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Modelling of Effects of Water Activity, pH and Temperature on the Growth Rate of *Mucor racemosus* Isolated from Soft Camembert Cheese

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Abstract: The prediction of the effect of the pH (4.0-6.0), NaCl (1.5-3.0%) corresponding to the values of aw (0.987-0.910) and of the temperature (10-30°C) on the growth of the colonies of *Mucor racemosus* isolated from Camembert cheese during the stage of refining was carried out on solid medium, the sabouraud with chloramphenicol. The fungic growth was obtained by the daily measurement of the diameter of the colonies (mm. Day-1). The primary predictive model of Baranyi was adopted to consider the growth rate maximum specific (μ_{max}). In one second approach, a secondary predictive models was developed, the polynomial model. The empirical models gave a satisfactory prediction of the experimental results. The effects of the temperature and the a_w on the growth of this fungic species were clearly shown, contrary to the pH which, with the experimental values tested, exerted any effect on the growth. This result was also shown by the variance analysis. The results of this study could be exploited by the industrialists of cheese dairy in order to predict the development of *Mucor racemosus* in Camembert cheese.

Key words: *Mucor racemosus* . Camembert cheese . Temperature . pH . Water activity . Predictive modelling

INTRODUCTION

Most of the food may be contaminated by moulds during their preparation, especially during their storing. Moulds are concern to the food industry as potential spoilage organisms [1], other sorts are mycotoxin producers [2]. For this reason, it is important to understand the growth kinetics of these organisms in the food context, in order to control the quality of the product from formulation to storage [3]. Predictive modelling has been extensively used mainly to predict bacterial growth as a function of environmental factors such as temperature, pH and activity of water [4-7]. However, model development of fungal growth has not received the same level of attention as that of bacterial growth [8, 9]. A few studies concerning fungal growth have dealt with the predictive modelling approach [10-12]. Several factors act on the growth of *Mucor* in the camembert cheese, the pH, the concentration in NaCl, the activity of the water, the relative humidity and the temperature practised during the stage of ripening [13]. The combination of these parameters quoted by the use of the modelling could serve in the context of the predictive microbiology to control the growth of *Mucor* during the ripening stage and all the duration of conservation. The aim of the present work was to develop and evaluate empirical models to the growth data of *Mucor racemosus.* Models resulting from this study could be used to predict and to prevent the germination and growth of *M.racemosus* as a function of temperature, pH and water activity.

MATERIALS AND METHODS

Fungal isolates: The fungus used in this study was isolated from the industrial camembert cheese during the ripening and was identified as *Mucor racemosus* according to the method of Le Mens [14].

Inoculum preparation: Fungi were grown on sabouraud agar added to chloramphenicol for 07 days at 24±0.5°C to obtain heavily sporulating cultures. Spores were then suspended in sterile distilled water containing 0.005% of wetting agent (tween 80). The final concentration of spores was in the range 1-5 10^6 spores ml^{-1}.

Growth media and incubation conditions: The standard growth medium used in all experiments was chloramphenicol sabouraud agar. Media of different water activity (a_w) were prepared by adding an adequate volume of sterile saturated solution of NaCl (Merck) in concentration of 1.5, 2, 2.5 and 3% (w/v). The final water activity was 0.987, 0.90, 0.85 and 0.80,

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respectively. Water activity was measured using a Novasina TH 200 (Novasina, Pfäffikon, Switzerland). The pH of medium was adjusted to the values of 4.0, 4.5, 5.0 and 6.0 with HCl (hydrochloric acid 0.1N).

Petri plates were inoculated in the center with 1 ml volume of spores suspension containing 20 ml solidified growth medium. The inoculated petri plates were incubated at 10, 15, 20, 25 and 30°C in high precision (±0.1°C), in polyethylene bags.

The assayed conditions were 4 a_w levels, 5 temperatures and 4 pH levels. All treatments were repeated twice.

Measurement: The diameters (y, expressed in mm) of the colony were measured in vertical and horizontal directions daily, at the same time (t, expressed in days) [15]. The experiment lasted 30 days.

Linear regression of colony radius against time was used to obtain growth rates (mm.days^{-1}) under each of conditions [16]. Microsoft Excel version 97 was used for this purpose. Analysis of variance for the different sets of results was carried out using statbox version 6.3.

