foodsim

# FOODSIM'2010

FOODSIM'2010

BRAGANÇA, PORTUGAL · JUNE 24-26, 2010

JUNE 24-26, 2010

CIMO RESEARCH CENTRE
BRAGANÇA, PORTUGAL

EDITED BY

VASCO CADAVEZ
AND
DANIEL THIEL

ORGANIZED BY

eti
European Technology Institute

IN COOPERATION WITH

UNIVERSITEIT
GENT

# 6TH INTERNATIONAL CONFERENCE

# ON

# SIMULATION AND MODELLING

# IN THE

# FOOD AND BIO-INDUSTRY

# 2010

# FOODSIM'2010


EDITED BY

Vasco Cadavez

and

Daniel Thiel



JUNE 24-26, 2010

BRAGANÇA, PORTUGAL

A Publication of EUROSIS-ETI

Printed in Ghent, Belgium

6th International Conference on
Simulation and Modelling
In the
Food and Bio-Industry

BRAGANÇA, PORTUGAL

JUNE 24-26, 2010

Organised by

ETI
The European Technology Institute

CIMO
Mountain Research Centre, Bragança

Sponsored by

Cámara Municipal Bragança

CoraNE

CVRTM

EUROSIS
The European Simulation Society

Ghent University

INATEL Fundação

IPB
Instituto Politécnico de Bragança
**Escola Superior Agraria**

Mirandela Municipio

Hosted by

**CIMO**

**Bragança, Portugal**

## EXECUTIVE EDITOR

**PHILIPPE GERIL**
**(BELGIUM)**

## EDITORS

### General Conference Chair

Vasco Cadavez
Mountain Research Centre (CIMO)
ESA - Instituto Politécnico de Bragança, Campus de Santa Apolónia,
Apartado 1172, 5301-855 Bragança,
Portugal

### General Program Chair

Daniel Thiel, University of Paris 13, Sciences Economiques
et Gestion, Paris, France

### Local Programme Committee

Joana Amaral, ESTiG-IPB, Bragança, Portugal
Vasco Cadavez, ESA-IPB, Bragança, Portugal
Elsa Ramalhosa, ESA-IPB, Bragança, Portugal

### International Programme Committee

**Simulation in Food Engineering and Processing**
Lionel Boillereaux, ONIRIS, Nantes, France
Cedric Brandam, INPT-ENSIACET, Toulouse, France
Matthew de Roode, NIZO, The Netherlands
Jean-Yves Monteau, ONIRIS, Nantes, France
Alcina Morais, ESBUCP, Porto, Portugal
Cristina Silva, ESBUCP, Porto, Portugal
Olivier Vitrac, INRA-UMR FARE, Reims, France

**Simulation in Food Sciences and Biotechnology**
Francis Butler, UCD, Dublin, Ireland
Enda Cummins, UCD, Dublin, Ireland
Ricardo Dias, ESTiG-IPB, Bragança, Portugal
Carla Fernandes, ESTiG-IPB, Bragança, Portugal
António Peres, ESA-IPB, Bragança, Portugal
José Teixeira, UM, Braga, Portugal

**Methods and Tools Applied to Food and Bio-Industries**
Kristel Bernaerts, Katholieke Universiteit, Leuven, Belgium
Paulo Cortez, UM, Guimarães, Portugal
Pierre-Sylvain Mirade, INRA-Theix, St Genes Champanelle, France
Leon Rothkrantz, Delft University of Technology, Delft, The Netherlands
Xavier Serra, IRTA, Girona, Spain

## International Programme Committee

**Methods and tools applied to Food Quality and Safety Evaluation**

Manuel Rui Alves, IPV, Viana Castelo, Portugal
Joana Amaral, ESTiG-IPB, Bragança, Portugal
Ursula Gonzales Barron, University College Dublin, Food Science and Veterinary Medicine, Ireland
Vasco Cadavez, ESA-IPB, Bragança, Portugal
Luis Dias, ESA-IPB, Bragança, Portugal
Isabel Ferreira, ESA-IPB, Bragança, Portugal
Isabel Mafra, REQUIMTE, Porto, Portugal
Beatriz Oliveira, FF-UP, Porto, Portugal
José Alberto Pereira, ESA-IPB, Bragança, Portugal
Isabel M. Vicariao, University of Sevilla, Spain
Miguel Vilas-Boas, ESA-IPB, Bragança, Portugal

