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## المسببة لفساد الأغذية *Debaryomyces hansenii* توقع نمو خميرة وذلك باستخدام التحليل الإحصائي المتعدد العوامل

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## Growth Prediction of the Food Spoilage Yeast *Debaryomyces Hansenii* using Multivariate Data Analysis

توقع نمو خميرة *Debaryomyces hansenii* المسببة لفساد الأغذية وذلك باستخدام التحليل الإحصائي المتعدد العوامل

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**Abstract:** The main aim of the present study was to predict the growth of the food spoilage yeast *Debaryomyces hansenii* by multivariate data analysis (MVDA) using temperature, pH and NaCl concentration as growth parameters. Growth of five strains of *D. hansenii* (DHI, DHII, DHIII, DHIV and DHV) was measured as optical density at 620 nm (OD620) at different values of temperature, pH and NaCl concentrations. It was found that salt was the most important factor, which affects yeast growth followed by temperature. The growth of all yeast strains was reduced by increasing salt concentration and decreasing temperature. On the other hand, pH was found to have a little effect on the growth of *D. hansenii*. Strain DHII was the most salt-tolerant strains among the five yeast strains investigated. Partial least squares (PLS) prediction model was created out using pH, temperature and NaCl concentration to predict the growth of *D. hansenii*. The model was acceptable with a correlation of 0.86. The developed PLS model will help in optimizing the food process conditions that will prevent food spoilage by *D. hansenii*.

**Keywords:** *Debaryomyces hansenii*, multivariate data analysis, food spoilage, partial least squares (PLS) model.

**المستخلص:** كان الهدف الرئيسي من هذه الدراسة هو التنبؤ بنمو خميرة *Debaryomyces hansenii* التي تسبب فساد الأغذية والطعام من خلال تحليل البيانات متعدد المتغيرات (MVDA) باستخدام درجة الحرارة، ودرجة الحموضة، وتركيز كلوريد الصوديوم كمؤشرات نمو. تم قياس نمو خمس سلالات من *Debaryomyces hansenii* (DHI, DHII, DHIII, DHIV and DHV) بواسطة الكثافة البصرية عند 620 نانومتر (OD620) بقيم مختلفة من درجات الحرارة ودرجة الحموضة وتركيزات كلوريد الصوديوم. وجد أن الملح كان أهم عامل يؤثر على نمو الخميرة تليها درجة الحرارة. تم تقليل نمو جميع سلالات الخميرة بزيادة تركيز الملح وخفض درجة الحرارة. من ناحية أخرى، وجد أن الأس الهيدروجيني (درجة الحموضة) له تأثير ضئيل على نمو *D. hansenii*. كانت سلالة DHII أكثر سلالات الخميرة مقاومة للملح من بين سلالات الخميرة الخمس التي تم فحصها. تم إنشاء نموذج التنبؤ بواسطة: Partial (PLS)

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Least Squares باستخدام الأس الهيدروجيني ودرجة الحرارة وتركيز كلوريد الصوديوم للتنبؤ بنمو *D. hansenii*. كان النموذج مقبولاً مع قيمة ارتباط 0.86. وسيساعد نموذج PLS المطور في تحسين ظروف عملية الغذاء التي ستمنع تلف الطعام بواسطة *D. hansenii*.

**الكلمات المفتاحية:** *Debaryomyces hansenii*، تحليل البيانات متعدد المتغيرات، فساد المواد الغذائية، نموذج المربعات الصغرى الجزئية (PLS).

