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Strategies to increase the stability of intermediate moisture foods towards *Zygosaccharomyces rouxii*: The effect of temperature, ethanol, pH and water activity, with or without the influence of organic acids

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ABSTRACT

Intermediate moisture foods (IMF) are in general microbiologically stable products. However, due to health concerns consumer demands are increasingly forcing producers to lower the fat, sugar and preservatives content, which impede the stability of the IMF products. One of the strategies to counteract these problems is the storage of IMF products at lower temperatures.

Thorough knowledge on growth/no growth boundaries of *Zygosaccharomyces rouxii* in IMF products, also at different storage temperatures is an important tool for ensuring microbiologically stability. In this study, growth/no growth models for *Z. rouxii*, developed by Vermeulen et al. (2012) were further extended by incorporating the factor temperature. Three different data sets were build: (*i*) without organic acids, (*ii*) with acetic acid (10,000 ppm on product basis) and (*iii*) with sorbic acid (1500 ppm on product basis). For each of these data sets three different growth/no growth models were developed after 30, 60 and 90 days.

The results show that the influence of temperature is only significant in the lower temperature range (8–15 °C). Also, the effect of pH is negligible (pH 5.0–6.2) unless organic acids are present. More specific, acetic acid had only an additive effect to ethanol and a_w at low pH, whereas sorbic acid had also an additive effect at the higher pH values. For incubation periods longer than 30 days the growth/no growth boundary remained stable but enlarged gradually between day 60 and 90, except for the lower temperature range (<12 °C) where the boundary shifts to more stringent environmental conditions.

1. Introduction

Sweet intermediate moisture foods (IMF) such as jams, bakery products, and chocolate fillings, generally contain between 20 and 50% (w/w) of water and a high amount of soluble compounds, which results in low water activity (a_w) values of 0.70–0.90. Consequently, these types of products are regarded as microbiologically stable. Due to health concerns and increased consumer demands for preservative free products, producers are more and more forced to lower the fat content, sugar content and use of preservatives. This can impede the stability of the products and cause spoilage of sweet IMF products by growth of osmophilic yeasts and xerophilic moulds, leading to e.g. cracked chocolates.

Zygosaccharomyces rouxii is the most notorious spoilage yeast in sweet IMF products causing problems with visible growth and fermentative spoilage (Fleet, 1992; Jermini and Schmidt-Lorenz, 1987; Martorell et al., 2005). This yeast needs a certain amount of sugar (lowered water activity) to achieve optimal growth and is able to grow at relatively low water activities (>0.65) (Tokuoka, 1993). Besides this, the yeast is also very acid resistant and tolerates low pH values (Praphailong and Fleet, 1997; Restaino et al., 1983; Vermeulen et al., 2012).

Growth/no growth (G/NG) models at 22 °C were previously developed for *Z. rouxii* including the factors a_w, pH, ethanol and acetic acid. However, it was demonstrated that at 22 °C the

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Table 1
Parameter estimates and the goodness-of-fit statistics for the growth/no growth models without organic.

Parameters	30 days		60 days		90 days	
	Estimate	St. dev.	Estimate	St. dev.	Estimate	St. dev.
a_w	2177.3	168.8	1847.0	165.5	716.8	77.7
Eth	8.7	0.9	15.3	1.3	1.0	0.4
pH	−36.8	7.8	−18.6	5.6	−19.9	4.5
Temp	2.1	0.1	4.9	0.5	4.6	0.3
a_w^2	−1302.5	102.0	−974.4	92.9	−372.7	47.1
Eth^2	−0.03	0.02	0.05	0.01	−0.07	0.005
pH^2	2.4	0.6	1.8	0.5	1.9	0.4
$Temp^2$	−0.02	0.01	−0.02	0.004	−0.02	0.002
$a_w \cdot Eth$	−8.4	1.0	−19.3	1.7	−1.9	0.5
$a_w \cdot pH$	13.3	4.6	–	–	–	–
$a_w \cdot Temp$	–	–	−2.6	0.5	−3.8	0.3
Eth·pH	−0.2	0.06	–	–	–	–
Eth·Temp	−0.1	0.02	−0.12	0.01	0.04	0.004
pH·Temp	–	–	−0.1	0.03	−0.1	0.02
Goodness-of-fit statistics						
−2log L	937.89		1166.77		1947.27	
AIC	961.89		1190.77		1971.27	
Hosmer–Lemeshow	26.422		19.055		63.881	
c-Value	0.991		0.986		0.958	
% Correct predicted	95.6		95		90	

microbial stability of sweet IMF cannot be guaranteed by simply lowering pH and a_w. Even with the addition of ethanol and acetic acid, it was impossible to prevent growth of *Z. rouxii* at the highest pH (6.2) and a_w (0.88) tested (Vermeulen et al., 2012). Therefore, the use of chemical preservatives seems to be inevitable or other factors need to be taken into consideration. IMF producers involved in this study were interested in the effect of lowering the storage temperature of e.g. chocolate fillings and chocolates, in order to achieve longer shelf-lives or less product loss due to spoilage.