**Simulation in Functional Foods**

Lourdes Amigo Garrido, CSIC, Madrid, Spain
Gianpaolo Ruocco, University of Basilicata, Potenza, Italy

**Simulation in Food Production Management, Economics and Traceability**

Martin Cloutier, UQAM, Montreal, Canada
Magda Aguiar Fontes, Faculdade de Medicina Veterinária, UTL, Lisboa, Portugal
Martin Grunow, Technical University of Denmark, Lyngby, Denmark
Vincent Hovelaque, INRA, Rennes, France

**Sustainable Food Production**

Albino Bento, ESA-IPB, Bragança, Portugal
Cesar de Prada, University of Valladolid, Spain
Federico Ferreres, Cebas-CSIC, Murcia, Spain
Björn Johansson, Chalmers University of Technology, Sweden
Jaime Pires, ESA-IPB, Bragança, Portugal
Manuel Ângelo Rodrigues, ESA-IPB, Bragança, Portugal

**Innovation in Traditional Food Products**

Maria J. Alcalde, University of Sevilla, Spain
Leticia Estevinho, ESA-IPB, Bragança, Portugal
Dietrich Knorr, Technische Universität, Berlin, Germany
Toomas Paalme, Tallinn University of Technology, Tallinn, Estonia
Elsa Ramalhosa, ESA-IPB, Bragança, Portugal

EUROSIS is a Division of ETI Bvba, The European Technology Institute, Torhoutsesteenweg 162, Box 4, B-8400 Ostend, Belgium

Printed in Belgium by Reproduct NV, Ghent, Belgium
Cover Design by Grafisch Bedrijf Lammaing, Ostend, Belgium

EUROSIS-ETI Publication

**ISBN: 978-90-77381-56-1**
**EAN: 978-90-77381-56-1**

# PREFACE

Dear colleagues,

Welcome to the 6th International Conference on Simulation and Modelling in Food and Bio Industries (FOODSIM'2010), which is held in Bragança, Portugal from 24 to 26 June 2010.

The FOODSIM'2010 brings together researchers, food experts and industrial users to present the state-of-art simulation research in the food industry, new research results and to exchange ideas and experiences about the modeling and simulation tools used in the food industry.

The main theme of FOODSIM'2010 is: "Simulation applied to food processes, quality, safety, and sustainability", and the success of the conference is already assured, as can be witnessed by the quality and scientific rigor of the 47 published papers. We also take this opportunity to challenge the researchers attending the FOODSIM'2010 to produce a seed for a FP7 project to be submitted at the next call for proposals, which is expected to open next July.

We present our recognition for the inestimable collaboration that we had in the FOODSIM'2010 organisation by Prof. Joana Amaral and Prof. Elsa Ramalhosa, and to all the reviewers for their professional work in the papers evaluation. We also present our recognition to all Institutions that contributed to prepare a pleasant social programme for FOODSIM'2010.

Finally, we wish you all a pleasant staying in Bragança and we are sure that you will have the opportunity to be delighted by the Portuguese hospitality.

Prof. Vasco Cadavez, Moutain Research Centre (CIMO), ESA - Instituto Politécnico de Bragança, Bragança, Portugal

Prof. Daniel Thiel, Universite de Paris 13, UFR Sciences Economiques et Gestion, Paris, France

# MEAD PRODUCTION: COMPARISON OF DIFFERENT PRODUCTION SCALES (PRELIMINARY RESULTS)

Teresa Gomes[a], Carla Barradas[a], Teresa Dias[ab], João Verdial[ab], Jorge Sá Morais[ab], Elsa Ramalhosa[ab], Letícia Estevinho[ab]

[a] Escola Superior Agrária, Instituto Politécnico de Bragança, Campus Santa Apolónia, Apartado 1172, 5301-885 Bragança, Portugal
[b] CIMO, Campus Santa Apolónia, Apartado 1172, 5301-855 Bragança, Portugal
Email: [teresa.md.gomes; carla.br.barradas]@alunos.ipb.pt;
[tdias; verdial; jsamorais; elsa; leticia]@ipb.pt