The maximum colony growth rates (μ_{max}) were obtained by applying Baranyi *et al*. model [17]. The average estimates of μ_{max} were then fitted to secondary models to describe the single and combined effects of pH, temperature and a_w on fungal growth.

A quadratic response surface was used and useful transformation of water activity was applied as introduced by Gibson *et al*. [8].

$$b_w = v(1-a_w) \quad (1)$$

The natural logarithm of maximum colony growth rates had following form:

$$\begin{aligned} \text{Ln } \mu_{max} = {} & a_1 + a_2\,T + a_3\,b_w + a_4\,pH + a_5\,T^2 \\ & + a_6\,b_w^2 + a_7\,pH^2 + a_8 T.b_w \\ & + a_9 T.pH + a_{10}\,b_w.pH \end{aligned} \quad (2)$$

The coefficients a_1……..a_{10} were estimated by linear regression.

RESULTS

The growth curves based on colony diameter were typical of fungal growth with a lag phase ranged from 6 to 7 days, followed by linear phase and upper asymptote. The estimated maximum colony growth (μ_{max}) rates for each combination of a_w, pH and temperature (T°C) are noted in Table 1.

The behaviour of *Mucor racemosus* according to the studied environmental parameters was quantified by the application of second model.

Table 1: Evaluation of specific growth rates of *Mucor racemosus* at various temperatures, a_w and pH

Temperature (°C)	pH	NaCl (%)	a_w	b_w	μ_{max}
10	4.0	1.5	0.987	0.114	2.640
10	4.5	1.5	0.987	0.114	2.110
10	5.0	1.5	0.987	0.114	2.280
10	6.0	1.5	0.987	0.114	1.431
15	4.0	1.5	0.987	0.114	5.231
15	4.5	1.5	0.987	0.114	6.340
15	5.0	1.5	0.987	0.114	6.452
15	6.0	1.5	0.987	0.114	6.221
20	4.0	1.5	0.987	0.114	8.751
20	4.5	1.5	0.987	0.114	10.622
20	5.0	1.5	0.987	0.114	10.540
20	6.0	1.5	0.987	0.114	9.281
25	4.0	1.5	0.987	0.114	10.469
25	4.5	1.5	0.987	0.114	11.052
25	5.0	1.5	0.987	0.114	14.118
25	6.0	1.5	0.987	0.114	12.109
30	4.0	1.5	0.987	0.114	8.119
30	4.5	1.5	0.987	0.114	7.220
30	5.0	1.5	0.987	0.114	7.455
30	6.0	1.5	0.987	0.114	6.356
10	4.0	2.0	0.950	0.223	2.542
10	4.5	2.0	0.950	0.223	2.210
10	5.0	2.0	0.950	0.223	2.042
10	6.0	2.0	0.950	0.223	1.344
15	4.0	2.0	0.950	0.223	4.783
15	4.5	2.0	0.950	0.223	5.630
15	5.0	2.0	0.950	0.223	5.784
15	6.0	2.0	0.950	0.223	4.796
20	4.0	2.0	0.950	0.223	7.980
20	4.5	2.0	0.950	0.223	9.875
20	5.0	2.0	0.950	0.223	10.438
20	6.0	2.0	0.950	0.223	6.875
25	4.0	2.0	0.950	0.223	9.872
25	4.5	2.0	0.950	0.223	10.324
25	5.0	2.0	0.950	0.223	11.530
25	6.0	2.0	0.950	0.223	6.480
30	4.0	2.0	0.950	0.223	8.688
30	4.5	2.0	0.950	0.223	9.124
30	5.0	2.0	0.950	0.223	9.230
30	6.0	2.0	0.950	0.223	5.756
10	4.0	2.5	0.935	0.254	2.320
10	4.5	2.5	0.935	0.254	2.410
10	5.0	2.5	0.935	0.254	2.465
10	6.0	2.5	0.935	0.254	1.115
15	4.0	2.5	0.935	0.254	4.156

