Lait (1998) 78, 241-250

Original article

Respiration of *Penicillium camemberti* during ripening and cold storage of semi-soft cheese

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(Received 10 March 1997; accepted 26 June 1997)

Abstract — The aim of the present paper was to study the respiration rate of *Penicillium camemberti* (Pc) growing on a semi-soft cheese. The respiration rate of Pc was measured during ripening and cold storage as a function of environmental parameters, such as partial pressures of oxygen and carbon dioxide, temperature and wrapping stress. Respiration rate decreased when oxygen partial pressure was reduced below 0.05 bar. A Michaelis-Menten equation was used to model O_2 consumption and CO_2 production as a function of O_2 partial pressure from 0.01 to 0.21 bar. During cold storage at 4.5 °C, Michaelis-Menten parameters $R_{O_2\text{-max}}$, $R_{CO_2\text{-max}}$, $K_{m\text{-}O_2}$ and $K_{m\text{-}CO_2}$ were 0.021 mmol/cm^2/24 h, 0.020 mmol/cm^2/24 h, 0.44 mmol/L and 0.70 mmol/L, respectively. From 3 °C to 12 °C, temperature had a large effect on respiration rate. This effect was modeled with an Arrhenius relationship. Moreover, respiration rate slightly increased with CO_2 partial pressure from 0.1 to 0.4 while relative humidity and wrapping did not affect Pc respiration. © Inra/Elsevier, Paris.

***Penicillium camemberti* / cheese / respiration rate / environmental parameter / cold storage**

Résumé — Étude de la respiration de *Penicillium camemberti* au cours de l'affinage et de la conservation à basse température d'un fromage à pâte molle. Le but de ces travaux est d'étudier les échanges respiratoires de *Penicillium camemberti* (Pc) implanté sur un fromage à pâte molle. La consommation d'O_2 et la production de CO_2 ont été mesurées pendant l'affinage et la conservation au froid en fonction de divers paramètres d'ambiance, tels que les pressions partielles en oxygène et en CO_2, la température, l'humidité relative et la pression exercée par l'emballage sur le fromage. Les échanges respiratoires chutent lorsque la pression partielle en O_2 autour de la flore descend au-dessous de 0,05 bar. Un modèle a été établi et a montré que l'activité respiratoire évolue selon une cinétique de type Michaelis-Menten. Les paramètres de cette cinétique $R_{O_2\text{-max}}$, $R_{CO_2\text{-max}}$, $K_{m\text{-}O_2}$ and $K_{m\text{-}CO_2}$ valent respectivement 0,021 mmol/cm^2/24 h, 0,020 mmol/cm^2/24 h, 0,44 mmol/L et 0,70 mmol/L, à 4,5 °C. Le CO_2, dans la limite de 0,1 à

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0,4 bar, a un léger effet activateur sur l'activité respiratoire. Les échanges respiratoires augmentent considérablement avec la température, entre 3 et 12 °C, selon une loi d'Arrhénius. En revanche, l'humidité relative de l'air et le tassement de la flore par l'emballage n'ont pas d'influence significative sur l'intensité des échanges gazeux.

***Penicillium camemberti* / fromage / respiration / paramètre de l'environnement / conservation au froid**

1. INTRODUCTION

Penicillium camemberti (Pc) is the most important surface flora of semi-soft cheeses like Camembert, Brie or Coulommiers. It confers particular appearance, texture, taste and flavor to the cheese [1, 3, 5, 7]. A better knowledge and control of this flora during cheese ripening and cold storage should allow improvement of cheese quality.

Several authors developed research concerning mould growth to limit contaminant spoilage [2, 8, 9]. They particularly studied the influence on cheese of environmental parameters such as pH, water activity (a_w), temperature or relative humidity (RH). In the particular case of Pc, respiratory exchanges were measured in standard ripening conditions (15 °C, 95% RH) by Wolfseder [10], but no data concerning *Penicillium camemberti* respiration on cheese as a function of environmental parameters are available in the literature.