**KEYWORDS**
Mead, Production scales, Ethanol, Glucose, Fructose, Glycerol, Acetic acid.

**ABSTRACT**

Mead production represents a possible economic alternative to honey producers that intend to obtain honey products with surplus value. From that the present work aims to study the influence of using different production scales on the quality of the final mead obtained and on the process performance. Increasing the production scale almost ten times (1.5 to 20 L), some differences were observed. Maximum specific growth rates equal to 0.045 and 0.038 $h^{-1}$ were obtained for fermentations carried out at 1.5 and 20 L, respectively. The time course of glucose and glycerol were similar for both production scales. Nevertheless, slight differences at the end of the fermentations were observed for fructose and acetic acid. In relation to ethanol, a higher final concentration was found in the pilot-scale, resulting in a higher ethanol yield. In conclusion, these preliminary results are a good promise to local honey producers who intent to obtain large-productions of mead.

## 1. INTRODUCTION

In the northeast of Portugal, the production of honey is an activity with significant economic importance, however, the excessive production lowers the honey price, acquiring great importance the study and development of new honey products in order to increase the value of honey (Pereira et al. 2009).

Honey is a natural complex product that is reported to contain at least 181 substances (Arráez-Román et al. 2006). These are mainly composed of carbohydrates and other minor substances, such as organic acids, amino acids, proteins, minerals, vitamins and lipids (Finola et al. 2007). Fructose and glucose are the predominate carbohydrates. These two sugars account for nearly 85–95% of the honey carbohydrates (Finola et al. 2007; Pereira et al. 2009). The composition of honey is rather variable and depends primarily on the floral source; however, certain external factors, such as seasonal and environmental factors and processing, also play an important role (Arráez-Román et al. 2006). Honey also contains volatile substances which are responsible for the characteristic flavour (Finola et al. 2007).

Mead production may be an activity with economical potential. In fact, mead is known as the drink of the gods, being one of the oldest alcoholic beverages in the world (Sroka and Tuszyński 2007). Mead contains between 9% to 18% (v/v) of ethanol. Mead fermentation is a time-consuming process, often taking several months. The fermentation rate depends on several factors, such as, honey variety, yeast strain, yeast nutrition, pH, among other factors (Navratil et al. 2001).

However, associated with its production several limitations have been documented, such as, relatively long time needed for wort fermentation and mead maturation (Sroka and Tuszyński 2007) and lack of uniformity in the final product, since the water content of honey changes every year, that can induce not only refermentations by yeasts, but also metabolisation of residual sugar by acetic acid bacteria and lactic acid bacteria. These reactions result in the production of volatile acidity and abnormal esters that decrease the organoleptic quality of the final product (O'Connor-Cox and Ingledew 1991). Other problems are encountered during mead production in the clarification and filtration stages. Although desirable, these steps may increase production costs. For these reasons, research work is needed in order to optimise the production process of this beverage (Sroka and Tuszyński 2007).

Generally, the studies related with mead production performed until now have only involved production at lab-scale (1.5 – 2 L). Thus, the aim of this work was to compare different production scales of mead in relation to the final product obtained and to the fermentations development. The production of mead was evaluated in a bioreactor of 1.5 L and subsequently in one of pilot-scale (20 L). Total reducing sugars, glucose, fructose, ethanol, acetic acid and glycerol, were evaluated and these were the parameters used to make the comparison between both production scales.

## 2. MATERIALS AND METHODS

### 2.1 Yeast strains and medium

A commercial wine yeast strain of *S. cerevisiae* (Fermol® Reims Champagne (Pascal Biotech®)) was used for all assays. Firstly, yeast cells (30g/hL) were hydrated in sugar water (50g/L) and incubated at 35°C for 20 min.

The growth medium was prepared by mixing honey with water (395g/L) and commercial nutrients (Enovit®) at a final concentration of 60g/hL, 6% (v/v) $SO_2$ at 8 g/hL and tartaric acid (Sigma–Aldrich) until obtaining a pH of 3.5.

### 2.2 Lab-scale mead fermentations

The hydrated commercial wine yeast strain was added to the growth medium. The fermentation progressed at 25°C with gentle agitation (120 rpm) and carried out in a 1.5L stirred tank reactor (Bioreactor Biostat A *plus*) for 313 hours. Along the fermentations, the pH and temperature were constantly monitored. Yeast cell biomass was determined by measuring the optical density at 640 nm in a UV–visible spectrometer (Jenway Genova). Glucose, fructose, ethanol, glycerol, and acetic acid were quantified along fermentation. These fermentations were carried out in duplicate.