Table 1: Continued

Temperature (°C)	pH	NaCl (%)	a_w	b_w	μ_{max}
15	4.5	2.5	0.935	0.254	4.142
15	5.0	2.5	0.935	0.254	4.210
15	6.0	2.5	0.935	0.254	3.854
20	4.0	2.5	0.935	0.254	6.652
20	4.5	2.5	0.935	0.254	8.243
20	5.0	2.5	0.935	0.254	8.540
20	6.0	2.5	0.935	0.254	6.112
25	4.0	2.5	0.935	0.254	7.878
25	4.5	2.5	0.935	0.254	9.545
25	5.0	2.5	0.935	0.254	10.010
25	6.0	2.5	0.935	0.254	8.532
30	4.0	2.5	0.935	0.254	6.731
30	4.5	2.5	0.935	0.254	7.012
30	5.0	2.5	0.935	0.254	7.154
30	6.0	2.5	0.935	0.254	6.520
10	4.0	3.0	0.91	0.3	1.980
10	4.5	3.0	0.91	0.3	1.985
10	5.0	3.0	0.91	0.3	2.045
10	6.0	3.0	0.91	0.3	1.008
15	4.0	3.0	0.91	0.3	3.285
15	4.5	3.0	0.91	0.3	3.310
15	5.0	3.0	0.91	0.3	3.657
15	6.0	3.0	0.91	0.3	2.855
20	4.0	3.0	0.91	0.3	1.718
20	4.5	3.0	0.91	0.3	1.908
20	5.0	3.0	0.91	0.3	1.919
20	6.0	3.0	0.91	0.3	1.049
25	4.0	3.0	0.91	0.3	1.795
25	4.5	3.0	0.91	0.3	1.967
25	5.0	3.0	0.91	0.3	1.978
25	6.0	3.0	0.91	0.3	1.774
30	4.0	3.0	0.91	0.3	1.354
30	4.5	3.0	0.91	0.3	1.443
30	5.0	3.0	0.91	0.3	1.429
30	6.0	3.0	0.91	0.3	1.149

Secondary models were obtained for prediction of the natural logarithm of maximum colony growth rate (ln μ_{max}) as a function of pH, temperature and activity of water expressed by b_w (polynomial multiple linear regression of equation (2).

The responses expressed by the surfaces (Fig. 1) according to the range of experimental conditions are represented by various curves having a shape of parabola with relatively parallel positions, what implies that the experimental factors (pH, a_w and temperature) act independently and put additive effects. It was also

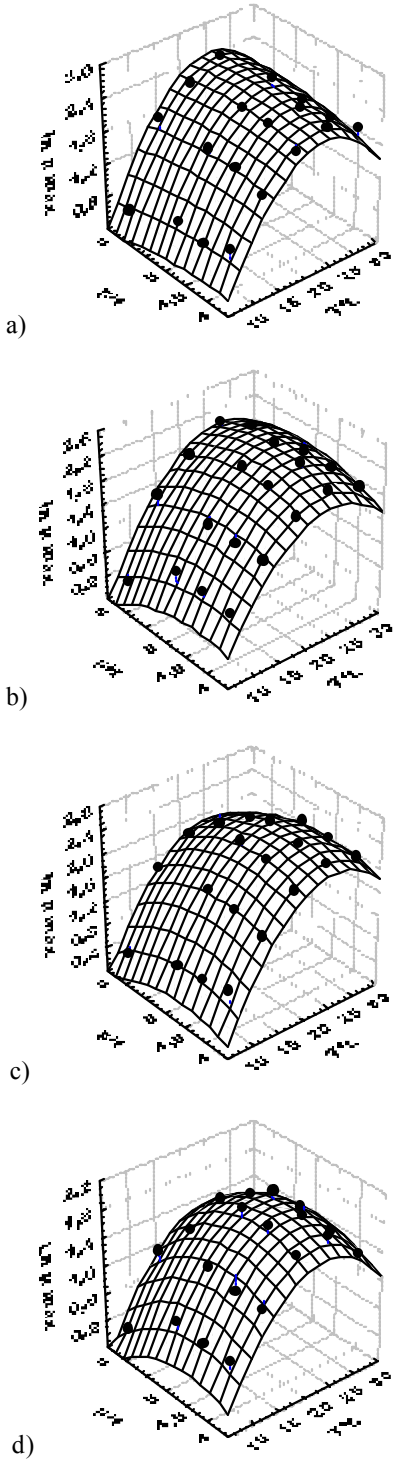

Fig. 1: Quadratic response surfaces predicting the effect of pH, temperature and Nacl concentration on the natural logarithm of maximum specific colony growth (ln μ_{max}) of *Mucor racemosus*. a) 1.5% NaCl; b) 2% NaCl; c) 2.5% NaCl; d) 3% NaCl