The aims of this paper were, on one hand, to measure respiration rate of surface flora during ripening and cold storage in the air (0.21 bar of oxygen and 0 bar of carbon dioxide). On the other hand, change in respiration rate was studied as a function of O_2 and CO_2 partial pressures, temperature, RH and wrapping.

2. MATERIALS AND METHODS

2.1. Cheeses

The day for processing of standard Coulommiers semi-soft cheeses was fixed as initial time (t_0). At day 1, time of inoculation with *Penicillium camemberti* (Texel, Dange Saint Romain, France), cheeses had a surface of 375 cm^2, a dry matter of 50%, a pH of 5.2, and a 'fat/dry matter' content of 60%. Immediately after inoculation cheeses were stored 9 days in a room regulated at 13 °C and 96% RH to allow Pc growth. At day 10, the dry matter and pH were 52% and 5.5, respectively, and cheeses were moved from storage room to experimental enclosures to study environmental parameters effects on cold storage phase during 8 weeks.

2.2. Experimental enclosures

Air-tight 5 L enclosures made from polycarbonate were equipped with two taps to evacuate, fill and replace the internal atmosphere (*figure 1*). N_2, O_2 and CO_2 partial pressures in enclosure were obtained by flushing a gas mixture (30 L/min during 2 min) obtained from a KEM100-3-M gas mixer (Air Liquide, Joueles-Tours, France). Humidity was controlled with supersaturated salt solutions agitated by a controlled velocity of circulating air. Because of the small volume of salt solution (400 mL), the amount of carbon dioxide soluble in the solution was neglected. A septum allowed to withdraw gas samples for gas chromatographic analysis. Temperature was maintained by storing enclosures in thermoregulated rooms (± 0.5 °C).

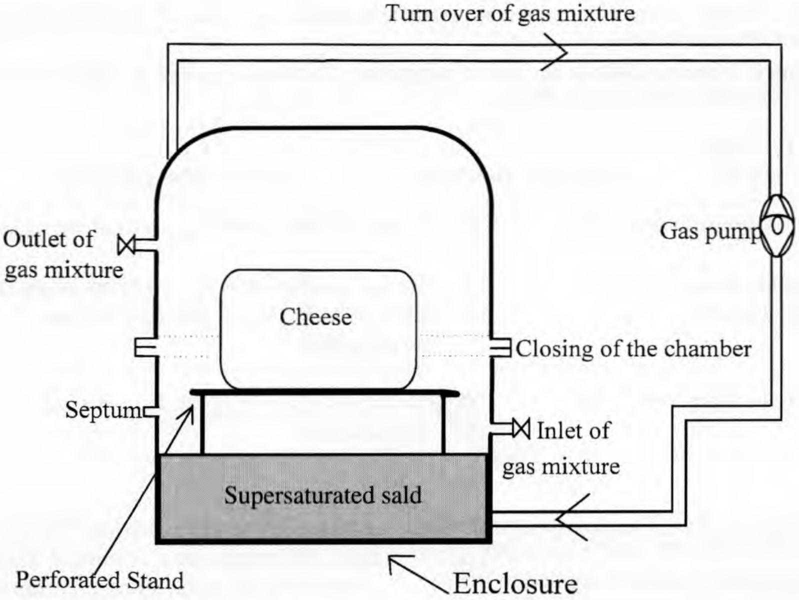

Figure 1. Experimental enclosure.
Figure 1. Enceinte expérimentale.

2.3. Analysis of the enclosure head space

200 µL samples of gas mixture (N_2, O_2 and CO_2) from enclosures were injected with a gas sampling syringe (Dynatec Pressure Lok, Baton Rouge, USA) and analyzed in a gas chromatograph equipped with a thermal conductivity detector and two columns (Hayesep for CO_2 and molecular sieve for O_2). Flow rate of helium carrier gas was 25 mL/min and column temperature was 40 °C. Gas partial pressures in test samples were determined from peak areas using the corrected area normalization method.

