

Fermentative Activity of Promising Yeasts for Cereal-based Beverages using CO₂ Headspace Analysis

FRANCESCO PIO CASANOVA, ANTONIO BEVILACQUA, LEONARDO PETRUZZI,
MILENA SINIGAGLIA and MARIA ROSARIA CORBO

Department of the Science of Agriculture, Food and Environment (SAFE),
University of Foggia, Foggia, Italy

Abstract

CASANOVA F.P., BEVILACQUA A., PETRUZZI L., SINIGAGLIA M., CORBO M.R. (2015): **Fermentative activity of promising yeasts for cereal-based beverages using CO₂ headspace analysis.** Czech J. Food Sci., 33: 8–12.

This article proposes an approach based on the evaluation of CO₂ produced by *Saccharomyces cerevisiae* var. *boulardii*, *Kluyveromyces lactis* × *Saccharomyces cerevisiae*, *Saccharomyces pastorianus* var. *pastorianus*, *Kazachstania exigua*, as a function of different media (laboratory media with glucose and maltose) and sugars to screen promising yeasts for cereal-based beverages. Data were modelled by the Gompertz equation to estimate the time of metabolic adaptation (λ), the rate of CO₂ production (k_{\max}), and the maximum concentration of CO₂ [(CO₂)_{max}]. *Kl. lactis* showed the lowest value of (CO₂)_{max}, which suggests an “attenuated” metabolic response in the medium containing glucose. *K. exigua* showed a reduced production of CO₂ in the presence of maltose; however, the decrease of (CO₂)_{max} was not related to an increase of λ .

Keywords: carbon dioxide; modelling; metabolic response; Gompertz equation; attenuation

Nowadays, cereals are used for the production of traditional fermented beverages as well as to design new foods with enhanced healthy properties (BLANDINO *et al.* 2003) for their high content of essential vitamins, dietary fibre, and minerals (CHARALAMPOPOULOS *et al.* 2002). Unfortunately, the low content of proteins and essential amino acids (lysine), the low starch availability, and anti-nutrients (phytic acid, tannins, and polyphenols) represent a drawback compared to milk and dairy products (BLANDINO *et al.* 2003). However, fermentation could improve the quality of whole grain and cereal-based products (GOBETTI *et al.* 2010).

A variety of yeasts and bacteria was found in some traditional cereal beverages such as kvass (WOOD & HODGE 1985), bouza (MORCOS *et al.* 1973), chichi (NICHOLSON 1960), and mahewu (HESSELTINE 1979); hereby indigenous microbiota significantly contributes to starch breakdown, acidification, detoxification, and flavour enhancement (OYEDEJI *et al.* 2013). Moreover, there is an increasing interest in cereal-based beverages produced by using starter cultures (ZANNINI *et al.* 2013), thus a focus on the

factors that regulate the metabolism of a starter culture is of great concern to optimise the production of this kind of fermented beverages (BLANDINO *et al.* 2003). An interesting approach relies upon the ability of some microorganisms to produce CO₂ from carbohydrates, as this compound can be assessed in a relatively easy way by some non-destructive and relatively low-cost sensors (BEVILACQUA *et al.* 2013). Nowadays the headspace gas analysis is a useful tool for routine analysis in packaged products, such as milk (BEVILACQUA *et al.* 2013), sausages (GØTTERUP *et al.* 2008), mushrooms (BORCHERT *et al.* 2014), ready-to-eat salads (BORCHERT *et al.* 2012), fresh-cut apples (ALTISENT *et al.* 2014); however no data are available for the production of CO₂ in cereal-based media by potentially beneficial yeasts.

The use of mathematical model is a great challenge in food microbiology to predict and describe microbial growth and inactivation through the use of some primary (cell count over time) and secondary models (effects of pH, temperature, a_w , and other parameters of growth and/or inactivation) (BEVILACQUA & SINIGAGLIA 2010). One of the most important

doi: 10.17221/258/2014-CJFS

growth models is the Gompertz equation, which is based on an assumption that microbial growth follows a sigmoidal trend and could be divided into three phases: lag, exponential, and stationary phases (ZWIETERING *et al.* 1990). Positive or negative Gompertz function can be also used to model physicochemical parameters (pH, colour, sensory acceptability), thus GARDINI *et al.* (1997) used it to model the evolution of CO₂ in the headspace of sealed systems, inoculated with some bacterial pathogens and *Saccharomyces cerevisiae*. This approach was also suitable to model the growth of *Pseudomonas* in laboratory media and in milk (BEVILACQUA *et al.* 2013). To the best of our knowledge, little is known about the suitability of this approach to yeasts and to the microorganisms of cereal beverages.

