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Section VI
Symposium
6th International CIGR Technical Symposium
TOWARDS A SUSTAINABLE FOOD CHAIN
Food Process,
Bioprocessing &
Food Quality
Management
Organized by
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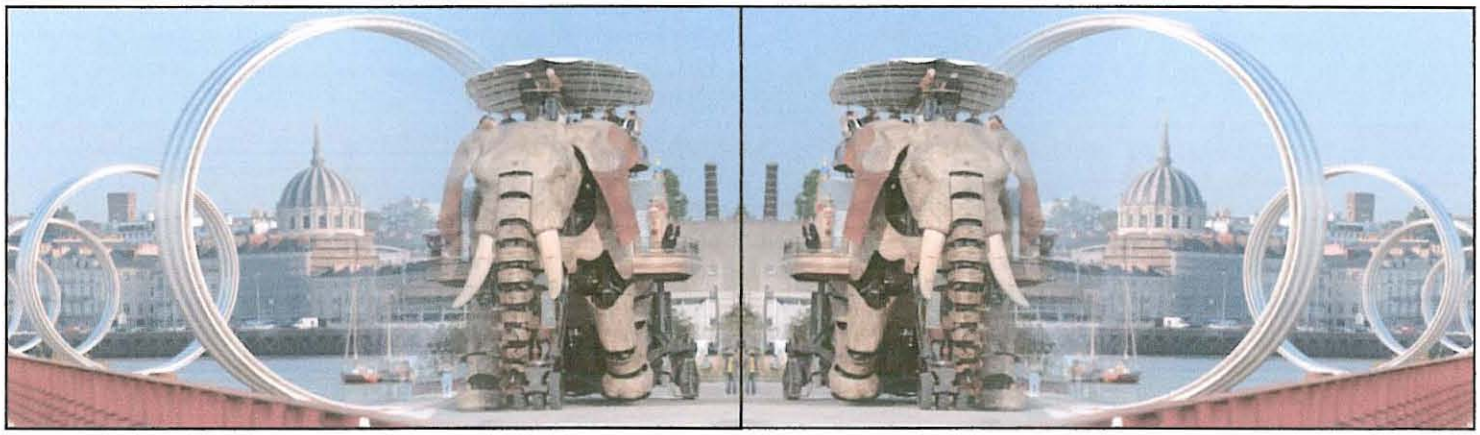

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Food Process, Bioprocessing and Food Quality Management

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Organized by :

Oniris
Nantes-Atlantic National College of Veterinary Medicine, Food Science and Engineering

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Introduction

Welcome in Nantes for the 6th issue of the CIGR Section VI symposium. CIGR Section VI (Postharvest Technology and Process Engineering) deals with the engineering principles and technologies in postharvest and processing of agri-food products. It is devoted to follow the trends, promote the advancement and enhance the dissemination and transfer of technology in postharvest and processing at a global scale.

To fulfil better its missions, Section VI of CIGR is organizing the 6th International Technical Symposium: «Toward a Sustainable Food Chain» in Nantes, France. The event follows 5 previous similar events held in each continent, demonstrating the international profile of CIGR: China (2004), Poland (2006), Italy (2007), Brazil (2008), and Germany (2009). As previous in Symposiums, selected papers presented at this Symposium will be published in a special issue of Food and Bioprocess Technology - An International Journal published by Springer and indexed by SCI.

I would like to thank all the colleagues who helped me to prepare this event, ONIRIS for its administrative support, the "Nantes Events Centre" for offering a great flexibility and understanding in adjusting the hosting capacity to the audience. Special thanks to Brigitte Poncelet from IMPASCIENCE, who designed the website and who also gave great technical support and advice in preparing the event.

Finally I would like to thank our partners, who supported us with different means; by financial support, by advertising the event, by bringing their Scientific recognition… they all greatly contributed to the success of this Symposium.

This book gathers the abstracts of the submissions of fully registered attendees; we apologize in advance for any errors or mistake we may have done when preparing this proceeding book. Theses abstracts correspond to a paper published in a CD (with ISBN) associated to the Symposium.

We hope that you will enjoy your visit in Nantes and the program of the symposium.

Pr Le BAIL – ONIRIS - Symposium Chairman

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Articles for

ORAL PRESENTATIONS

SESSION 3-6

Food Quality 6 - General

Mead Production Improvements After Using A Factorial Design

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Abstract. *In the north of Portugal, and in particular in Trás-os-Montes region, there is sometimes an overproduction of honey. Mead is one of the possibilities to overcome this problem. However, most of the time, mead is not produced in a standardized, but empirical and handmade form.*

In this work it was evaluated in what way factors, such as, temperature and salt concentration, as well as, the yeast strain used, affect the fermentation process linked to mead production. Temperatures of 20, 25 and 30°C and salt concentrations of 60, 90 and 120 g/hL were used, as well as, two commercial strains of Saccharomyces cerevisiae, namely, Fermol® Reims Champagne and ICV® D47, used in oenological fermentations. A 3^2 factorial design was applied to each yeast strain, and the experimental data were analyzed by the Response Surface Methodology.