2.4. Oxygen consumption rate and carbon dioxide production rate determination

Each cheese was placed in an enclosure of which internal atmosphere was flushed with adequate gas mixture and the initial partial pressure of each gas checked by chromatography. After 2 days, gas samples were analyzed in duplicate. Rates of O_2 consumption (R_{O_2}) and CO_2 production (R_{CO_2}) were calculated from:

$$R_{O_2} = \frac{V \ \Delta P_{O_2}}{S_f \ \ \Delta t}$$

$$R_{CO_2} = \frac{V \ \Delta P_{CO_2}}{S_f \ \ \Delta t}$$

Respiratory quotient (RQ), defined as the ratio of CO_2 production to O_2 consumption (R_{CO_2}/RO_2), was also calculated with a sampling period of 2 days.

2.5. Range of environmental parameters studied

The respiration rate was measured separately as a function of four parameters (*table I*).

Table I. Range of four environmental parameters studied and value of the other parameters fixed for the experiments.

Tableau I. Domaine d'étude des quatre paramètres d'ambiance étudiés et valeurs fixées des autres paramètres dans chaque étude.

Parameter studied	Minimum	Maximum	Level of other parameters
O_2 partial pressure (bar)	0.01	0.21	T = 4.5 °C, RH = 96%, P_{CO_2} = 0 bar, no packaging-
CO_2 partial pressure (bar)	0	0.4	T = 4.5 °C, RH = 96%, P_{O_2} = 0.21 bar, no packaging
Temperature (°C)	3	12	RH = 96%, P_{O_2} = 0.21 bar, P_{CO_2} = 0 bar, no packaging
Relative humidity (%)	88	100	T = 4.5 °C, P_{O_2} = 0.21 bar, P_{CO_2} = 0 bar, no packaging

Moreover, effect of wrapping was studied at 4.5 °C, 96% RH, PO_2 = 0.21 bar and P_{CO_2}= 0 bar with three treatments applied to surface flora of cheese: i) unwrapped and unpressed flora (control); ii) flora pressed for 1 min on both sides by placing a 500 g iron disk on the cheese once a day; and iii) flora wrapped in highly permeable packaging (microperforated cellophane), assuming that packaging continuously press the flora. The first measurement of respiratory activity was carried out after 2 days of storage with the packaging. Then, it was carried out every week for 8 weeks.

Analyses were made in duplicate and carried out on two different cheeses. The data presented in *table II* are average values of the measurements.

3. RESULTS AND DISCUSSION

3.1. Measurement of gas exchange during ripening and cold storage

The respiration rate (RO_2 and R_{CO_2}) of Pc during 10 days of ripening at 13 °C and 96% RH is presented in *figure 2*. At t = 6 days, respiration rates increase, corresponding to mycelium development as shown by Molimard et al. [6] who counted a constant population of 3.6 ± 0.1 log FCU/g during 7 days at 12 °C and 95% RH and then observed a rapid population increase up to 5.2 ± 0.5 log FCU/g at 14 days. This population remained stable for 3 weeks in the same storage conditions.

The gas exchanges are maximal at 9 days reaching 1.9 $cm^3/cm^2/d$ for O_2 and 2.0 $cm^3/cm^2/d$ for CO_2. During ripening average RQ is equal to 1.07. Our results are compared to those obtained at 15 °C and 95% RH by Wolfseder [10]. In *figure 2*, the curve shapes are in agreement but the maximum of gas exchanges is higher and obtained more rapidly in Wolfseder's study (6 days after manufacturing). This can be due to differences in cheese technology or micro-organism, which are not specified in Wolfseders' study.

At 10 days, Pc was developed and cheese was placed in cold storage with unmodified air (0.21 bar of O_2 and 0 bar of CO_2). The effect of temperature change from 13 °C to 4.5 °C was a dramatical decrease of gas exchanges (*figure 3*). Then, respiration was stable during 8 weeks of storage at 4.5 °C with a respiratory quotient of 0.8. Moving cheeses from the ripening chamber to the cold storage chamber hardly reduces the growing and activity of Pc. Industrially, if for economical reasons temperature changes too early or too late, aspect, taste and texture will be largely modified.

