International Food Research Journal 22(3): 1288-1292 (2015)
Journal homepage: *http://www.ifrj.upm.edu.my*

Influence of the addition of pollen and brewer's yeast on growth of *Saccharomyces cerevisiae* in honey-must

*Kempka, A.P., Frühauf, M., Pagliarini, M.A., Matiello, J.A., Fachinello, F. and Prestes, R.C.

[1]*Universidade do Estado de Santa Catarina, Department of Food Engineering and Chemical Engineering, BR 282 Km 573.7, s/n, 89.870-000, Pinhalzinho-SC, Brazil*
[2] *Universidade Federal de Santa Maria, Department of Science and Food Technology, Avenida Roraima, 1000, 97.105-900, Santa Maria-RS, Brazil*

Article history

Received: 15 May 2014
Received in revised form: 23 September 2014
Accepted: 27 September 2014

Keywords

Honey
Supplement of nitrogen
Cellular growth
S. cerevisiae

Abstract

Honey can be considered an excellent source of carbohydrates for fermentation processes, but low concentrations of nitrogen are found in this substrate, having therefore, a possible need for supplemental. This study aimed to verify the growth profiles of *Saccharomyces cerevisiae* in honey-must supplemented with pollen or brewer's yeast. Were made experiments with concentrations 15-75 g/L of supplements and these were compared to an experiment without addition of supplements. The elemental analysis showed that the amount of nitrogen present in the brewer's yeast is 2.85 times higher than the pollen present in the content. In relation to the growth of *S. cerevisiae* it has been found that the lower concentrations tested led to higher growth both for the pollen as for brewer's yeast. The experiments added with brewer's yeast reached the maximum growth in 8 hours of fermentation and the experiments added pollen reached the maximum growth in 12 hours of fermentation. The results showed that supplementation favors the cell growth when compared to the experiment without supplementation.

Introduction

Honey, one of the oldest and most traditional sweetening foods, has been reported to contain about 200 substances. This natural product is essentially a concentrated aqueous solution of different carbohydrates, including fructose, glucose, maltose, sucrose, and other oligo- and polysaccharides (Escuredo *et al*., 2013). The quality of honey is determined by their sensory, physical and chemical properties. Its physical and chemical properties depend on the nectar and pollen floral source, color, flavor, moisture and protein content and sugars (Azeredo *et al.*, 2003).

All honeys share certain general characteristics, including a moisture content below 20%, a sugar content of 70–80%, an ash content ranging from 0.1% to 0.2%, and a pH between 3.8 and 4.7. Proteins, free amino acids (principally proline), organic acids, aromatics, and vitamins and minerals are minor components and several enzymes are important components of honey such as α-glucosidase, β-glucosidase, amylase and glucose oxidase (Won *et al.*, 2008; Roldán *et al*., 2011).

Honey is a substance that has been used for centuries to make beverages can be fermented to produce different types of mead and spirits that may have different flavors depending on the floral source of honey and additives and yeast used in fermentation (Gupta & Sharma, 2009). For contain low concentrations of protein and amino acids, in fermentation processes may be necessary the supplement these compounds.

For supplementation of protein content with the purpose to produce fermented honey can be used as pollen, which is collected from flowers as a source of proteins, lipids, vitamins and minerals for the survival of bees (Roldán *et al*., 2011). Commercial brewer's yeast is inactive yeast (dead yeast cells with no leaving power) remaining after the brewing process. It is an inexpensive nitrogen source with good nutritional characteristics and a very bitter taste, generally recognized as safe (GRAS) (Ferreira *et al*., 2010).

The yeast *(Saccharomyces cerevisiae)* is used by brewers repeatedly (usually 4-6 times) and is the second main byproduct of the brewing industry (brewer's yeast). Its use is still limited, being basically used as animal feed, receiving little attention as a marketable commodity, and its disposal is often an environmental problem. Various attempts have been made to utilize them in biotechnological processes such as, for example, the production of value-added compounds such as ethanol, or as substrates for

*Corresponding author.
Email: *aniela.kempka@udesc.br*
Tel: +55 49 33663971

the cultivation of micro-organisms, or simply raw material for the extraction of compounds (Ferreira *et al*., 2010). The objective of this study was to verify the growth profiles of *S. cerevisiae* using different concentrations of pollen and brewer's yeast in the honey-must and evaluate these possible nutritional supplements as alternatives to the micro-organism.