## INTRODUCTION:

*Debaryomyces hansenii* (*D. hansenii*) is a perfect haploid yeast that reproduced by vegetative multilateral budding. *D. hansenii* was isolated from various food products. This yeast species was the main yeast present in the microbiota of surface-ripened cheeses (Petersen et al., 2002). It contributes to the ripening process of cheeses by assimilation of lactose, lactic and citric acids (Valdés-Stauber et al., 1997; Viljoen & Greyling, 1995; Welthagen & Viljoen, 1998). Besides, *D. hansenii* was reported to promote the growth of *Brevibacterium linens* and increase the yellow colour intensity in Danish surface-ripened cheeses (Masoud & Jakobsen, 2003). Furthermore, the role of *D. hansenii* in meat fermentation has been investigated by many researchers. These yeast species were found to contribute to proteolytic and lipolytic activities in dry fermented sausage (Patrignani et al., 2007). Andrade et al. (2010) demonstrated that *D. hansenii* produced 3-methylbutanol, 3-methylbutanal and 2-propanone in dry-cured meat products contributing to their flavour. The antagonist effect of *D. hansenii* against *Penicillium verrucosum* in the dry fermented sausage was examined and showed that *D. hansenii* significantly reduces mold growth (Núñez et al., 2015). On the other hand, *D. hansenii* was reported as spoilage yeast in processed food. Westall and Filtenborg (1998) reported that *D. hansenii* was among the spoilage yeasts in soft cheese. It was also found that *D. hansenii* was one of the spoilage microorganisms in meat (Jofré et al., 2009).

*Debaryomyces hansenii* is an osmotolerant yeast that can grow at high salt concentration. *D. hansenii* has been isolated from cheese brines (Besançon et al., 1992; Kaminarides & Laskos, 1992). However, the growth of different strains of *D. hansenii* was found to be reduced by increasing salt concentrations (Masoud & Jakobsen, 2005). *D. hansenii* can grow at pH values of 4-6 showing very little effect on the amount of its growth at this pH range (Van Den Tempel & Jakobsen, 2000). The optimum temperature for growth of *D. hansenii* was reported to be 25°C (Sørensen & Jakobsen, 1997).

The environment of microorganism is complex and difficult to control due to the influence of many factors in the growth process. To save time and cost for measurements, multivariate data analysis (MVDA), i.e. chemometrics, can be used for classification and prediction of microorganisms in many foodborne applications (Koutsoumanis et al., 2016).

MVDA has several techniques for classification and prediction. Some of these methods are principal component analysis (PCA) and hierarchical cluster analysis (HCA), which have been used as unsupervised methods in several applications (Taha & Abu-Khalaf, 2020; Zaid et al., 2020), and

discrimination function analysis (DFA) that has been used as a supervised identification method (Al Ramahi et al., 2019). For prediction/modelling, partial least squares (PLS) can be used as a linear supervised technique (Abu-Khalaf & Hmidat, 2020; Zaid et al., 2020) and artificial neural network (ANN) as a non-linear method (Abu-Khalaf & Iversen, 2007).

MVDA techniques were used to predict the development of spoilage microorganisms linked to propolis extracts (Pobiega et al., 2019), also it was used for identification of the most important variables for different mold species causing food damage caused by *Fusarium* (Marín et al., 2005). Moreover, Da Costa et al. (2014) built a PCA model to interpret the nutritional status related to a bacterial progression in plants. Benitez-Cabello et al. (2020) used PCA to study the effect of different parameters of lactobacilli strains on the fermentation on table olives. Valerio et al. (2020) used PLS model to study the microbiological factors affecting the quality of beef meat in different storage situations. The amino acids in yeast extract for simulating *Escherichia coli* growth were studied using PCA and PLS models (Tachibana et al., 2019).

The aim of this work was to create a combination of salt concentration, pH and temperature in a culture medium that can be used to prevent the growth of *D. hansenii* in processed food and prevent its spoilage. The MVDA will be applied to study the relationship between different environment factors (NaCl, pH and temperature) that influence the different yeast strains growth i.e. optical density (OD) and build a growth model for yeast.

## **MATERIAL AND METHODS:**

### ***Debaryomyces hansenii* strains:**

Five strains of *D. hansenii* (DHI, DHII, DHIII, DHIV, DHV) were obtained from Arla Foods, Denmark. They were isolated from the surface of red smear cheeses.