Table II. Influence of packaging on *P. camemberti* respiration rate and respiratory quotient.

Tableau II. Influence de l'emballage sur l'activité respiratoire de *P. camemberti* et sur son quotient respiratoire.

	O_2 consumption rate ($cm^3/day/cm^2$)	CO_2 production rate ($cm^3/day/cm^2$)	Respiratory quotient (R_{CO_2}/R_{O_2})
Unwrapped flora	0.51 ± 0.06	0.4 ± 0.06	0.79 ± 0.03
Pressed flora	0.57 ± 0.08	0.47 ± 0.08	0.83 ± 0.10
Wrapped flora	0.58 ± 0.05	0.49 ± 0.06	0.84 ± 0.07

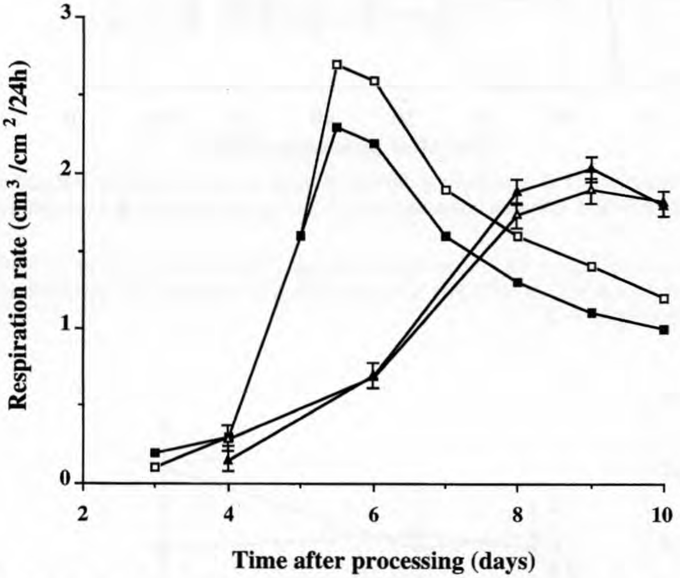

Figure 2. Respiration rate of *P. camemberti* during ripening. Oxygen consumption (Δ) and CO_2 production (▲) measured in the present paper at 13 °C and 96% RH; oxygen consumption (□) and CO_2 production (■) measured by Wolfseder [10] at 15 °C and 95% RH.

Figure 2. Activité respiratoire de *P. camemberti* durant l'affinage. Consommation d'oxygène (Δ) et production de CO_2 (▲) mesurées dans cette étude à 13 °C et 96 % HR ; consommation d'oxygène (□) et production de CO_2 (■) mesurées par Wolfseder (1973) à 15 °C et 95 % HR.

3.2. Effect of O_2 partial pressure during cold storage

For the eight different P_{O_2} applied around the cheese, gas exchanges and respiratory quotient were measured as constant during 8 weeks of cold storage. Nevertheless, respiration rate increased with increasing O_2 partial pressure in the enclosure (*figure 4*). At high O_2 partial pressure, the reaction kinetic was maximum and remained fairly constant over a range of partial pressures from 0.21 bar to 0.05 bar. Respiration rates decreased rapidly when O_2 partial pressure level fell below 0.05 bar and vanished for an O_2