Materials and Method

The experimental analyzes were performed at the Bioprocess Laboratory and Microbiology Laboratory in Department of Food Engineering - State University of Santa Catarina. The Elemental Analysis (Elementar CHNS) was performed by combustion in the Laboratory of Soil and Sustainability of the Department of Animal Science - State University of Santa Catarina. The method is based on complete combustion of a sample (1g) of known mass of organic material, which contains mainly carbon (C), hydrogen (H), nitrogen (N), sulfur (S) and oxygen (O) and subsequent analysis of gas resulting from the combustion process, essentially carbon dioxide (CO_2), water (H_2O), nitrogen oxides (NO_x) and sulfur dioxide (SO_2). For obtain the values of proteins, we multiplied the value of the percentage of nitrogen by 6.25 (reference value).

Supplementation and determination of growth of Saccharomyces cerevisiae

For verify the influence of pollen concentration and the concentration of brewer's yeast, the following experiments were performed: control- C (without pollen or brewer's yeast), E1, E2, E3, E4 and E5 containing 15 g/L, 30 g/L, 45 g/L, 60 g/L and 75 g/L of the pollen or brewer's yeast , respectively, totaling 11 experiments. For obtaining of the fermentation medium, was made the dilution of the honey, obtaining a percentage of soluble solids of the 18°Brix. After, has made the correct of the acidity to pH 3.6. The concentrations of the pollen or brewer's yeast were added to each experiment and subsequently it was inoculated with 15 g/hL of the *S. cerevisiae*. The incubation occurred in an incubator at 25ºC for 12 hours (Roldan *et al*., 2011). Samples were withdrawn each 2 hours to determine the growth of yeast.

The growth of *S. cerevisiae* was accomplished using the methodology proposed by Mendes-Ferreira *et al*. (2010) with modifications. Samples of the 10 mL were centrifuged for 10 minutes at 3,000 rpm in pre-weighed tubes. Subsequently, the supernatant was exhausted and dried in an incubator for 24 hours at 100°C. The samples, after cooling, were weighted and the mass of cells determined by the weight difference. It was determined the mass of cells before the start of fermentation to be discounted this value.

Determination of kinetic parameters

The kinetic parameters determined for the growth of S. cerevisiae in pollen and in brewer's yeast were: the specific growth rate, which is the change of the natural log of the cell number density with time and the time required to double the population, called the doubling time, shown in Equations 1 and 2, respectively (Doran, 1995).

$$lnX = lnX_0 + \mu.t \quad (1)$$

$$t_d = \frac{ln2}{\mu_m} \quad (2)$$

where X is the cell concentration (g/L); X_0 is the initial cell concentration (g/L), μ is the specific growth rate (h^{-1}), μ_m is the maximum specific growth rate (h^{-1}), t_d is the time (hours), td is the doubling time.

Statistical analysis

Data were analyzed using the Statistica 10.0® (Statsoft Inc.). Significant differences ($p<0.05$) between means were identified using Tukey procedures. All the experiments were carried out in duplicate.

Results and Discussion

It is found that in relation to the nitrogen content (results of elemental analysis) the brewer's yeast corresponded to 46.56% of protein and the pollen corresponded to 16.31% of protein. As for the remaining of the elements, the values found were presented near. According to Roldan *et al.* (2011), the pollen contains, in addition to sugars, 7.4% of the moisture, approximately 20% of the protein (close to that found in the present study), 6% fat and 2.2% ash, plus vitamins, minerals and carotenoids. The proline, aspartic acid, phenylalanine and glutamic acid are primary amino acid existing in the pollen. Cells from yeast (brewer's yeast) are used as a supplement because it contains 45% to 60% of the protein. Yeast cells also contain lipids, vitamins and minerals (Chae *et al.* 2001).