### **Culture Media:**

Malt Yeast Glucose Peptone broth (MYGP) was used to inoculate strains of *D. hansenii*. This medium was prepared by adding 3 g of malt extract (Difco 0186-17), 3 g of yeast extract (Difco 0127-17), and 3 g of bactopectone (Difco 0118-17) and 10 g D(+)-glucose monohydrate (Merck 8342) to 1 L of distilled water. MYGP broth was adjusted to pH 5.6 with the addition of 1 M HCl or 1 M NaOH.

Diluent saline peptone (SPO) was prepared by dissolving 8.5 g NaCl (Merck 6404), 0.3 g disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ ; Merck 6579) and 1 g bactopectone (Difco 0118-17) in 1 L distilled water and adjusted to pH 5.6 with the addition of 1 M HCl or 1 M NaOH. The two media were sterilized by autoclaving at 121°C for 15 min.

### **Preparation of Yeast Inocula:**

Stains of *D. hansenii* were inoculated in 25 mL of MYGP broth and incubated at 25 °C for 48 h. Yeast cultures were centrifuged at 3000 x g for 10 min, then the supernatant was discarded and the pellet was

resuspended in SPO. The concentration of each yeast was determined under the microscope using a haemocytometer (Neubauer) and the cell concentration was adjusted to 107 cells / mL.

#### **Experimental Design:**

In this experimental work, a complete factorial design was established, in which a combination of pH, NaCl and temperature was used. The effects of pH (5.5, 6.0, 6.5, 7.0, 7.5), NaCl (0, 4, 8, 12, 16 % w/v) and incubation temperature (10, 15, 20, 25 °C) on growth of the five strains of *D. hansenii* were examined. Hundred treatments for each strain were performed with four replicates for each treatment (i.e. with a total numbers of samples of 20000 = 100 treatments \* 5 strains \* 4 replicates).

#### **Growth Determination:**

Yeast strains were inoculated in microtitre plates by adding 10 µL of each yeast (107 cells / mL) into 190 µL of MYGP. Growth was determined after 24 h of incubation at the four different temperatures by measuring the absorbance at 620 nm (OD620) using a Multiskan diffusing-wave spectroscopy (DWS) (Lab system, Helsinki, Finland). The initial OD620 for the five strains of *D. hansenii* was measured before incubation and it was between 0.190 and 0.220, Yeast growth was determined by subtracting the average of the initial OD620 from OD620 obtained after 24 h of incubation.

#### **Data Analysis**

For MVDA, Unscrambler software (ver. 10.3, Camo, Norway) was used for building PLS model for predicting the yeast growth using the other three parameters (pH, temperature and % NaCl w/v), and study the relationship between different factors. Moreover, correlation loading model was carried out to study the most important factors affecting the PLS model. To investigate the significance (at  $p = 0.05$ ), between tested strains at different levels of salt concentrations, Anova two factors (Excel Microsoft Office, 2010) was used.

## **RESULTS AND DISCUSSION:**

#### **Descriptive statistics**

It can be seen from Table 1 that the range of growth was between 0.16 and 0.67, with a standard deviation of 0.11. The high value of the standard deviation can be explained due to the experimental design structure in which the influence of different values of parameters was tested in the trials, i.e. NaCl%, pH and temperature

**Table (1): The minimum, maximum, mean and standard deviation obtained from growth trials of *Debaryomyces hansenii* strains at different values of NaCl%, pH and temperatures**

Value	NaCl% w/v	pH	Temperature	OD620
Min	0.00	5.50	10.00	0.16
Max	16.84	7.50	25.00	0.67
Mean	8.42	6.50	17.50	0.37
Std Dev.	5.96	0.71	5.59	0.11

