling meat fermentations. Only recently lipolysis of pork fat by the use of cell-free extract of *D. hansenii* has been investigated at different combinations of temperature, pH and NaCl concentrations (Sørensen and Samuelsen, 1996).

The purpose of the present paper has been to examine lipolysis of pork fat by whole cell inocula of the commercial meat starter culture *D. hansenii* at combinations of temperature, pH and NaCl relevant to meat fermentations, and to compare with previous results obtained using cell free extracts of *D. hansenii* (Sørensen and Samuelsen, 1996).

2. Materials and methods

2.1. Culture

The culture used was *D. hansenii*, supplied by Rudolph Müller, Pohlheim, Germany. Before use the freeze dried culture was grown at 30°C overnight in yeast-malt extract broth (YM, Difco, Detroit, MI). Cells were inoculated into 36 different pork fat emulsions (Table 1) at a rate of 3.5×10^6 cells/ml pork fat estimated by phase contrast microscopy.

2.2. Lipolysis of pork fat emulsions

The pork fat emulsions used as model systems to evaluate lipolysis by whole cell inocula of *D. hansenii* were prepared in YM broth (Difco) containing phosphate buffer, 0.2 mol/l, according to the pH value required (Table 1). The YM broth was supplemented with NaCl as required (Table 1), and gum arabic (Sigma), 10% (w/v). Melted pork fat, 10% (v/v), was added slowly during homogenizing (Ultra-Turrax, Janke and Kunkel IKA Labortechnik, Staufen, Germany) at a speed of 24 000 rpm for 4×1 min with 1 min intervals. The pH value of the emulsions was measured with a combined pH electrode (U402-88TE-S7, Ingold, Urdorf, Switzerland) con-

nected to a pH meter (PHM 80, Radiometer, Copenhagen, Denmark). The pH was adjusted to the required value using 0.1 M HCl or 0.1 M NaOH. The emulsions were autoclaved for 15 min at 121°C. Each assay mixture consisted of 0.9 ml

Table 1
Results and the experimental design used to investigate the lipolysis of pork fat by *D. hansenii* at different environmental conditions; the increase in the total amount of free fatty acids (FFA), and the cell count at the end of incubation are shown

Temperature (°C)	pH	NaCl (% w/v)	Incubation time (days)	Lipolysis by *D. hansenii* (μmol FFA)	Log_{10} (cells/ml)
10	4.70	2.5	5	0.00	8.14
10	4.70	2.5	15	0.46	9.47
10	4.70	7.5	5	0.00	7.66
10	4.70	7.5	15	0.09	9.39
10	6.00	2.5	5	0.00	8.20
10	6.00	2.5	15	1.48	9.08
10	6.00	7.5	5	0.00	7.89
10	6.00	7.5	15	2.73	9.13
30	4.70	2.5	5	5.55	9.08
30	4.70	2.5	15	11.19	9.58
30	4.70	7.5	5	1.73	9.07
30	4.70	7.5	15	10.89	9.48
30	6.00	2.5	5	7.40	8.85
30	6.00	2.5	15	13.78	9.48
30	6.00	7.5	5	9.44	9.13
30	6.00	7.5	15	13.88	9.46
0[a]	5.35	5.0	10	0.00[b]	6.97
40[a]	5.35	5.0	10	0.00[b]	7.07
20	4.05[a]	5.0	10	2.59	9.27
20	6.65[a]	5.0	10	11.96	9.23
20	5.35	0.0[a]	10	3.61	9.08
20	5.35	10.0[a]	10	3.38	9.01
20	5.35	5.0	0[a]	0.00	6.54
20	5.35	5.0	20[a]	7.74	9.42
20	5.35	5.0	10	4.23	9.11
20	5.35	5.0	10	3.85	9.14
20	5.35	5.0	10	5.37	9.20
20	5.35	5.0	10	4.02	9.22
20	5.35	5.0	10	4.23	9.14
20	5.35	5.0	10	3.58	9.22
20	5.35	5.0	10	3.85	9.27
20	5.35	5.0	10	4.14	9.13
20	5.35	5.0	10	4.07	9.14
20	5.35	5.0	10	3.79	9.06
20	5.35	5.0	10	3.86	9.04
20	5.35	5.0	10	3.72	9.12

[a] These values exceed the chosen ranges of the environmental factors due to the experimental design used.