Therefore, the present study was aimed to: (a) investigate CO₂ production by four target strains (*Saccharomyces cerevisiae* var. *boulardii*, *Kluyveromyces lactis* × *Saccharomyces cerevisiae*, *Saccharomyces pastorianus* var. *pastorianus*, *Kazachstania exigua*) in two laboratory media, different in a carbon source (glucose and maltose); (b) evaluate the amount of CO₂ in model systems simulating cereal-based beverages (diluted Malt Extract broth) and model CO₂ through the Gompertz equation to describe this phenomenon by using some simple parameters.

MATERIAL AND METHODS

Strains. Four yeasts were used throughout this research: *Saccharomyces cerevisiae* var. *boulardii* ATCC MYA-796 (GenBank: JQ070086.1) was purchased from the American Type Culture Collection (Manassas, USA), whilst *Kluyveromyces lactis* × *Saccharomyces cerevisiae* DBVPG 6530 (previously known as *Saccharomyces distaticus* and proposed for brewing; FONTANA *et al.* 1992), *Saccharomyces pastorianus* var. *pastorianus* DBVPG 6033 (type strain of *Saccharomyces carlsbergensis* E.C. Hansen, isolated from brewery), and *Kazachstania exigua* DBVPG 4384 (previously known as *S. exiguus*, isolated from sea water) were from the Industrial Yeast Collection, University of Perugia (Perugia, Italy).

Media. The following media were used throughout the research: YPG broth (Yeast Peptone Glucose: bacteriological peptone 20 g/l; yeast extract 10 g/l; glucose 20 g/l; all the ingredients were from Oxoid, Milan, Italy); Malt Extract broth (Oxoid), and Malt Extract broth diluted to 15%.

Inoculum preparation. Yeast strains were grown in YPG broth incubated at 25°C for 48–72 h; then, 20 ml of each strain were centrifuged at 1000 g for 10 min at 4°C. The supernatant was discarded, and yeast cells were suspended in 2 ml of distilled water (7 log CFU/ml).

CO₂ production. The experiments were performed in glass vials (volume 20 ml; Dani Instruments, Cologno Monzese, Italy) containing 10 ml of media (YPG broth, Malt Extract broth, diluted Malt Extract broth). After yeast inoculation (ca. 5 log CFU/ml), vials were sealed with a butyl cap and a metal ring and stored at 15 and 25°C (for 48–96 h); the content of CO₂ in the headspace (% v/v) was evaluated through a headspace gas analyser Checkmate II (PBI Dansensor, Ringsted, Denmark). The initial level of yeasts was assessed through spread plating on YPG agar, incubated at 25°C for 72 hours.

The analyses were performed over at least four different batches for each time and sample. CO₂ values were fitted through a positive Gompertz equation, reparameterised by ZWIETERING *et al.* (1990) and BEVILACQUA *et al.* (2013) and cast in the following form:

$$\text{CO}_2 = (\text{CO}_2)_0 + (\text{CO}_2)_{\max} \times \exp \left\{ - \exp \left[\left(k_{\max} \times 2.71 \right) \frac{\lambda - \text{time}}{(\text{CO}_2)_{\max}} + 1 \right] \right\}$$

where: (CO₂)₀, (CO₂)_{max} (v/v) – initial and the maximum contents of CO₂ in the headspace; k_{max} – rate of CO₂ production in the exponential phase (CO₂/h); λ (h) – time before the beginning of CO₂ production; time – independent variable, i.e. the time of sampling

Statistical analysis. For each parameter, the statistical differences were determined by one- and two-way ANOVA and Tukey's test as the *post-hoc* comparison test (*P* < 0.05). Data analysis and fitting were performed by the STATISTICA software for Windows Ver. 10.0.1011.0 (StatSoft, Inc., Tulsa, USA).