**Cross-Correlation:**

**Table (2)** summarizes the overall relationship between factors. It can be observed that NaCl% has the highest correlation with growth with an absolute value of 0.75. The correlation with pH was very low i.e. absolute value of 0.05. However, for temperature, the correlation was 0.43. This indicates that the most important factor, which affects yeast growth was NaCl% followed by temperature. This was confirmed by studying the most important factors in the PLS model, the correlation loading plot was carried out (**Figure 1**). The outer circle represents 100% of explained variance and the inner circle 50% explained variance for each variable. It can be seen the pH is not an important factor in the model, which means that changing the pH from 5.5 to 7.5 does not affect the growth of *D. hansenii*. It has been reported that changes of pH in a range of 4.7 to 6.0 (Sørensen & Jakobsen, 1997), 4.0 to 6.0 (Van Den Tempel & Jakobsen, 2000) and 5.5-7.5 (Masoud & Jakobsen, 2005) have no significant effect on the growth of *D. hansenii*. On the other hand, it appears that NaCl% is the most important factor due to its location in the plot. This can help in controlling the safety and quality of food by optimizing the conditions during food processing. Moreover, this information can reduce the time required for measuring different parameters and therefore save the cost of running the process.

**Table( 2):.The cross-correlation of NaCl%, temperature and pH with the growth of *Debaryomyces hansenii* strains (OD620)**

Parameter	OD620
NaCl %	-0.75
pH	-0.05
Temperature	0.43

**Tolerance of *Debaryomyces hansenii* strains to NaCl:**

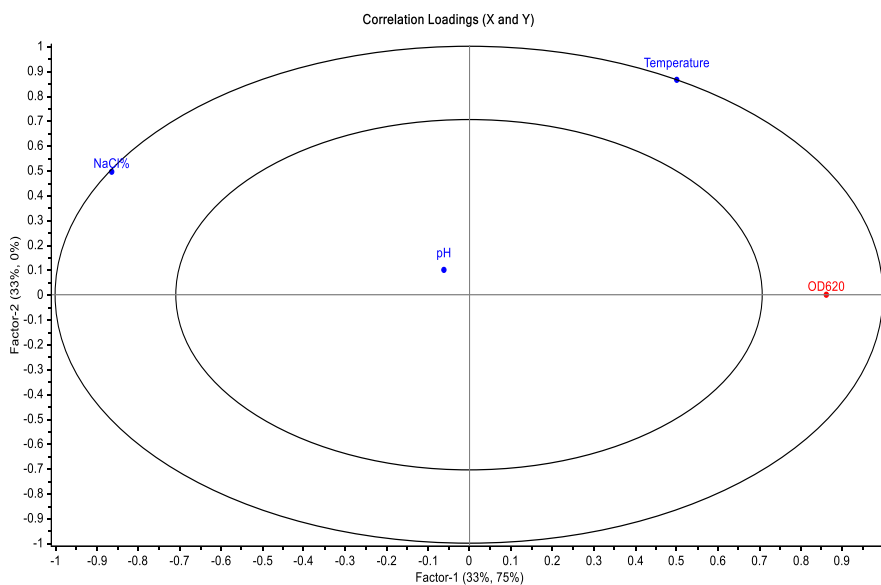
There was a significant difference ( $p < 0.05$ ) using Anova two factors (Excel Microsoft Office, 2010) on the growth of all tested strains at different levels of NaCl% concentrations and temperature.

**Figure (2)** shows the growth of the five strains of *D. hansenii* at 25°C in MYGP medium with different salt concentrations (0, 4, 8, 12, 16 % w/v). It can be seen that the best growth of all yeast strains was at 25°C without adding salt. The growth of yeast strains decreased as NaCl% concentrations increased over zero. Strain DHIII seems to be more salt-tolerant than the other 4 strains. Some strains of *D.*

*hansenii* has been isolated from cheese brines where there is high salt concentrations (Petersen et al., 2002). **Table (3)** represents the total growth change ((new value – value at 0 NaCl%) / (value at 0 NaCl%) \*100%) for the 5 strains of *D. hansenii*. The maximum percentage change for D.HII was from 7 to 26% growth reduction at 4-16 of NaCl% w/v. This confirms that DHII is the most salt-tolerant strain. The other strains have a higher growth reduction than strain DHII when salt concentrations increase (Table 3). It has been reported that the growth of *D. hansenii* was reduced by decreasing temperature from 25 to 10oC (Masoud & Jakobsen, 2005; Sørensen & Jakobsen, 1997). The growth of *D. hansenii* strains can be reduced by increasing salt concentrations and reducing temperature.