[b] It was not possible to fit the quadratic polynomial model to the results of these observations and therefore they were excluded from the model development.

emulsion added to 0.1 ml whole cell inocula of *D. hansenii*. Control emulsions were added to 50 mM phosphate buffer pH 6.0 instead of cell inocula. Incubations were carried out as stated in Table 1. Each experiment was carried out in triplicate. The amount of free fatty acids was determined by titration as described previously (Sørensen and Samuelsen, 1996).

2.3. Experimental design

The experimental design chosen to investigate the combined effects of environmental conditions on lipolysis was constructed using SAS/STAT for Windows 6.10 (SAS, Cary, NC, USA). The experiment was a response surface methodology (RSM), central composite design (CCD-O) with the four variables being temperature (T, °C), pH (P), NaCl (S, % w/v) and incubation time (I, days). The combinations of the variables are shown in Table 1. The effects of the independent variables on lipolysis of pork fat (μmol FFA) by *D. hansenii* was modelled using the REG and the RSREG procedures in SAS/STAT.

3. Results and discussion

The results showed that the meat starter culture *D. hansenii* is able to hydrolyse a natural fatty substrate like pork fat and release fatty acids. The amounts of fatty acids released at the different environmental conditions are shown in Table 1. Growth of *D. hansenii* occurred at all environmental conditions investigated and the cell numbers at the end of incubation are listed in Table 1. The regression analysis is presented in Table 2. The statistics for the quadratic polynomial model of lipolysis is shown in Table 2. The values of R^2 and $\bar{R}^2$ (Table 2) indicate that 96% and 93%, respectively, of the total variance in the response is explained by the model. Temperature, pH and time of incubation all affected the lipolytic activity significantly whereas NaCl concentration had no significant effect ($P > 0.05$).

From Fig. 1 it can be seen that lipolysis increased when temperature increased and during the entire incubation period. At 10°C, lipolysis was very restricted during the whole incubation period, even though the yeast strain was able to grow (Table 1). However, from previous results it is known that the hydrolysing capacity of *D. hansenii* lipase is very limited at 10°C (Sørensen and Samuelsen, 1996), explaining the restricted lipolysis caused by whole cells of *D. hansenii* observed in the present study. An increase in pH resulted in higher lipolysis, the effect being most pronounced at high temperatures (Fig. 2). At the lowest pH examined, pH 4.7, pronounced lipolysis only occurred at temperatures above 10°C. No increase in cell count was observed by increasing pH (Table 1) but the increased lipolysis is likely to be caused by the increased hydrolysing capacity of the lipase at higher pH values (Sørensen and Samuelsen, 1996). Altering NaCl concentration in the range examined (2.5–7.5%) had no significant effect on lipolysis (Table 2) and the cell count of *D. hansenii* was not affected either (Table 1). Previous results on the effect of NaCl addition on the hydrolysing capacity of *D. hansenil* lipase support the

Table 2
Parameter estimates and statistics derived from the results of the lipolysis (μmol FFA) of pork fat by *Debaryomyces hansenii* at different conditions of temperature (T), pH (P), NaCl concentration (S) and incubation time (I)