In Figures 1 and 2 are shown the growth profiles of *S. cerevisiae* in honey-must added of pollen and brewer's yeast, respectively. It is observed in Figure 1 that after 12 hours of fermentation, the experiment containing 30 g/L of pollen presented the highest growth of yeast, followed by the experiment with 15 g/L. The experiment showed the lowest growth in 12 hours was with the addition of 75 g/L of pollen. It is also verified that the experiments with 45, 60 and 75

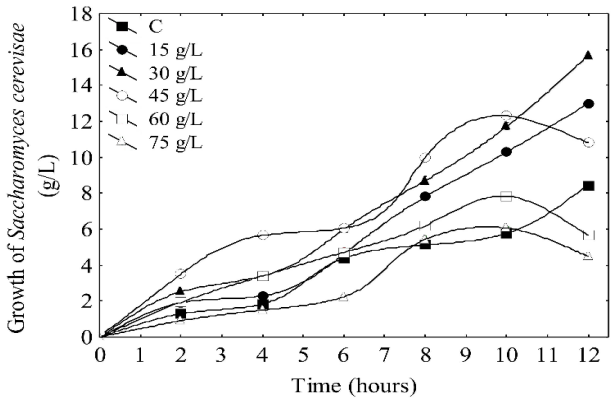

Figure 1. Profiles of the growth of *Saccharomyces cerevisiae* (g/L) in honey-must containing different concentrations of pollen

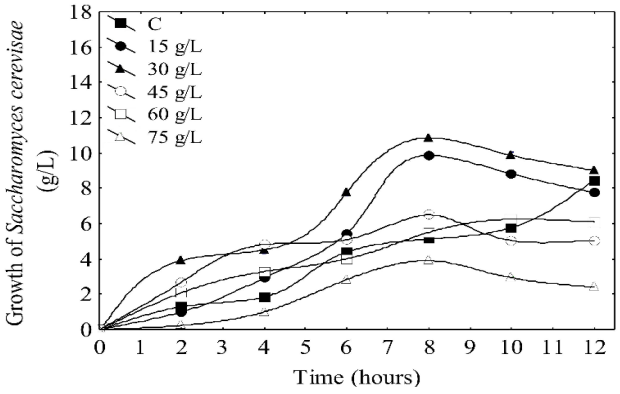

Figure 2. Profiles of the growth of *Saccharomyces cerevisiae* (g/L) in honey-must containing different concentrations of brewer's yeast

g/L of pollen there was a decrease in the growth of the 10th to the 12th hour.

In Figure 2, for growth in 12 hours, the experiment that showed higher cell mass was the experiment with 30 g/L with brewer's yeast followed by the C and the experiment with addition of 15 g/L of the brewer's yeast. To verify the statistical difference between results obtained using pollen and brewer's yeast in 8, 10 and 12 hours, was held the Tukey Test, with 95% reliability.

Table 1 shows the results for the experiments with addition of pollen. It was found that between the times of 8, 10 and 12 hours (for the same experiment), there was statistical difference ($p<0.05$) between the averages of the results of cell growth. For the experiments C, with addition of 15 g/L of pollen and with addition of 30 g/L of pollen, the highest value of the cell growth was obtained for the 12-hour time, and the values statistically different between themselves ($p<0.05$). For the experiments with addition of 45 g/L, 60 g/L and 75 g/L of pollen, the maximum values of cell growth were attained in 10 hours, also statistically different between themselves ($p<0.05$).

The results of the experiments with addition of

Table 1. Medium of growth of *Saccharomyces cerevisiae* in honey-must (control- C) and the honey-must added pollen to 8, 10 and 12 hours.

Experiment	Growth of *Saccharomyces cerevisiae* (g/L)*		
	8 hours	10 hours	12 hours
C	$5.13^{aA} \pm 0.06$	$5.77^{aB} \pm 0.14$	$8.45^{aC} \pm 0.16$
15 g/L	$7.81^{bA} \pm 0.02$	$10.30^{bB} \pm 0.09$	$13.02^{bC} \pm 0.09$
30 g/L	$8.71^{cA} \pm 0.23$	$11.72^{cB} \pm 0.21$	$15.65^{cC} \pm 0.13$
45 g/L	$10.00^{dA} \pm 0.03$	$12.31^{dB} \pm 0.21$	$10.83^{dC} \pm 0.23$
60 g/L	$6.21^{eA} \pm 0.04$	$7.85^{eB} \pm 0.11$	$5.67^{eC} \pm 0.16$
75 g/L	$5.44^{aA} \pm 0.17$	$6.07^{aB} \pm 0.05$	$4.50^{fC} \pm 0.08$