### 2.3 Pilot-scale mead fermentations

The growth of *Saccharomyces cerevisiae* was performed in a fermentation cube of 20 L, and incubated at 25°C for 334 hours. The agitation of the culture was performed twice a day. During the fermentation, pH and temperature were periodically measured, as well as the parameters reported before (Section 2.2).

### 2.4 Glucose, fructose, ethanol, glycerol and acetic acid quantification

Glucose, fructose, ethanol, glycerol, and acetic acid were analyzed individually, using a Varian HPLC system, equipped with a Rheodyne injector with 20 µL loop, a Supelco Gel C-610H column (300x7.8 mm) at 35°C and a refractive index detector RI-4 (Varian). Isocratic elution was employed with a mobile phase consisting of 0.1% (v/v) phosphoric acid (Fisher Scientific, p.a.) at a flow rate of 0.5 mL/min. Data was recorded and integrated using the Star Chromatography Workstation software (Varian). Glucose, fructose, ethanol, glycerol and acetic acid were quantified by external standard calibration.

All values reported in this work correspond to averages of the results obtained from triplicate determinations, being the percentage relative standard deviations of which less than 5%.

## 3. RESULTS AND DISCUSSION

The process of mead fermentation is quite difficult to perform due to several factors, such as, high sugar concentrations and low nitrogen, vitamins, minerals contents. Furthermore, when the production scale is changed other aspects must be considered, such as pH, temperature, dissolved oxygen, homogeneity of the culture medium, etc., as these parameters are more difficult to control in larger production scales. This will lead to differences in the fermentation performance and in changes on the organoleptical characteristics of the final product. As a result, it is important to guarantee that in large-scale production the final product still has the desirable characteristics and the maximum process yield.

### 3.1 Mead production in Lab-scale

The fermentation performance during the lab-scale production is presented in Figure 1. In relation to biomass, almost no lag-phase was observed, having the exponential phase a duration of around 90 hours. After that the stationary phase was observed.

In terms of glucose and fructose, both sugars were metabolized by the yeasts during the exponential and stationary phases. The glucose content decreased from 101 to 5.13 g/L and fructose from 125 to 11.1 g/L. These results indicate that both sugars were almost completely consumed. Moreover, the glucose consumption rate was higher than fructose, as observed by the slopes of the curves, showing a preferential consumption of glucose over fructose.

The final ethanol concentrations were equal to $99.4 \pm 0.9$ g/L. Glycerol and acetic acid were also produced along fermentations, reaching the following values: $6.42 \pm 0.03$ and $0.60 \pm 0.05$ g/L, respectively.

### 3.2 Mead production in Pilot-scale

The fermentation performance during the pilot-scale production is presented in Figure 2. In general terms, some differences were detected when comparing these results with the ones obtained in the lab-scale production. In relation to biomass, a lag-phase around 28 hours was observed, suggesting that yeasts cells due to the high sugar concentration in growth medium and the lower $O_2$ concentration were under stress conditions. Only after that period, the exponential phase started with a total duration of around 90 hours.

At 125 hours after inoculation the biomass decreased. This might be due to two phenomena: *i*) Difficulties in promoting the desirable agitation of the medium when sample collection was being performed; *ii*) Cell sedimentation.

