

Effect of Anoxia on Respiration Rate (Fermentative Index) and Ethanol Production of Onion Bulbs (*Allium cepa* L.)

N. Benkeblia* and N. Shiomi

Department of Food and Nutrition Sciences, Graduate School of Dairy Science Research,
Rakuno Gakuen University, 582 Bunkyo-dai Midorimachi, Ebetsu,
Hokkaido 069-8501, Japan

تأثير انعدام الأوكسجين على معدل التنفس (المؤشر التخميري) وإنتاج الايثانول من أبصال البصل (*Allium cepa* L.)

نور الدين بنكيبلية ونوريو شيومي

خلاصة: تمت دراسة المنحني الفسيولوجي لأبصال البصل ممتلاً في إنتاج ثاني اوكسيد الكربون والمؤشر التخميري وإنتاج الايثانول من خلال وضعها تحت ظروف انعدام الأوكسجين الكامل (100% نيتروجين). بينت النتائج أزداد إنتاج ثاني أكسيد الكربون من 0.01 إلى 1.56 كيلو باسكال خلال الأربع والعشرين ساعة الأولى كما بلغ معدل الزيادة في متوسط إنتاج ثاني أكسيد الكربون بين صفر وأربع وعشرين ساعة 0.09 كيلو باسكال/ساعة. وقد بلغت قيمة Q₁₀ للمؤشر التخميري 1.9. بينت النتائج إن إنتاج الأبصال التي وضعت تحت ظروف انعدام الأوكسجين بعد 6 ساعات معتمداً على درجات الحرارة حيث بلغ 0.563 و 0.760 pmol kg⁻¹ h⁻¹ تحت 10 و 20 درجة مئوية على التوالي. ولم يمكن تحديد الكمية المنتجة تحت 4 درجات مئوية. يستنتج من ذلك إن البصل قد يكون أقل تحملاً لانعدام الأوكسجين عن أنواع الخضراوات الأخرى مثل الخرشوف والزهرة والطماطم والبطاطس والاسبركس.

ABSTRACT: The physiological behavior, including carbon dioxide production, fermentative index (FI) and ethanolic production of onion bulbs kept under total anoxia (100% N₂) was investigated. During the first 24 hours, carbon dioxide production increased from 0.01 to 1.56 kPa CO₂, and the average rate of the increase in CO₂ production between 0 and 24 hours was 0.09 kPa/h. The Q₁₀ of the fermentative index was 1.9. Ethanol produced by onion bulbs kept under anoxia during 6 hours was temperature dependent, and was 0.563 and 0.760 pmol kg⁻¹ h⁻¹ at 10 and 20°C respectively, while at 4°C the quantity produced was not detected. It is concluded that onion seems to be less tolerant to anoxia than other vegetables such as artichoke, cauliflower, tomato, potato and asparagus.

Keywords: Anoxia, fermentative index, ethanol, onion, storage conditions.

Onion may be one of the oldest cultivated crops due to its growing versatility and portability and the fact that it could be dried and preserved for long times. Onions are usually held for long periods between harvesting and marketing so as to match the market demand. However, during their storage, onion bulbs are exposed to environmental and atmospheric conditions which can affect their physiology and biochemistry. During this period, high catabolism is considered the main cause of changes in quality, such as sprouting and rotting (Benkeblia, 2003). Higher plants are aerobic organisms and rely on oxygen to survive. Occasionally, plants and stored crops experience lower oxygen availability (hypoxia) and, less frequently, total absence of oxygen (anoxia), due mainly to environmental

factors such as modified atmosphere storage or packaging, or anatomical structure of some tissues. The characteristics of tissues such as the dry outer scales of the bulbs may severely limit the permeability of oxygen. The main final products of anaerobic metabolism (decomposition of sugars) in higher plants are lactate and ethanol (Mohr and Schopfer, 1995). However, investigation showed that a peak of lactate formation preceded ethanol accumulation (Andreev and Vartapetian, 1992; Davies *et al.*, 1974). These authors considered lactic acid formation to be a prerequisite for subsequent activation of alcoholic formation. Moreover, plant tolerance to low oxygen availability differs considerably with species and organs (Gibbs and Greenway, 2003; Greenway and Gibbs, 2003). Some

*Corresponding author.

tissues are able to withstand days of hypoxia while others die in a few hours and some seeds can germinate in anoxia while others require full oxygen availability (Perata and Alpi, 1993). However, despite the wide range of response of plants to hypoxia or anoxia, some responses are common while other are more typical of the tolerance of the plants which are classified as tolerant or intolerant to anoxia (Kennedy *et al.*, 1992; Perata and Alpi, 1993; Ratcliffe, 1995).