*Debaryomyces hansenii* was detected in different kinds of processed food causing their spoilage. According to the present results, changes in the environmental conditions during processing of food can be a helpful tool to eliminate the growth of this yeast species in food causing its spoilage. For example, increasing salt concentration and decreasing incubating temperature in cheeses will prevent the growth of *D. hansenii*.

In this study, a complete factorial design was applied in which it was possible to study the combined effects of salt, pH and temperature on the growth of *D. hansenii*. As mentioned above, pH was found to have no significant effect on the growth of *D. hansenii* but a combined effect was of salt concentration and temperature affected the growth of this yeast. Those findings seem to make it possible to set food processing conditions, i.e. salt concentration and temperature, which will not suit the growth of this yeast species in processed food.



**Figure(1):** The correlation loading plot for the PLS prediction model, two principal components explained 66% and 75% of temperature, pH and NaCl% (X) and optical density at 620 nm (Y) matrixes, respectively.

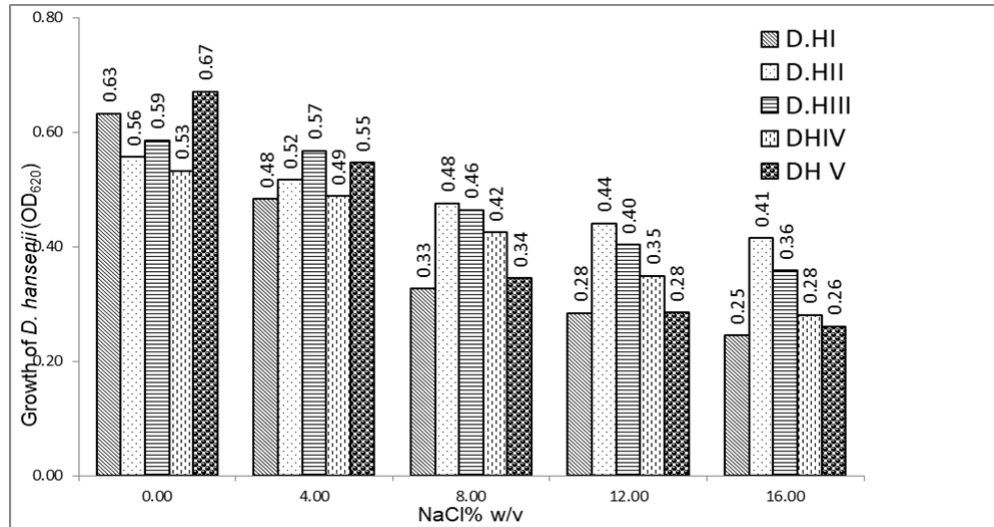


Figure (2): The effect of NaCl concentrations (0, 4, 8, 12, 16% w/v) on the growth of the five strains of *Debaryomyces hansenii* (DHI, DHII, DHIII, DHIV, DHV) in MYGP medium after 24 h of incubation at 25°C.

Table (3): Percentages of the total growth changes ((new value – value at 0 NaCl%)/(value at 0 NaCl%)\*100%) of *Debaryomyces hansenii* strains at different salt concentrations.