RESULTS AND DISCUSSION

CO₂ production in laboratory media. The starting point of this research was the article of BEVILACQUA *et al.* (2013); they proposed a headspace gas analysing approach for the evaluation of the level of *Pseudomonas* spp. in milk. The method is based upon the fact that pseudomonads consume O₂ and produce CO₂, and these changes can be easily evaluated. In the present paper, the amount of CO₂ produced by four yeast strains was assessed in two

Table 1. Production of CO₂ in the headspace of sealed vials, containing YPG broth or Malt Extract broth at 25°C and 15°C (initial inoculum, 5 log CFU/ml)

Strain	25°C				15°C			
	(CO ₂) _{max}	k _{max}	λ	R ²	(CO ₂) _{max}	k _{max}	λ	R ²
YPG broth								
A	62.19 ± 2.06 ^b	5.08 ± 0.28 ^b	6.96 ± 0.48 ^{a,b}	0.996	62.19 ± 1.43 ^a	2.91 ± 0.34 ^{bc}	12.01 ± 1.71 ^a	0.998
B	36.11 ± 1.64 ^a	2.00 ± 0.07 ^a	7.39 ± 0.46 ^b	0.998	68.55 ± 4.05 ^b	1.39 ± 0.22 ^a	13.11 ± 3.52 ^a	0.993
C	62.02 ± 0.99 ^b	5.21 ± 0.15 ^b	6.47 ± 0.22 ^{a,b}	0.999	66.08 ± 0.53 ^{ab}	2.34 ± 0.09 ^b	12.41 ± 0.64 ^a	0.999
D	64.21 ± 1.65 ^b	5.16 ± 0.23 ^b	6.36 ± 0.34 ^a	0.997	66.79 ± 1.10 ^{ab}	3.19 ± 0.23 ^c	16.53 ± 0.75 ^a	0.999
Malt extract broth								
A	41.78 ± 1.02 ^b	3.18 ± 0.12 ^{b,c}	7.47 ± 0.33 ^b	0.999	23.32 ± 0.34 ^c	ns	21.24 ± 0.37 ^a	0.999
B	19.08 ± 1.55 ^a	1.12 ± 0.08 ^a	8.41 ± 0.77 ^b	0.996	7.74 ± 0.57 ^a	0.86 ± 0.24 ^a	20.54 ± 1.24 ^a	0.978
C	45.04 ± 1.34 ^b	2.91 ± 0.09 ^c	7.10 ± 0.32 ^b	0.998	14.92 ± 1.32 ^b	0.83 ± 0.99 ^a	–	0.993
D	18.44 ± 0.80 ^a	1.47 ± 0.13 ^b	4.24 ± 0.51 ^a	0.996	13.88 ± 1.04 ^b	0.82 ± 0.17 ^a	–	0.994

Strain: A – *S. cerevisiae* var. *bouardii*; B – *Kl. lactis*; C – *S. pastorianus*; D – *K. exigua*; ns – not significant; Fitting parameters of Gompertz equation ± standard error; (CO₂)_{max} – maximum concentration of CO₂ (% v/v); k_{max} – maximum rate of CO₂ production (%/h); λ – time before the beginning of the exponential phase in CO₂ trend (h); ^{a–c} letters indicate significant differences among yeasts (one-way ANOVA and Tukey's test, *P* < 0.05)

different laboratory media (YPG broth containing glucose, and Malt Extract broth with maltose) or in diluted Malt Extract broth, at 15 and 25°C. The target yeasts were selected on the basis of some beneficial effects on human health reported in the literature such as probiotic activity (*S. cerevisiae* var. *bouardii*), improvement of bioavailability of minerals (*S. pastorianus* and *K. exigua*) and folate biofortification (*Kl. lactis*) (MOSLEHI-JENABIAN *et al.* 2010).