Based on this, the present study extended the model developed by Vermeulen et al. (2012) by incorporating the factor temperature, next to pH, a_w and ethanol. In addition, the effect of a constant concentration of organic acids was tested leading to three different data sets: (*i*) without organic acids, (*ii*) with acetic acid (10,000 ppm on product basis) and (*iii*) with sorbic acid (1,500 ppm on product basis). For each of these data sets three different G/NG models were developed after 30, 60 as well as after 90 days.

2. Material and methods

2.1. Preparation of growth media

Sabouraud broth (Oxoid, Basingstoke, UK) was used as basic medium for the growth of *Z. rouxii*. This medium was modified to mimic chocolate fillings as described in Vermeulen et al. (2012). In short, high amounts of sugar (50% (w/w), glucose (G-8270, Sigma–Aldrich, Steinheim, Germany) and fructose (F-0127, Sigma–Aldrich) in a 1:1 ratio) were added. The media were varying in (*i*) a_w (0.76–0.88, four equidistant levels) by adding glycerol (Sigma–Aldrich), (*ii*) pH (5.0–6.2, four equidistant levels) by adding HCl (UN 1789, Merck, Darmstadt, Germany) (*iii*) ethanol concentration (0–4.5% (w/w) on total medium basis, four equidistant levels) (Merck). All these media were made without organic acids, with acetic acid (10,000 ppm on product basis) (Sigma–Aldrich) or with sorbic acid (1,500 ppm on product basis) added as potassium sorbate (Sigma–Aldrich). The 10,000 ppm acetic acid on total medium basis was chosen to build up a data set at the same conditions as in the article of Vermeulen et al. (2012) at 22 °C, only. To establish the water activity, calibration curves were used describing a_w as a function of the added concentrations of glycerol to the modified Sabouraud medium (with glucose and fructose) (Vermeulen et al., 2012). The exact a_w value was determined by a Labmaster a_w equipment (Novasina, Stork Intermes NV, Berchem, Belgium). The pH was measured by a pH electrode (Knick pH meter, Berlin, Germany).

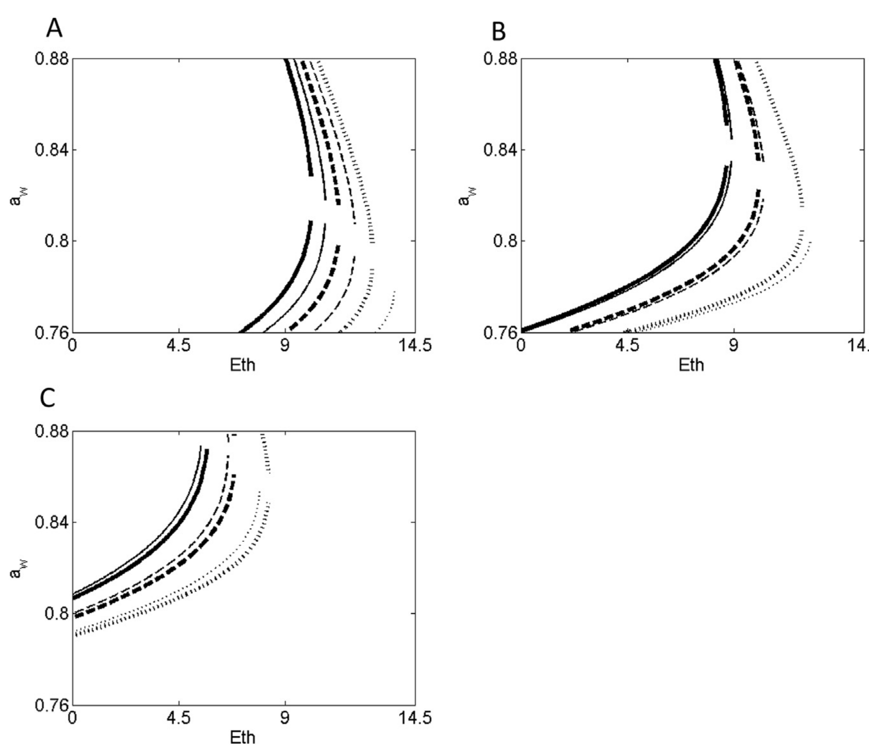

Fig. 1. Growth/no growth boundary after 60 days of incubation at 22 °C (A), 15 °C (B) and 8 °C (C) in media without acetic acid at pH 5.0 (thin lines) and 6.2 (bold lines). Lines represent the ordinary logistic regression model predictions $p = 0.9$ (—), $p = 0.5$ (- -), $p = 0.1$ (…).