Parameter	Estimate	t value	Significance level
Intercept	58.2300	4.0040	0.0008
T	−0.4790	−1.7790	0.0912
P	−21.6200	−4.3370	0.0004
S	−1.4020	−1.5270	0.1434
I	−0.1300	−0.2830	0.7800
$T \times T$	0.0024	0.6000	0.5558
$P \times P$	1.9030	4.2460	0.0004
$S \times S$	−0.0230	−0.7450	0.4655
$I \times I$	−0.0020	−0.2500	0.8055
$T \times P$	0.1100	2.8940	0.0093
$T \times S$	−0.0070	−0.7210	0.4797
$T \times I$	0.0261	5.2590	0.0000
$P \times S$	0.3030	1.9870	0.0616
$P \times I$	−0.0060	−0.0810	0.9365
$S \times I$	0.0123	0.6200	0.5425
F test	36.900	(significant with 14, 19 df at a 1% level of significance)	
R^2	0.965		
$\bar{R}^2$	0.938		
Lack of fit test[a]			

[a] Test not performed since preparation of the center point emulsions was not fully randomized.

present results on lipolysis by whole cell inocula (Sørensen and Samuelsen, 1996). Generally, in comparison with previous examinations using cell free extracts of *D. hansenii* produced at optimal conditions, no pronounced difference were observed for the combined effects of the environmental conditions on lipolysis.

Lipolysis caused by *D. hansenii* was not affected by NaCl and it was still significant at pH 4.7, indicating that this commercially available starter culture may hydrolyse pork fat during processing of fermented meat products. Compared to another meat starter culture, *Staphylococcus xylosus* (Sørensen and Samuelsen, 1996), lipolysis by *D. hansenii* seems to be much more resistant to the decrease of pH occurring during fermentation of meat products like sausages.

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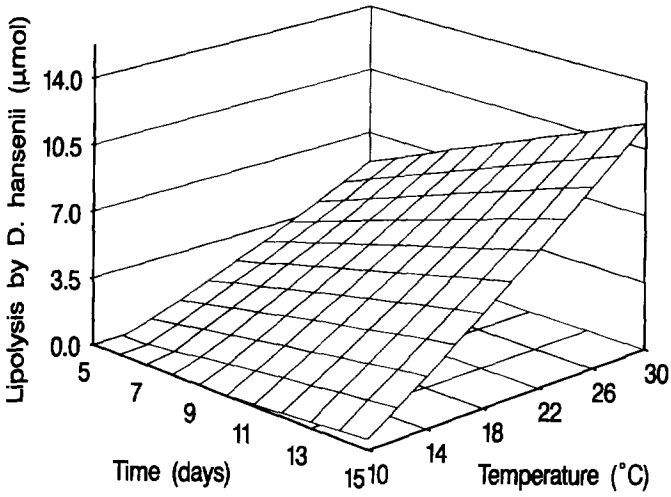

Fig. 1. Three dimensional response surface curve of the predicted pork fat lipolysis caused by *D. hansenii* as a function of incubation time and temperature at pH 5.35 and at 7.5% (w/v) NaCl.

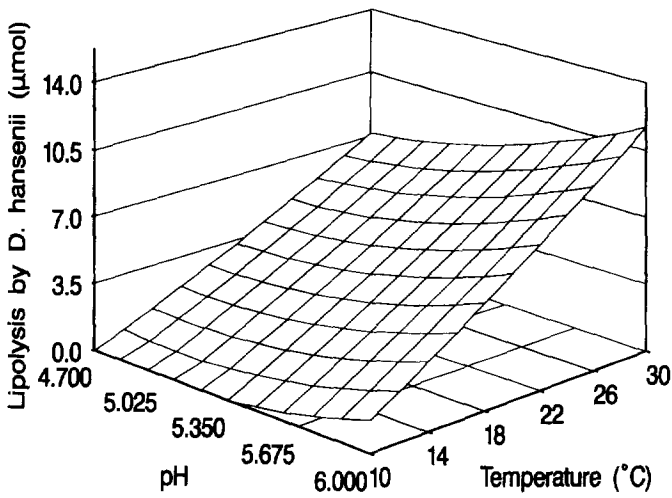

Fig. 2. Three dimensional response surface curve of the predicted pork fat lipolysis caused by *D. hansenii* as a function of pH and temperature at 5% (w/v) NaCl and after 10 days of incubation.

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