Table 2: Analysis of variance of the effects of pH, temperature and water activity on growth of *M.racemosus* on chloramphenicol sabouraud agar medium

Source of variation	df	M.S	F value
Total variance	79	744.258	
Temperature (T)	4	58.848	22.616**
pH	3	0.620	0.318^{ns}
a_w	3	32.536	16.672**
TxpH	12	616.930	79.03*
Txa_w	12	7.916	1.014**
pH x a_w	9	3.989	0.681^{ns}

df: degrees of freedom; M.S: mean square, ns: not significant, (*): significant $p<0.05$, (**): significant $p<0.01$

Table 3: Coefficients and statistical parameters for the polynomial growth model of *Mucor racemosus*

Model	Parameter	Estimated value	R^2	d.f	RMSE
Polynomial	a1	-16.2405	0.953	66	0.192
	a2	1.5334			
	a3	5.1914			
	a4	-0.1232^{ns}			
	a5	-1.2465			
	a6	0.1044			
	a7	0.0029^{ns}			
	a8	0.0907			
	a9	-0.001			
	a10	-0.0067			

d.f: degree of freedom; RMSE: root mean square error, R: correlation coefficient

observed that the growth rates are better in the optimal region of temperature (20-25°C), of concentration in NaCl (1.5%) and of pH (4.5-5) (Fig. 1a,b). On the other hand, the growth rates are for a low level in not optimal conditions such as the temperature of 10-15°C, a concentration in NaCl of 2.5 3% and a pH = 6 (Fig. 1c,d).

Statistical analysis of variance showed significant differences due to a_w, temperature and also the interaction a_w x temperature (Table 2), but no effect of the pH was obtained.

DISCUSSION

Mucor is a rather fast growing fungus. Since the fungus was isolated from soft cheese camembert, it would be important to investigate experimental conditions closer to the brine environment of cheese dairy. Thus, the NaCl concentration used in manufacturing vary generally from 1.5 to 3%.

The temperature varies from 10 to 30°C since the cheese in its manufacture involves several steps that happen at ambient and at low temperature generally ranged from 8 to 15°C (drying and ripening). Finally for pH, it ranged from 4 to 6 because after fermentation, pH values are ranged from 4.3 to 5.5 and increased to about 7 at end of ripening (12^{th} days).

The results corroborate those obtained experimentally by Le Bars-Bailly *et al.* [18] which showed that the optimal conditions for growth of *Mucor racemosus*, are at a temperature of 22°C, pH 4.5 and low salt content (< 1.5%). according Furthermore Pitt and Hocking [19], most of the mould grow below 0.80 a_w, however, as zygomycetes *Rhizopus* and *Mucor* and some Deuteromycetes as *Fusarium* and *Trichoderma* can not develop into below 0.90 a_w. In another studies,the optimum growth of several strains of *Mucor* contamination on experimental cheese is ranged between 20 and 24°C [14].

The quantification of results was obtained through predictive models. Using the primary model developed by Baranyi *et al.* [17] determined the maximum growth rate of *Mucor racemosus* depending on different combinations of parameters (T °, pH and salt levels). The same model has been applied for fungal species as *Penicillium roqueforti* [10], *Aspergillus flavus* [8] and *Penicillium brevicompactum* [11].

The polynomial model is an approach to empirical models for fungus and is often preferable that the mechanistic models [8].

In the case of our tests, this model has shown good performance in terms of r^2 (correlation coefficient) and RMSE (root mean square error) between the experimental and predicted values (Table 3)

Similar results were obtained by Panagou *et al.* [12]; Vali'k *et al.* [10]; Vali'k and Pieckova [20]. However, the secondary model shows no significant effect of pH on the rate maximum growth of *Mucor racemosus*, this has been confirmed both by the results of the variance analysis.

Predictive microbiology studies the behaviour of micro-organisms under different physico-chemical conditions such as temperature, water activity, pH. It can help the identification of critical points of production and distribution process and optimisation of production and distribution chains [6]. The modelling approach introduced in this paper can contribute to predict the development of *Mucor racemosus* in camembert cheese.

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