Both yeast strains seem to be appropriate for mead production; however the models developed for the Fermol® Reims Champagne predict better the fermentation development than the ones for ICV® D47. Thus, it was concluded that in the future when using the Fermol® Reims Champagne yeast in mead production, it's advisable to work at a temperature between 24°C and 29°C, and with a salt concentration between 85 and 115 g/hL.

Keywords. Mead, Factorial Design, Response Surface Methodology, Ethanol, Sugars, Glycerol, Acetic acid.

Introduction

Trás-os-Montes, northeast of Portugal, is one of the most important Portuguese region for honey production; however, sometimes it is difficult to sell all the production. In spite of this, mead production appears as a possible alternative to overcome this problem. Nevertheless, handmade production of mead is most of the time an empirical task and has not been often successful due to problems during fermentations. So, it is necessary to optimize it.

Mathematical models used in the process conception of optimization and control are useful tools in this field. The Response Surface Methodology (RSM) is one possibility (Chung, Ma & Braatz, 2000; Wu, 2002). It is appropriate to identify the effect of individual variables and to find out the best conditions for a multivariable system.

As temperature and salt concentration, as well as, yeast strain are factors that influence fermentation performance, the main objective of this study was to use RSM to determine the most favorable conditions for mead production.

Materials and methods

Microoganisms and preparation of musts

In all assays, two commercial strains of *Saccharomyces cerevisiae* were used, namely Fermol® Reims Champagne (Pascal Biotech®) and ICV® D47, both used in wine production. Freeze-dried yeast cells (30g/hL) were hydrated in sugar water (50g/L) and incubated at 35ºC for twenty minutes. The growth medium was prepared by mixing honey with water (395g/L) and commercial nutrients (Enovit®) (60, 90 or 120 g/hL), 6% (v/v) of SO_2 (8 g/hL) and tartaric acid (Sigma–Aldrich) until obtaining a pH of 3.5.

Fermentation conditions

The fermentations occurred in Erlenmeyer flasks of 500 ml using a working volume of 250 ml. The fermentations progressed at 20, 25 or 30ºC with salt concentrations of 60, 90 or 120 g/hL, indicated by the factorial design. The fermentations developed during 216 hours (approximately 15 days). Throughout the fermentations, the temperature was constantly controlled. Biomass was determined periodically by optical density at 640 nm. Ethanol, acetic acid, glycerol, fructose and glucose were quantified by HPLC-RI. Each assay was performed in duplicate.

Experimental design

To study the effect of temperature and salt concentration for each of the oenological yeasts used, a factorial design 3^2 was applied. The experimental data were analyzed by RSM, using the software "Minitab 15.0". The relationship between dependent and operational variables was established by the following model:

$$y = B_0 + B_1x_1 + B_2x_2 + B_{11}x_1^2 + B_{22}x_2^2 + B_{12}x_1x_2$$

, where y is the dependent variable, B corresponds to the regression coefficients (calculated using the experimental data by multiple regression using the least-squares

method), and x corresponds to the independent variables. Regarding the parameter B: i) B_0 is a constant; ii) B_1 and B_2 are the linear coefficients; iii) B_{11} and B_{22} are the quadratic coefficients; and iv) B_{12} is the interaction coefficient between variables 1 and 2.

The independent variables used in this study were temperature and concentration of nutrients - Salt Enovit (Table 1).

Table 1- Independent variables used in this study.

Variable	Nomenclature	Units	Levels
Temperature	**T**	°C	20-25-30
Enovit Salt Concentration	**C**	g/hL	60-90-120

Twelve experiments were performed, as indicated in Table 2, showing the set of the experimental conditions assayed (expressed in terms of coded variables). The sequence was randomly established to limit the influence of systematic errors in the interpretation of results. It should be noted that the experiments 1-9, allowed the calculation of the regression coefficients, while the assays 10-12 were replicas at the central point of the experimental design in order to estimate the influence of experimental errors.

Table 2 – Experimental conditions used in mead production.

Assay	Temperature (°C)	Concentration of nutrients – Enovit salt	Assay	Temperature (°C)	Concentration of nutrients – Enovit salt
1	0	1	**7**	0	0
2	1	-1	**8**	-1	0
3	0	-1	**9**	1	0
4	-1	-1	**10**	0	0
5	1	1	**11**	0	0
6	-1	1	**12**	0	0

The dependent variables studied in this work were ethanol, acetic acid, glycerol, and the final concentrations of glucose and fructose, allowing the determination of five models.