*Means ± standard deviation followed by different lowercase letters, in the column, are different at the 95% level of confidence between hours. Means followed by different capital letters, in the line, are different at the 95% level of confidence between samples (for the same concentration of the pollen)

the brewer's yeast are shown in Table 2. For times of 8, 10 and 12 hours, we found (as compared to the same experiment) that only statistically different ($p<0.05$) between the mean values for cell growth for the experiment with addition of the 45 g/L with brewer's yeast (maximum growth at 8 hours). For other experiments, it can be affirmed that the time of 8 hours is sufficient in order to obtain the maximum in cells of *S. cerevisiae*. Furthermore, comparing the experiments at time for 8 hours, it is found that maximum growth occurred for the experiment with addition of 30 g/L of brewer's yeast, with the highest value obtained statistically different the other values, followed the experiment with addition of 15 g/L of brewer's yeast.

Therefore, the experiments with addition of pollen reached the maximum concentration of cells in 12 hours and process for experiments with addition of brewer's yeast, the maximum concentration of cells was obtained for the 8 hour time corresponding to the experiment adding 30 g/L for both. According Mendes-Ferreira *et al*. (2010), although more studies are needed, the results of their study suggest a reduced yeast fermentative ability, and thereby increased risk of delayed or arrested fermentations, is due to factors other than nitrogen limitation in honey-musts, the same occurred in the present study when compared the experiment C with the experiments supplemented.

It was found by elemental analysis that the amount of nitrogen present in the brewer's yeast is 2.85 times higher than the amount of nitrogen present in the

Table 2. Medium of growth of *Saccharomyces cerevisiae* in honey-must (control-C) and the honey-must added brewer's yeast to 8, 10 and 12 hours.

Experiment	Growth of *Saccharomyces cerevisiae* (g/L)*		
	8 hours	10 hours	12 hours
C	$5.13^{aA} \pm 0.06$	$5.77^{aA} \pm 0.14$	$8.45^{aA} \pm 0.16$
15 g/L	$9.87^{cA} \pm 0.16$	$8.82^{bA} \pm 0.08$	$7.75^{bA} \pm 0.13$
30 g/L	$10.86^{dA} \pm 0.11$	$9.87^{cA} \pm 0.16$	$9.01^{cA} \pm 0.02$
45 g/L	$6.50^{eA} \pm 0.23$	$5.04^{dB} \pm 0.03$	$5.05^{dB} \pm 0.06$
60 g/L	$5.52^{aA} \pm 0.05$	$6.25^{eB} \pm 0.06$	$6.10^{eB} \pm 0.14$
75 g/L	$3.90^{bA} \pm 0.10$	$2.93^{fA} \pm 0.08$	$2.40^{fA} \pm 0.08$

*Means ± standard deviation followed by different lowercase letters, in the column, are different at the 95% level of confidence between hours. Means followed by different capital letters, in the line, are different at the 95% level of confidence between samples (for the same concentration of the brewer's yeast).

pollen. In relation to the growth of *S. cerevisiae*, the lowest concentrations tested of the supplements (15 g/L and 30 g/L) led to higher growth, regardless of supplementation with pollen or brewer's yeast. The experiments added with brewer's yeast reached the maximum growth at 8 hours of fermentation. For the experiments added pollen, the maximum growth of was reached at 12 hours.

Kempka and Mantovani (2013) in a study on the production of mead observed a reduction in 96-hour of the fermentation with addition of 1% of pollen compared to experiments without added, demonstrating that nutrients, especially the protein contained in pollen can increase the velocity the fermentation process. Table 3 shows the kinetic parameters of growth of *S. cerevisiae* in the supplemented media. It is found that the values of the specific growth rate for the experiments supplemented with pollen were higher than the control experiment (C) for concentrations of 15 g/L and 75 g/L. For the experiments supplemented with brewer's yeast, the values of the specific growth rate for the experiments supplemented with 15g/L, 30 g/L and 75 g/L were higher than experiment C.

Regarding the doubling time, it is found in Table 3 that for the experiments with 15 g/L and 75 g/L of pollen, the values of this parameter were lower compared to the experiment C, the same occurring to those supplemented with experiments 15 g/L, 30g/L and 75 g/L of brewer's yeast. It is also verified that the experiment with 45 g/L of brewer's yeast had the highest doubling time (approximately 3 times greater than the C), which may indicate an inhibitory effect of this supplement on an intermediate concentration. The lowest doubling time also occurred for the experiment with the addition of brewers yeast (30 g/L), with doubling times 6 times lower than the time shown by the experiment C and 2.5 times lower than the experiment with 75 g/L (second lowest doubling time).