The biochemical and physiological responses of vegetable crops to oxygen-deprived environments are not well documented and advancement in the knowledge of the physiological parameters of fruits and vegetables would be helpful for post harvest technologies, thus allowing good and long preservation of the shelf life of the produce. Unfortunately, little is known of physiological parameters of fruits and vegetables kept under anoxia and no data are available on the respiratory parameters of bulbs or other fresh produce. The purpose of this investigation was to study the physiological behavior of onion bulbs kept under total anoxia, including evaluation of some physiological parameters.

Materials and Methods

ONIONS: bulbs, *Allium cepa* var. Rouge Amposta (organic product, free of any preharvest chemical treatments), which had been freshly harvested and dried in the field for two weeks, were obtained from the local market (MIN, Avignon, France). They were sorted for uniformity and absence of defects, packed in commercial plastic (PVC) trays each of 12 kg and placed at 20°C prior to treatments.

ANOXIC TREATMENT: Samples of onion bulbs (10 ± 0.05 kg) were placed in 120 L vessels, the lid of which was tightly secured. The vessels were flushed with pure nitrogen (N₂) and the final gas composition within the flushed vessels was checked with a gas chromatograph as described below. This averaged from 99.5 to 99.7 kPa N₂, and less than 0.2 kPa O₂.

CO₂ MEASUREMENT AND FERMENTATIVE INDEX (FI) DETERMINATION: Carbon dioxide (CO₂) produced within the vessels was measured according to the method of Benkeblia *et al.* (2002), using a gas chromatograph. At specific time intervals (after 3, 6, 12, 24 and 48 hours), gas samples (50 µl) were taken from the vessels through a silicone septum and analyzed by a gas chromatograph (model M200, Microsensor Technology Inc., Fremont, USA). The chromatograph involved two manifolds: one fitted with an MS-5A, 4 m capillary column set at 80°C with argon as carrier gas at a flow rate of 20 ml min⁻¹, and the other fitted with a capillary Poraplot 4, 6 m column set at 30°C with helium as carrier gas at a flow rate of 30 ml min⁻¹. Both manifolds were coupled with a thermal

conductivity detector (TCD). In such conditions argon does not interfere with O₂ peaks. The gas standard used (L'Air Liquide, France) was 10:10:80 % of CO₂:O₂:N₂, respectively. The production of CO₂ is expressed in kPa, and FI was calculated by linear regression from the CO₂ depletion curve as described by the following equation:

$$FI \text{ (mmol kg}^{-1} \text{ h}^{-1}\text{)} = \frac{\frac{S}{100} (V - P) \times 273}{(273 + T) \times 22.2 \times \frac{P}{1000}}$$

S = slope of the CO₂ depletion curve
 V = volume of the vessel (liter)
 P = weight of the sample (kg)
 T = temperature of measurement (°C)

$$\left[S = \frac{\Delta CO_2}{\Delta t} \right]$$

and expressed as mmol kg⁻¹ h⁻¹. The mean value of the CO₂ produced was determined from triplicate measurements.

MEASUREMENT OF ETHANOL PRODUCTION: Ethanol concentration was measured on the headspace of the vessels by a gas chromatograph (DI.121FL model, IGC Instruments, Saulx les Chartreux, France) fitted with a Porapack T column (4 mm x 2 m) (Alltech, Deerfield, IL, USA) set at 100°C, and equipped with a flame ionizing detector at 150°C. The carrier gas was N₂ with a flow rate of 30 ml min⁻¹, and the integrator was an Intersmat (model ICR-1B, IGC Instruments, Saulx les Chartreux, France). A sample of 100 µl of a standard solution of ethanol (10⁻⁴ M) was injected prior the analysis. The production of ethanol was compared with the standard solution, taking into account the volume of the vessels. Ethanol production was calculated as pmol kg⁻¹ h⁻¹ using an equation similar to the one for fermentative index.

All determinations were carried out in triplicate and the experiment was repeated twice. The data were averaged and compared using Statistica 5.0 software (StatSoft, Maisons-Alfort, France).