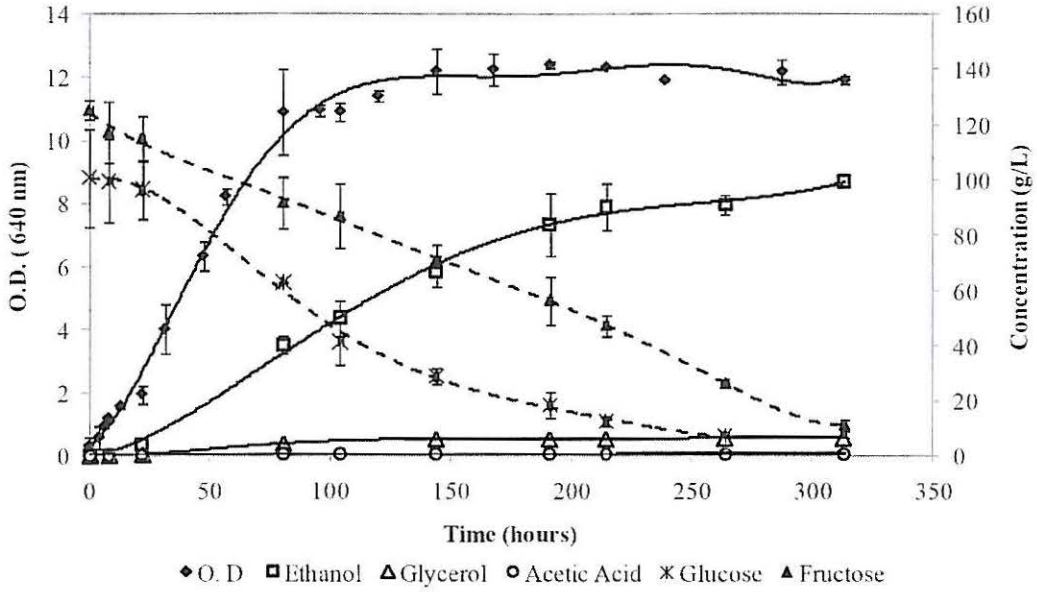


Figure 1 – Fermentation performance in mead production at lab-scale.

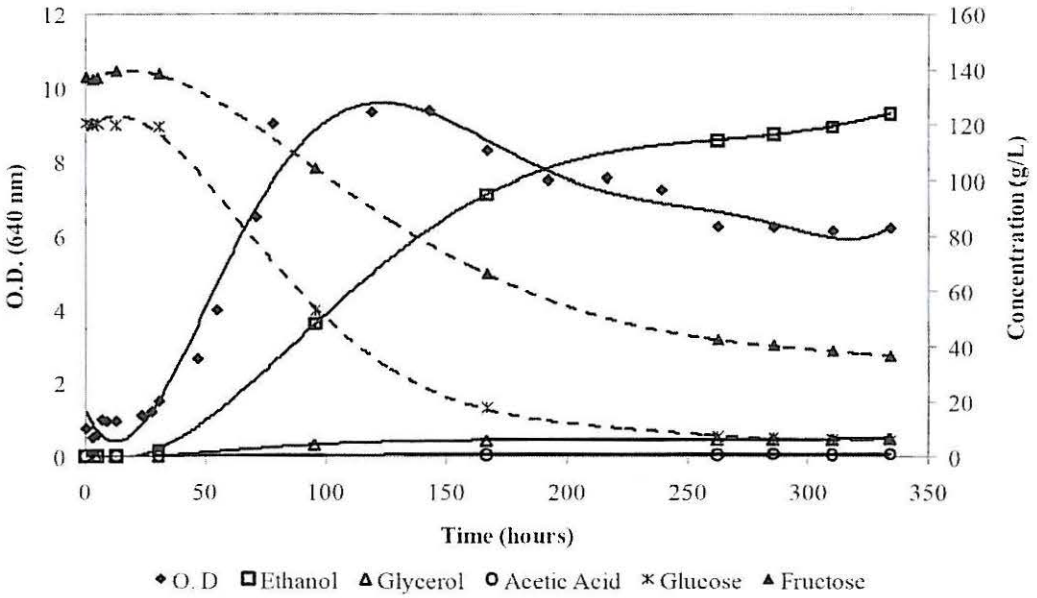


Figure 2 – Fermentation performance in mead production at pilot-scale.

In terms of glucose and fructose, both sugars were metabolized too by the yeasts. The glucose content decreased from 121 to 5.09 g/L and fructose from 137 to 36.4 g/L. When comparing the final values with the ones obtained in the previous assay, similar results were obtained for glucose. On contrary, higher final fructose concentrations were determined in the pilot-scale production. Moreover, a higher glucose consumption rate was again observed.

The final ethanol concentration was equal to 124 g/L. Glycerol and acetic acid were also produced along fermentations, reaching the following values: 6.84 and 0.94 g/L, respectively.

### 3.3 Comparison between both production scales

In order to compare both production scales, the following parameters were evaluated: maximum specific rates ($\mu$), sugars consumed, ethanol, glycerol and acetic acid productions (Table 1).