Table 3. Maximum specific growth rates, doubling time and yields for the experiments with addition of pollen and brewer's yeast

Experiment	Pollen		Brewer's yeast	
	μ_{max} (h^{-1})	h_d (h)	μ_{max} (h^{-1})	h_d (h)
15 g/L	0.2093	3.3117	0.3735	1.8558
30 g/L	0.1889	3.6694	1.2060	0.5747
45 g/L	0.1541	4.4980	0.0748	9.2667
60 g/L	0.1395	4.9688	0.1354	5.1193
75 g/L	0.2884	2.4034	0.4731	1.4651
C	$\mu_{max} = 0.1915\ h^{-1}$ and $h_d = 3.6196$ h			

For the two supplements, as there is an increased concentration, is also an increase of the doubling time and thereafter a decline, indicating that intermediate concentrations (the concentrations tested) can lead to the development of some inhibitory effect on *S. cerevisiae*. In this way, it appears that both the pollen, a product with higher added value, such as brewer's yeast, featured as a byproduct, can be used to produce biomass of *S. cerevisiae*. Nevertheless, research is still needed on the physiology and metabolism of *Saccharomyces cerevisiae* under the particular harsh honey-musts environment (Mendes-Ferreira *et al*., 2010).

Conclusion

It was verified by elemental analysis that the amount of nitrogen present in the brewer's yeast is 2.85 times higher than the pollen present in the content. In relation to the growth of *S. cerevisiae*, the lowest concentrations tested (15 and 30 g/L) have led to higher growth, regardless of supplementation with pollen or brewer's yeast. The experiments added with brewer's yeast growth reached maximum at 8 hours of fermentation and for the experiments added pollen, maximum growth was reached at 12 hours. The pollen and brewer's yeast can be considered promising supplements for fermentation processes

where it requires nitrogen addition. The results of the growth using brewer's yeast as supplement presented themselves in less time compared with pollen. This product, often discarded or sent to animal feed, can be used in fermentative processes, with satisfactory results.

References

Azeredo, L.C., Azeredo, M.A.A., Souza, S.R. and Dutra, V.M.L. 2003. Protein contents and physicochemical properties in honey samples of Apis mellifera of different floral origins Food Chemistry 80(2): 249-254.

Chae, H.J., Joo, H. and In, M-J. 2001. Utilization of brewer's yeast cells for the production of food-grade yeast extract. Part 1: effects of different enzymatic treatments on solid and protein recovery and flavor characteristics. Bioresource Technology 76(3):253-258.

Doran, P.M. 1995. Bioprocess Engineering Principles. Elsevier Science and Technology Books 430p.

Escuredo, O., Miguez, M., Fernandez-Gonzalez, M, and Seijo, M.C. 2013. Nutritional value and antioxidant activity of honeys produced in a European Atlantic area. Food Chemistry 138(2-3): 851-856.

Ferreira, I.M.P.L.V.O., Pinho, O., Vieira, E. and Tavarela, J.G. 2010. Brewer's *Saccharomyces* yeast biomass: characteristics and potential applications. Trends in Food Science and Technology 21(2): 77-84.

Gupta, J.K. and Sharma, R. 2009. Production technology and quality characteristics of mead and fruit-honey wines: A review. Natural Product Radiance 8(4): 345–355.

Kempka, A.P. and Mantovani, G.Z. 2013. Produção de hidromel utilizando méis de diferentes qualidades. Revista Brasileira de Produtos Agroindustriais 15(3):273-281.

Mendes-Ferreira, A., Cosme, F., Barbosa, C., Falco, V., Inês, A. and Mendes-Faia, A. 2010. Optimization of honey-must preparation and alcoholic fermentation by *Saccharomyces cerevisiae* for mead production. International Journal of Food Microbiology 144(1): 193–198.

Roldán, A., Muiswinkel, G.C.J. van, Lasanta, C., Palacios, V. and Caro, I. 2011. Influence of pollen addition on mead elaboration: Physicochemical and sensory characteristics. Food Chemistry 126(2): 574–582.

Won, S-R., Lee, D-C., Ko, S.H., Kim, J-W. and Rhee, H-I. 2008. Honey major protein characterization and its application to adulteration detection. Food Research International 41(10): 952–956.