Results and discussion

Fermentations development

Due to the significant number of fermentations carried out, it was decided to only discuss the fermentation progress of five concrete situations, namely:

A) T = 20°C (level -1) + salt concentration = 120 g/hL (Level 1)
B) T = 20°C (level -1) + salt concentration = 60 g/hL (level -1)
C) T = 30°C (level 1) + salt concentration = 120 g/hL (Level 1)
D) T = 30°C (level 1) + salt concentration = 60 g/hL (level -1)

} Extreme conditions

E) T = 25°C (level 0) + salt concentration = 90 g/hL (level 0) } Central condition

When analyzing the results obtained for Fermol® Reims Champagne yeast strain in relation to biomass, it was found that the highest concentrations were obtained in assays 1A, 1C and 1E. In terms of the length of the exponential phase, it was found that in most situations the stationary phase began after approximately 70 hours, except for C and D experiments that started around 50 hours.

In relation to ethanol, the final concentrations determined were similar in all assays, being equal to 91.4±0.06, 107±0.02, 113±0.03, 115±0.22 and 119±0.43 g/L for A, B, C, D and E cases, respectively. The lowest concentration was determined in the assay corresponding to temperature of 20°C and a salt concentration of 120 g/hL.

Acetic acid and glycerol and were also produced during the fermentations. In the case of acetic acid production, a minimum value of 0.12±0.06 g/L and a maximum value of 0.78±0.003 g/L, were observed. For glycerol, values between 5.40±0.04 and 7.04±0.05 g/L were determined. Nevertheless, the acetic acid concentrations were always below the legal limit (<18 meq/L which corresponds to about 1.1 g/L) (Regulation (EC) Nº. 1493/1999, Annex VB -1b), and the glycerol concentrations were within the values reported by Rankine & Bridson (1971) for Australian wines that should be between 1.4 and 9.9 g/L.

In relation to sugars, it was observed that in all assays, fructose and glucose were consumed during the fermentations. Generally, the rate consumption of glucose was slightly higher (for example in assay 1C) or identical (assay 1A) to fructose. The final values for glucose were equal to 2.55±0.51 to 5.11± 0.12 g/L and for fructose to 1.51±0.31 to 27.61±0.16 g/L.

The results of ICV D47® yeast strain were identical to the ones obtained with Fermol® Reims Champagne (Pascal Biotech®), not being discussed.

Statistical treatment of the results

In terms of the model fits, better results were observed for the yeast *Saccharomyces cerevisiae* Fermol® Reims Champagne (Pascal Biotech®) than for ICV D47®. In fact, the "lack-of-fit" was not significant ($p>0.05$) in four models – ethanol, acetic acid, glycerol and glucose – determined for the former yeast, unlike the observed for the yeast *Saccharomyces cerevisiae* ICV D47®. For this yeast strain, the "lack-of-fit" was significant ($p<0.05$) in all the models determined, meaning that the mathematical models developed for this last yeast strain were not as suitable as those determined for the yeast Fermol® Reims Champagne and so, the equations used to predict the responses for ICV D47® should be interpreted with some caution.

In relation to *Saccharomyces cerevisiae* Fermol® Reims Champagne fermentations, it was observed that the addition of Enovit salt resulted in only small effects on the variables studied, with the exception of fructose ($p<0.05$). The temperature caused a positive significant effect ($p<0.05$) in the production of acetic acid and glycerol, as demonstrated by the values of the B_2 (linear) and B_{22} (quadratic) coefficients. This last parameter was also significant for the consumption of glucose. In relation to the interactive term, relative to the Enovit® salt concentration and temperature, it was significant for acetic acid, glycerol and fructose.

In general terms, the effects of temperature and salt concentration on the dependent variables studied in the present work for the Fermol® Reims Champagne are illustrated in Figure 1. In relation to ethanol, in most situations the ranges of temperature and salt concentration evaluated had little influence on the production of ethanol (Figure 1A),

since it varies predictably between 10 and 12.5%. For the acetic acid (Figure 1B), it was verified that temperatures above 25°C and nutrient concentrations between 72 and 120 g/hL, may cause an increase in the production of this compound; however, the results suggest that acetic acid concentrations above 0.8 g/L are difficult to obtain, not existing the risk of exceeding the maximum permissible value. In terms of glycerol (Figure 1C), an increase in temperature favors it's production.

To avoid refermentations it is desirable to have low levels of glucose and fructose at the end of the fermentations. High salt concentrations in conjunction with temperatures below 27°C favor the consumption of these sugars. On contrary, low levels of salt and temperatures below 25ºC can lead to final concentrations exceeding 3.5 and 10 g/L of glucose and fructose (Figures 1D and 1E), respectively, which is undesirable.

Conclusions

The present work showed that both yeast strains seem to be appropriate for mead production; however, the quadratic regression models developed for the yeast Fermol® Reims Champagne predict better the fermentation parameters linked to mead production than the models developed for the ICV® D47.