Data were fitted through a common primary model (Gompertz equation) and three fitting parameters were pointed out: lag phase or time of metabolic adaptation (λ), rate of CO₂ production in the exponential phase of metabolism (k_{max}), and the maximum concentration of CO₂ in the headspace [(CO₂)_{max}]. The model fitted the experimental data very well, as shown by R² values (0.978–0.999) (Table 1); *S. cerevisiae* var. *bouardii*, *S. pastorianus*, and *K. exigua* showed the highest values of (CO₂)_{max} (ca. 60%) in YPG broth at 25°C. Otherwise, the parameters k_{max} (2%/h) and λ (7.39 h), as well as (CO₂)_{max} (36%), suggested an attenuated metabolism in *Kl. lactis*. At 15°C the highest value of (CO₂)_{max} was observed for *Kl. lactis* (68.55%), which also showed the lowest value for k_{max} (1.39%/h). No differences were found for the fitting parameter λ.

S. cerevisiae var. *bouardii* and *S. pastorianus* showed the highest (CO₂)_{max} (ca. 40%) in Malt Extract broth, although the amount of the gas was lower than in YPG broth. On the other hand, *Kl. lactis* and *K. exigua* experienced lower values of (CO₂)_{max}

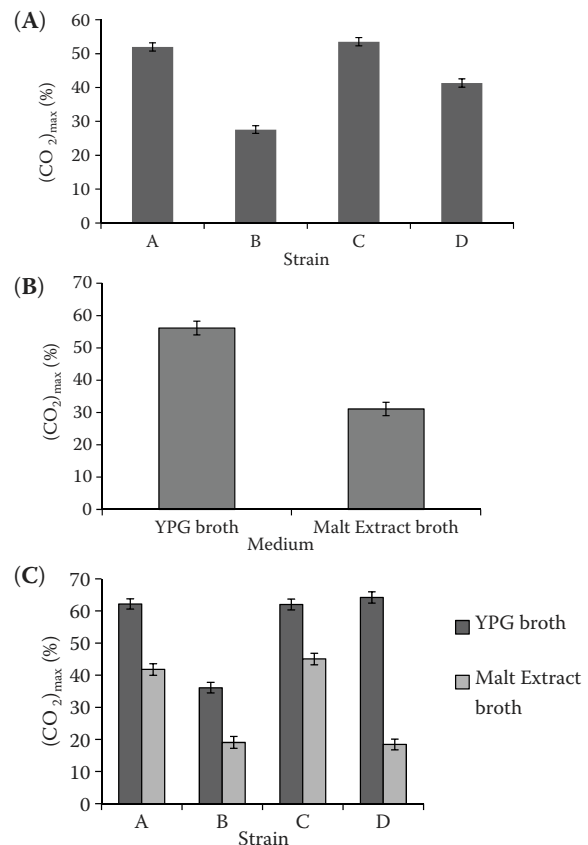


Figure 1. Two-way ANOVA for the effects of strain (A), medium (B), and strain vs medium (C) on (CO₂)_{max} graphs for the decomposition of the effects of the factors; vertical bars denote 95%-confidence; strain A – *S. cerevisiae* var. *bouardii*; B – *Kl. lactis*; C – *S. pastorianus*; D – *K. exigua*