Yeast Strains	NaCl% w/v			
	4	8	12	16
DHI	-24%	-48%	-55%	-61%
DHII	-7%	-15%	-21%	-26%
DHIII	-3%	-21%	-31%	-39%
DHIV	-8%	-20%	-34%	-47%
DHV	-19%	-49%	-58%	-61%

**Prediction Model:**

PLS prediction model for the growth of *D. hansenii* (Y matrix) using the other three factors (X matrix) was established (Figure 3. for calibration and validation sets). Full cross-validation and two principal components were used in the model. They later explained 66% and 75% of X and Y matrixes, respectively. It can be seen that the model was robust, since it has an acceptable correlation (R2) and slope of 0.75. Moreover, the relative error for both calibration set (i.e. (RMSEC/range)\*100%) and validation set (i.e. (RMSECV/range)\*100%) was about 16% for both sets, which is an acceptable range of error (Mudalal et al., 2020).

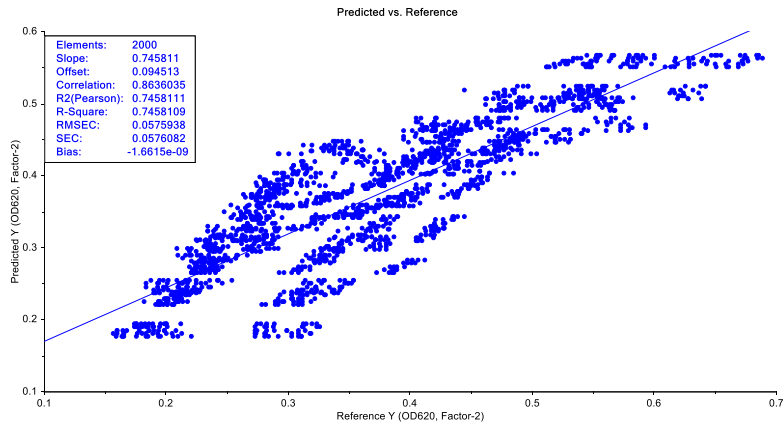
The prediction model can be used to predict the growth of yeast for any future application, which can save time and money, by providing the values of temperature, salt concentration and pH for any type of processed food. This will help in optimizing the food process conditions that will prevent food spoilage i.e. growth of yeast in food.



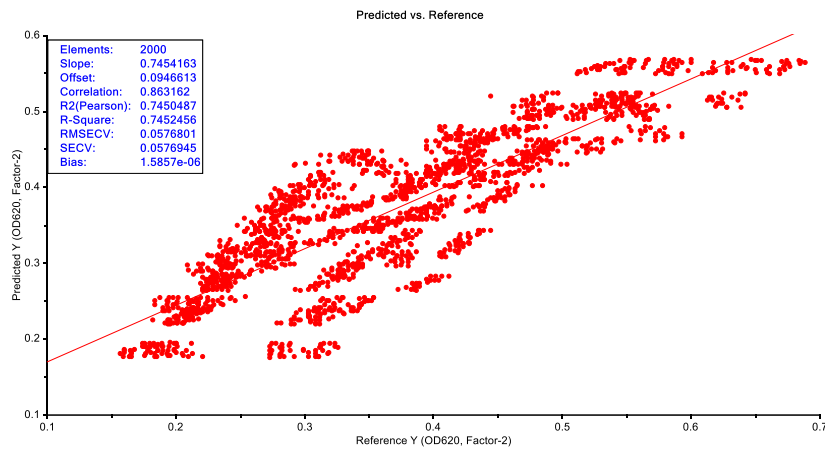
**CONCLUSION:**

The present study demonstrated that the growth of *D. hansenii* can be reduced by increasing salt concentration and reducing the temperature in different processed food products to prevent their spoilage. Furthermore, the PLS prediction model seems to be a good tool to predict the growth of *D. hansenii* in processed food using the parameters: salt, temperature and pH. Moreover, the correlation loading managed to reveal the relationship between different measured parameters. Further research is needed.

**A**



**B**



**Figure (3): PLS model for prediction yeast strains using two principal components and full cross-validation. The calibration set (A) and validation set (B) have almost the same results with a high correlation, which indicates a good and robust model.**

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