Table 1 - Mead production - Parameters determined for the alcoholic fermentations carried out in bioreactors of 1.5L (lab-scale production) and inox cube of 20L (pilot-scale production).

| Parameter | Bioreactor (1.5L) | Inox cube (20L) |
|---|---|---|
| Total time of fermentation (h) | 315±22 | 334 |
| $\mu_{máx}$ ($h^{-1}$) | 0.045±0.000 | 0.038 |
| Sugars consumed (g/L)* | 218±16 | 216 |
| Ethanol (%) | 9.69±0.02 | 12.4 |
| $Y_{Ethanol/Sugars}$ (%) | 35.3±2.2 | 45.5 |
| Glycerol (g/L) | 6.36±0.09 | 6.84 |
| Acetic acid (g/L) | 0.56±0.02 | 0.94 |

*Evaluated as Σ(Glucose±Fructose).

Values presented correspond to median±amplitude/2

The maximum growth specific rate obtained for the inox cube was lower than the obtained in 1.5L bioreactor. However, the sugars consumed in both production scales were comparable. In relation to ethanol, a higher final concentration was observed in the pilot-scale, resulting in a higher ethanol yield.

Another important aspect that must be referred to is the uncommon behavior of ethanol production. In fact, this is a primary metabolite that is expected to be produced along the exponential phase. However, as stated previously by Pereira et al. (2009), ethanol production in mead making might be still observed along the stationary phase. This behavior occurred in both production scales.

Glycerol concentrations obtained in both assays are in agreement with values published in the literature for wines. Rankine and Bridson (1971) refer that glycerol in Australian wines range between 1.4 and 9.9 g/l. For inox cube fermentation, a slight higher value of glycerol was obtained; however, the values are identical for both assays.

In relation to acetic acid, higher concentrations were obtained at the pilot-scale production, being observed approximately a two-fold increase; however, in the two cases the values still remain lower than the legal limit (inferior to 18 meq/L that is almost 1.1 g/L) (Council Regulation (EC) Nº 1493/1999, Annex V-B-1b).

## 4. CONCLUSIONS

With this work it was verified that changing from lab-scale to pilot-scale production (an increase of more than ten times fold), differences among the fermentations were observed. A higher lag-phase and a lower maximum specific growth rate were determined for the pilot-scale production. However, higher final ethanol concentrations were obtained in this assay, resulting in an increase in ethanol yield.

In the future we intend to perform organoleptic assays and to study the role of some parameters, such as temperature, salts, etc., on the quality of the mead produced.

## REFERENCES


Arráez-Román, D., Gómez-Caravaca, A.M., Gómez-Romero, M., Segura- Carretero, A. and Fernández-Gutiérrez, A. (2006). Identification of phenolic compounds in rosemary honey using solid-phase extraction by capillary electrophoresis–electrospray ionization-mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*, 41, 1648–1656.

Council Regulation (EC) Nº 1493/1999 of 17 May, on the common organisation of the market in wine, Annex V-B-1b.

Finola, M. S., Lasagno, M. C. and Marioli, J. M. (2007). Microbiological and chemical characterization of honeys from central Argentina. *Food Chemistry*, 100, 1649–1653.

Kahoun, D., Řezková, S., Veškrnová, K., Královský, J. and Holčapek, M. (2008). Determination of phenolic compounds and hydroxymethylfurfural in meads using high performance liquid chromatography with coulometric-array and UV detection. *Journal of Chromatography A*, 1202, 19–33.

Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Analycal Chemistry*, 31, 426-428.

Navrátil, M., Šturdík, E. and Gemeiner, P., (2001). Batch and continuous mead production with pectate immobilised, ethanol-tolerant yeast. *Biotechnology Letters*, 23, 977–982.

O'Connor-Cox, E.S. and Ingledew, W.M. (1991). Alleviation of the effects of nitrogen limitation in high gravity worts through increased inoculation rates. *Journal of Industrial Microbiology*, 7, 89–96.

Pereira, A. P, Dias, T., Andrade J., Ramalhosa, E. and Estevinho L. M. (2009). Mead production: Selection and characterization assays of *Saccharomyces cerevisiae* strains. *Food and Chemical Toxicology*, 47, 2057–2063.

Rankine, B.C. and Bridson D.A. (1971), Glycerol in Australian wines and factors influencing its formation. *American Journal of Enology and Viticulture*, 22, 6–12.

Sroka, P. and Tuszyński, T. (2007). Changes in organic acid contents during mead wort fermentation. *Food Chemistry*, 104, 1250–1257.