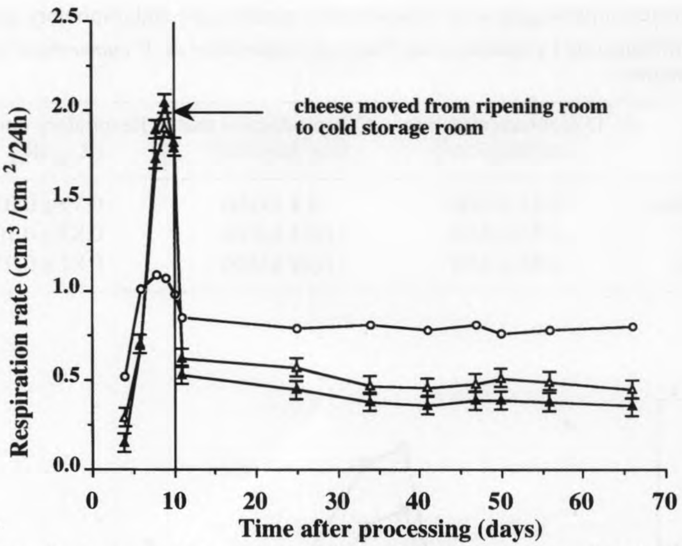

Figure 3. Respiration rate of *P. camemberti* during ripening at 13 °C and 96% RH and cold storage at 4.5 °C and 96% RH. Oxygen consumption (Δ), CO_2 production (▲), respiratory quotient (O).

Figure 3. Activité respiratoire de *P. camemberti* durant l'affinage à 13 °C et 96 % HR et la conservation au froid à 4,5 °C et 96 % HR. Consommation d'oxygène (Δ), production de CO_2 (▲), quotient respiratoire (O).

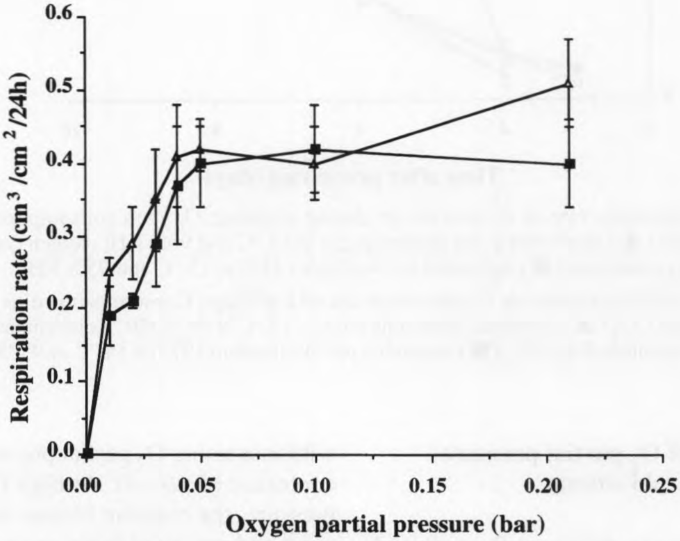

Figure 4. Respiration rate of *P. camemberti* versus oxygen partial pressure during cold storage at 4.5 °C and 96 % RH. Oxygen consumption (Δ), CO_2 production (■).

Figure 4. Activité respiratoire de *P. camemberti* en fonction de la pression partielle en O_2 durant la conservation au froid à 4,5 °C et 96 % HR. Consommation d'oxygène (Δ), production de CO_2 (■).

below 0.05 bar and vanished for an O_2 partial pressure equal to zero.

Curve shape appeared characteristic of an enzymatic reaction. The dependence of the rate of respiration of *Penicillium camemberti* with O_2 partial pressure could be expressed by the Michaelis-Menten equation:

$$R_{O_2} = \frac{(R_{O_2\text{-max}} \cdot P_{O_2})}{(K_{m\text{-}O_2} + P_{O_2})}$$

$$R_{CO_2} = \frac{(R_{CO_2\text{-max}} \cdot P_{O_2})}{(K_{m\text{-}CO_2} + P_{O_2})}$$

From the curves presented in *figure 4*, the constants describing respiration rate dependence on oxygen partial pressures were determined with a linear representation of the Michaelis-Menten equation:

$$\frac{1}{R} = f\left(\frac{1}{P_{O_2}}\right)$$

The regression coefficients were $R^2 = 0.91$ for oxygen and 0.86 for carbon dioxide (*figure 5*). These high values of regression coefficients showed that the respiration can be model by a Michaelis-Menten equation. Values of $R_{O_2\text{-max}}$, $RC_{O_2\text{-max}}$, $K_{m\text{-}O_2}$ and $K_{m\text{-}CO_2}$ were 0.48 $cm^3/cm^2/24$ h or 0.021 $mmol/cm^2/24$ h, 0.46 $cm^3/cm^2/24$ h or 0.020 $mmol/cm^2/24$ h, 0.44 mmol/L and 0.70 mmol/L, respectively. These values allow to predict oxygen consumption rate and carbon dioxide production rate for a given partial pressure, which can be useful for storage with modified or controlled atmosphere packaging.