By the results obtained for Fermol® Reims Champagne the ranges of temperature and salt concentration seem not to have much influence in the production of ethanol that varied between 10 and 12.5%. However, an increase in temperature above 24ºC and a salt concentration between 72 and 112 g/hL can cause an increase in the production of glycerol and acetic acid. This last specie is undesirable, nevertheless, in all cases the acetic acid concentration was below 0.8 g/L, which is lower than 1.1 g/L, the maximum value permissible.

Thus, in the future when using Fermol® Reims Champagne in mead production the following conditions should be used: temperature between 24 and 29ºC and a salt concentration between 85 and 110 g/hL, in order to not exceed the legal limit for acetic acid, to promote the production of ethanol and glycerol, and for obtaining low concentrations of fructose at the end (<4g/L) to avoid refermentations.

Acknowledgements

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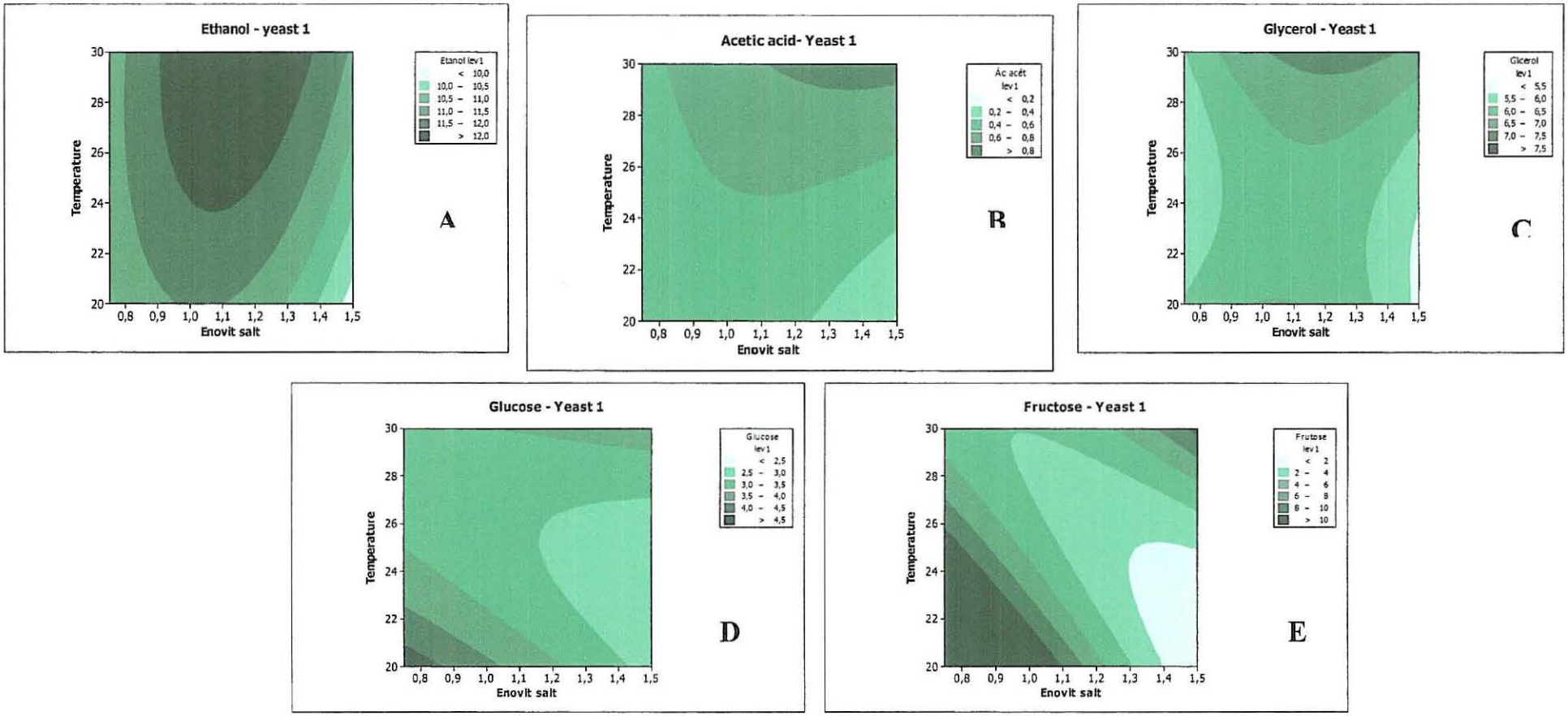

Figure 1 – Contour plots of the effect of T (ºC) and Enovit salt concentration (g/L) on ethanol (A), acetic acid (B) and glycerol (C) productions, as well as, on glucose (D) and fructose (E) consumptions for the yeast Fermol® Reims Champagne.