Results and Discussion

The CO₂ production of onion bulbs under anoxia is shown in Figure 1. The CO₂ measured during the first 24 hours increased from 0.01 to 1.56 kPa CO₂. The average rate of the increase in carbon dioxide production between 0 and 24 hours was determined by fitting linear regression lines to the CO₂ production vs. time data in Figure 1. The increase of CO₂ production was 0.09 kPa per hour and the coefficient of determination (R²) of the regression was 0.93. Beyond 24 hours, CO₂ production increased sharply and was likely due to anaerobic or aero-anaerobic micro organisms

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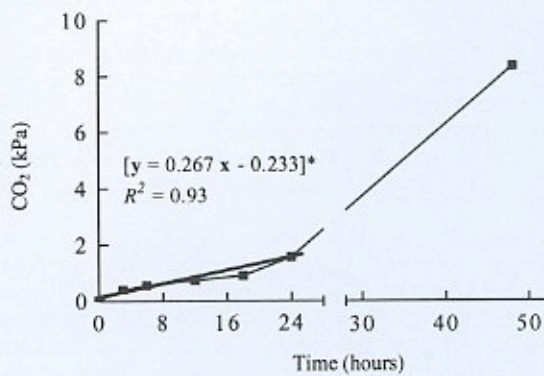


Figure 1. Evolution of CO₂ production of onion bulbs kept under anoxia at 20°C (* regression curve was calculated from 0 to 24 hours).

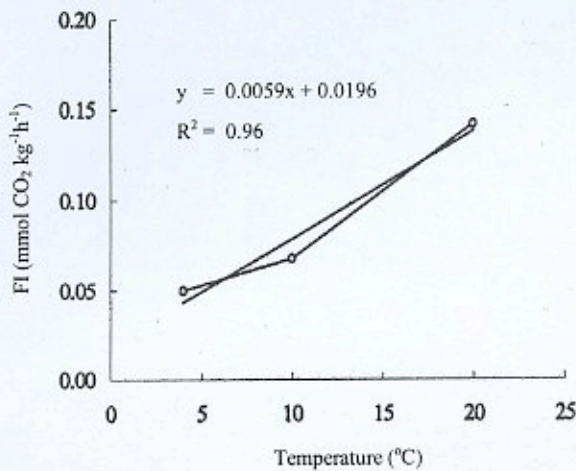


Figure 2. Effect of temperature on Fermentative Index (FI) of onion bulbs kept under anoxia.

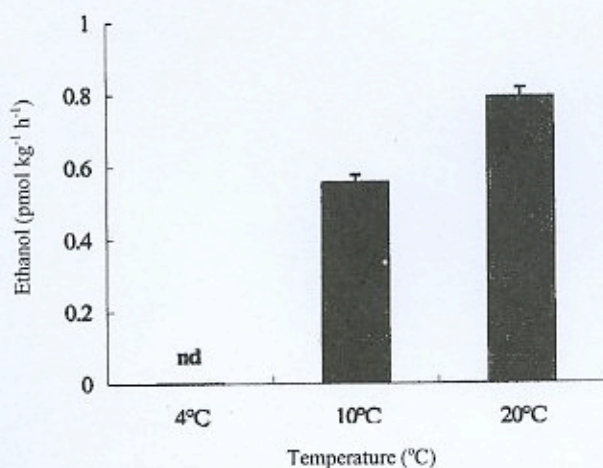


Figure 3. Effect of temperature on ethanol production of onion bulbs kept 6 hours under anoxia (nd = not detected).

such as yeasts and lactic acid bacteria. This pattern could be interpreted as anaerobic metabolism being diverted towards ethanolic fermentation after a limited period. The carbon dioxide produced then did not come totally from the metabolism of bulb tissues,

which is low in aerobic conditions, with a respiration rate (RR_{O₂}) of 0.21 mmol kg⁻¹ h⁻¹ at 20°C (Benkeblia *et al.*, 2000). Furthermore, the increase of carbon dioxide observed after 24 hours could be explained by the saturated levels of fermentative products in the onion bulbs, although knowledge of physiological response of onion bulb tissues to anoxia at the level of primary and secondary metabolism is unknown.

The fermentative index (FI) of onion bulbs under anoxia is shown in Figure 2. The fermentative process was temperature dependent. The velocity of the fermentative index vs. temperature could be calculated from the following equation - $Q_{10} = 10^{10a}$ (a is the slope of the curve); the estimated Q₁₀ is 1.9 and the coefficient of determination (R²) of the regression is 0.96 (Figure 2). Anaerobic catabolism of onion bulbs is rather moderate and much lower than for other vegetable crops (Peppelenbos *et al.*, 1996). This low anaerobic catabolism could also be due to the slight diffusion of CO₂ out of the inner bulb tissues, and this diffusion could be markedly influenced by the tightness of the dry outer scales (Hoftun, 1993).