doi: 10.17221/258/2014-CJFS

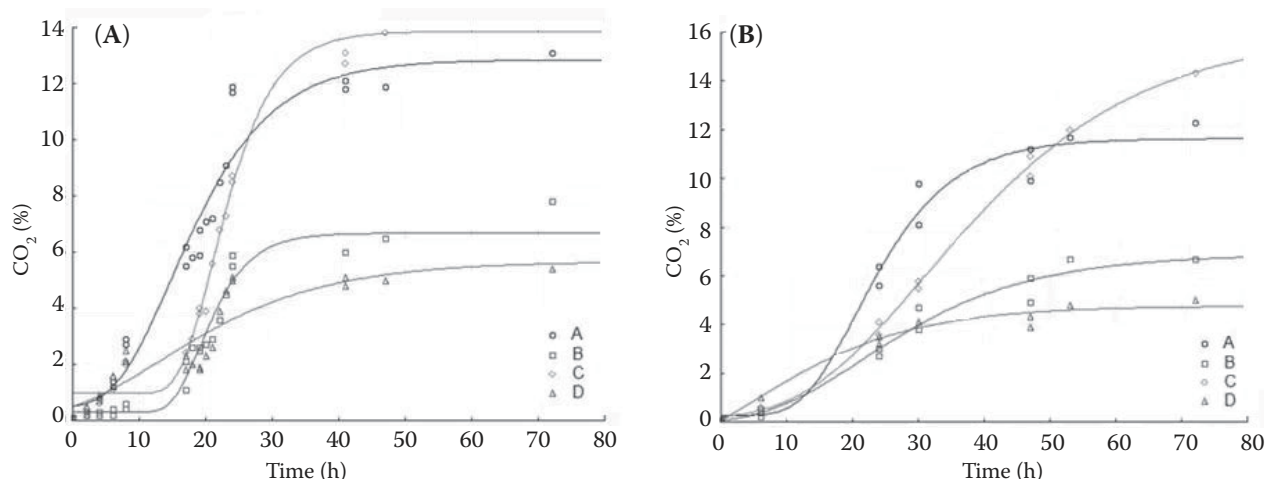


Figure 2. CO₂ production in 15% Malt Extract broth at 25°C (A) and 15°C (B)

A – *S. cerevisiae* var. *bouardii*; B – *Kl. lactis*; C – *S. pastorianus*; D – *K. exigua*; data point are the mean values of two replicates

(ca. 19%); *K. exigua* showed the lowest value of k_{\max} (4.24/h), too.

At 15°C *S. cerevisiae* var. *bouardii* produced the highest concentration of CO₂ (23.32%) followed by *S. pastorianus* and *K. exigua* (14.92–13.88%, respectively), and finally by *Kl. lactis* (7.74%).

The fitting parameter $(CO_2)_{\max}$ was analysed by two-way ANOVA and the factor “strain” exerted a strong effect (Figure 1A); moreover maltose caused an attenuation of the metabolic response (Figure 1B), probably related to microbial inability to fully utilise this sugar (ROMANO *et al.* 2006). Some additional interactive effects of temperature × substrate were also observed (Figure 1C).

CO₂ production in diluted Malt Extract broth. Figure 2 shows the evolution of CO₂ in the headspace of vials containing Malt Extract broth diluted to 15%; this medium was used as a model system to simulate a beverage containing a low amount of sugars. At 25°C the yeasts attained the maximum concentration of CO₂ after ca. 40 h (13% in *S. cerevisiae* var. *bouardii* and *S. pastorianus* and 6% in *Kl. lactis* and *K. exigua*). The lag phase was 6 h for *S. cerevisiae* var. *bouardii* and *K. exigua* and 15 h for *Kl. lactis* and *S. pastorianus*. Yeasts experienced similar trends at 15°C, although the differences for the lag phase (ca. 12–13 h) were not significant ($P > 0.05$).

CONCLUSIONS

This paper proposes a headspace gas analysing approach for the evaluation of CO₂ produced by four yeasts (*S. cerevisiae* var. *bouardii*, *Kl. lactis* ×

S. cerevisiae, *S. pastorianus*, and *K. exigua*) to screen some promising target strains for the production of cereal-based beverages. *Kl. lactis* showed a lower value of $(CO_2)_{\max}$ in YPG and Malt Extract broth, whilst *K. exigua* produced a reduced amount of CO₂ in the presence of maltose; however, $(CO_2)_{\max}$ was not related to the duration of the lag phase, thus suggesting a partial uncoupling between these parameters.

References

- Altisent R., Plaza L., Alegre I., Viñas I., Abadias M. (2014): Comparative study of improved vs. traditional apple cultivars and their aptitude to be minimally processed as ‘ready to eat’ apple wedges. *Food Science and Technology*, 58: 541–549.
- Bevilacqua A., Sinigaglia M. (2010): Food shelf life and safety: challenge tests, prediction and mathematical tools. In: Bevilacqua A., Corbo M.R., Sinigaglia M. (eds): *Application of Alternative Food-Preservation Technologies to Enhance Food Safety and Stability*. Sharjah (UAE), Bentham Publisher: 161–187.
- Bevilacqua A., Corbo M.R., Martino G., Sinigaglia M. (2013): Evaluation of *Pseudomonas* spp. through O₂ and CO₂ head-space analysis. *International Journal of Food Science and Technology*, 48: 1618–1625.
- Blandino A., El-Aseeri M.E., Pandiella S.S., Cantero D., Webb C. (2003): Cereal based fermented foods and beverages. *Food Research International*, 36: 527–543.
- Borchert N., Hempel A., Walsh H., Kerry J.P., Papkovsky D.B. (2012): High throughput quality and safety assessment of packaged green produce using two optical oxygen sensor based systems. *Food Control*, 28: 87–93.