Ethanol production of onion bulbs kept under anoxia for 6 hours is shown in Figure 3. Results showed that ethanol production was temperature dependent, and after six hours the amount of ethanol was 0.563 and 0.760 pmol kg⁻¹ h⁻¹ at 10 and 20°C, respectively, while at 4°C the quantity was not detected. However, it is known that the assessment of ethanol in a headspace of the jars used in the experiment was not rigorous. The evaporated ethanol was not in balance with the ethanol dissolved in a dynamic system and its partial pressure is highly temperature dependent. Thus, the measured quantity of ethanol does not reflect the real produced quantity, which is higher. Despite the large literature available on the recent advances in storage technology, especially modified atmosphere packaging, and determination of some physiological parameters to predict gas exchanges of tissues and the behavior of the fresh produces under different commercial atmospheres, there is a lack of data on these factors concerning fruits and vegetables under anoxia.

Finally, onion seems relatively less tolerant to anoxia due to the CO₂ production observed after 24 h (CO₂ > 1 kPa). These results are in agreement with the classification reported by Baudry (2000) considering that 1% O₂ is the limit below which injury can occur, although this limit does not always refer to the fermentation threshold. Comparatively, these O₂-limits are 2% for artichoke and cauliflower, 3% for potato and 10% for asparagus. It was also noted that during the first 24 hours under anoxia, ethanol production was relatively higher after only six hours. This indicates that the response of onion bulbs to oxygen deprivation is rapid. Thus, the first response, characterized by a burst of lactate production, is short. This acid production

causes a shift from lactic acid to ethanolic fermentation. These results are also in agreement with the observation that onion bulbs are sensitive to carbon dioxide concentration above 1%.

Conclusions

The results of this study indicate that onion seems to be less tolerant to anoxia than other vegetables. These results could be helpful for storage conditions of onion bulbs and other similar edible bulbs. However, further physiological and biochemical investigations are needed to study the response of onion bulb tissues to anoxia at the level of primary and secondary metabolism.

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References

- Andreev, V.Y. and B.B. Vartapetian. 1992. Induction of alcoholic and lactic fermentation in the early stages of aerobic incubation of higher plants. *Phytochemistry* 31(6):1859-1861.
- Beaudry, R.M. 2000. Responses of horticultural commodities to low oxygen: limits to the expanded use of modified atmosphere packaging. *HorTechnology* 10(3):491-500.
- Benkeblia, N., P. Varoquaux, B. Gouble and G. Selsclet-Attou. 2000. Respiratory parameters of onion bulbs: effects of ionizing radiation and temperature. *Journal of the Science of Food and Agriculture* 80:1772-1778.
- Benkeblia, N., P. Varoquaux, N. Shiomi and H. Sakai. 2002. Effect of irradiation, MH and CIP treatments on respiration rate and oligosaccharides variations in onion bulbs in store. *International Journal of Food Science and Technology* 37:169-176.
- Benkeblia, N. 2003. Postharvest technology of onions. In: *Crop Management and Postharvest Handling of Horticultural Products*, R. Driss, R. Niskanen and S. Mohan-Jain (Editors), 107-137. Science Publishers, Enfield, New Hampshire.
- Davis, D.D., S. Grego and P. Kenworth. 1974. The control of the production of lactate and ethanol in higher plants. *Planta* 118:297-310.
- Gibbs, J. and H. Greenway. 2003. Mechanisms of anoxia tolerance in plants. I. Growth, survival and anaerobic catabolism. *Functional Plant Biology* 30(1):1-47.
- Greenway, H. and J. Gibbs. 2003. Mechanisms of anoxia tolerance in plants. II. Energy requirements for maintenance and energy distribution to essential processes. *Functional Plant Biology* 30(10):999-1036.
- Hoftun, H. 1993. Internal atmosphere and watery scales in onion bulbs (*Allium cepa* L.). *Acta Horticulturae* 343:135-140.
- Kennedy, R.A., M.E. Rumpho and T.C. Fox. 1992. Anaerobic metabolism in plants. *Plant Physiology* 100(1):1-6.
- Mohr, H. and P. Schopfer. 1995. *Plant Physiology*. Springer Verlag, Berlin, Germany.
- Peppelenbos, H.W., L.M.M. Tijssens, J. Vant'Leven and E.C. Wilkinson. 1996. Modelling oxidative and fermentation carbon dioxide production of fruits and vegetables. *Postharvest Biology and Technology* 9(3):283-295.
- Perata, P. and A. Alpi. 1993. Plant responses to anaerobiosis. *Plant Science* 93:1-17.
- Ratcliffe, R.G. 1995. Metabolic aspects of the anoxic response in plant tissue. In: *Environment and Plant Metabolism*, N. Smirnoff (Editor), 111-127. Bios Scientific Publishers, London.

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