- Borchert N.B., Cruz-Romero M.C., Mahajan P.V., Ren M., Papkovsky D.B., Kerr J.P. (2014): Application of gas sensing technologies for non-destructive monitoring of headspace gases (O₂ and CO₂) during chilled storage of packaged mushrooms (*Agaricus bisporus*) and their correlation with product quality parameters. *Food Packaging and Shelf Life*, 2: 17–29.
- Charalampopoulos D., Wang R., Pandiella S.S., Webb C. (2002): Application of cereals and cereal components in functional foods: a review. *International Journal of Food Microbiology*, 79: 131–141.
- Fontana A., Bidenne C., Ghommidh C., Guiraud J.P., Vezinhet F. (1992): Study of the flocculation of *Saccharomyces diastolicus* NCYC 625. *Journal of the Institute of Brewing*, 98: 401–407.
- Gardini F., Lanciotti R., Sinigaglia M., Guerzoni M.E. (1997): A head space gas chromatographic approach for the monitoring of the microbial cell activity and the cell viability evaluation. *Journal of Microbiological Methods*, 29: 103–114.
- Gobbetti M., Di Cagno R., De Angelis M. (2010): Functional microorganisms for functional food quality. *Critical Reviews in Food Science and Nutrition*, 50: 716–727.
- Gøtterup J., Olsen K., Knøchel S., Tjener K., Stahnke L.H., Møller J.K.S. (2008): Colour formation in fermented sausages by meat-associated staphylococci with different nitrite- and nitrate-reductase activities. *Meat Science*, 78: 492–501.
- Hesseltine C.W. (1979): Some important fermented foods in mid-Asia, the Middle East and Africa. *Journal of the American Oil Chemists' Society*, 56: 367–374.
- Morcos S.R., Hegazi S.M., EL-Damhougy S.T. (1973): Fermented foods in common use in Egypt II. The chemical composition of bouza and its ingredients. *Journal of the Science of Food and Agriculture*, 24: 1157–1161.
- Moslehi-Jenabian S., Pedersen L.L., Jespersen L. (2010): Beneficial effects of probiotic and food borne yeasts on human health. *Nutrients*, 2: 449–473.
- Nicholson G.E. (1960): Chicha maize types and chichi manufacture in Peru. *Economic Botany*, 14: 290–299.
- Oyedemi O., Ogunbanwo S.T., Onilude A.A. (2013): Predominant lactic acid bacteria involved in the traditional fermentation of *Fufu* and *Ogi*, two Nigerian fermented food products. *Food Nutrition and Science*, 4: 40–46.
- Romano P., Capece A., Jespersen L. (2006): Taxonomic and ecological diversity of food and beverage yeasts. In: Querol A., Fleet G.H. (eds): *Yeasts in Food and Beverages*. New-York, Springer: 13–53.
- Wood B.J.B., Hodge M.M. (1985): Yeast-lactic acid bacteria interactions and their contribution to fermented food-stuffs. In Wood B.J.B. (ed.): *Microbiology of Fermented Foods*. Vol. 1. London, Elsevier Applied Science Publication: 263–293.
- Zannini E., Mauch A., Galle S., Gänzle M., Coffey A., Arendt E.K., Taylor J.P., Waters D.M. (2013): Barley malt wort fermentation by exopolysaccharide-forming *Weissella cibaria* MG1 for the production of a novel beverage. *Journal of Applied Microbiology*, 115: 1379–1387.
- Zwietering M.H., Jongenburger I., Rombouts F.M., van't Riet K. (1990): Modeling the bacterial growth curve. *Applied and Environmental Microbiology*, 56: 1875–1881.

Received: 2014–05–13

Accepted after corrections: 2014–09–25

Corresponding author:

Dr ANTONIO BEVILACQUA, Ph.D., Università degli Studi di Foggia, Dipartimento di Scienze Agrarie, degli Alimenti e dell'Ambiente, Via Napoli 25, 71122, Foggia, Italy; E-mail: antonio.bevilacqua@unifg.it; abevi@libero.it