3.3. Effect of partial pressure of CO_2, relative humidity and wrapping during cold storage

Effects of CO_2 partial pressure, relative humidity (RH) or wrapping on Pc respiration were evaluated under conditions in which O_2 was maintained at a partial pressure high enough to have no limiting

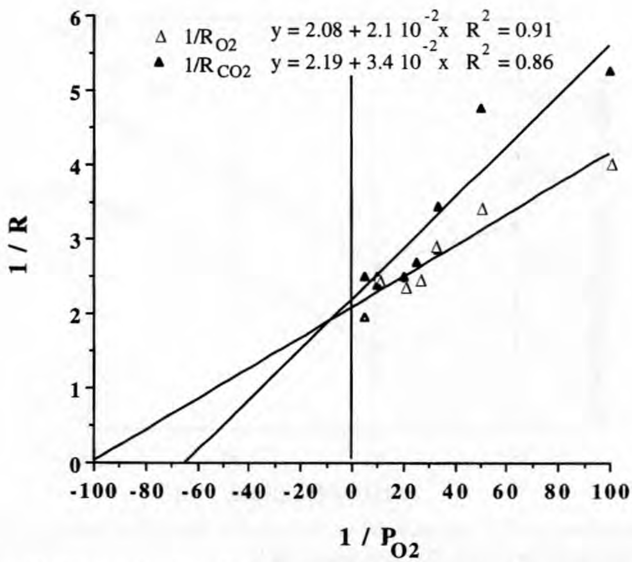

Figure 5. Graphical determination of Michaelis-Menten parameters from data presented in *figure 4*.

Figure 5. Détermination graphique des paramètres de Michaelis-Menten à partir des données de la *figure 4*.

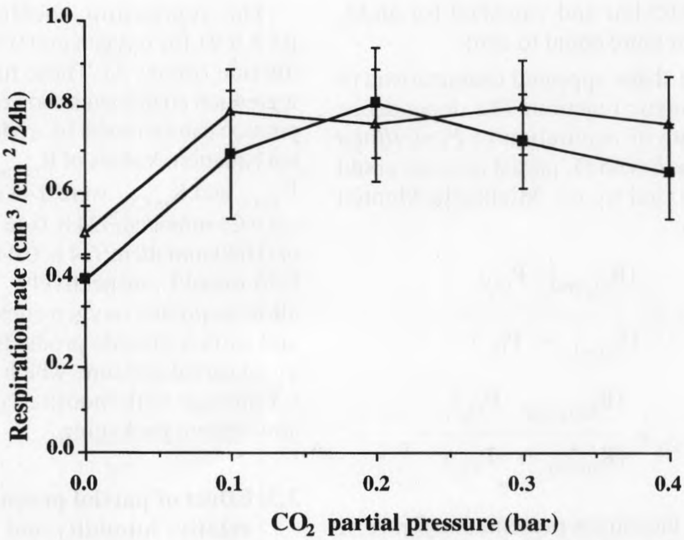

Figure 6. Respiration rate of *P. camemberti* versus CO_2 partial pressure during cold storage at 4.5 °C and 96% RH oxygen consumption (Δ), CO_2 production (■).

Figure 6. Activité respiratoire de *P. camemberti* en fonction de la pression partielle en CO_2 durant la conservation au froid à 4,5 °C et 96% HR. Consommation d'oxygène (Δ), production de CO_2 (■).

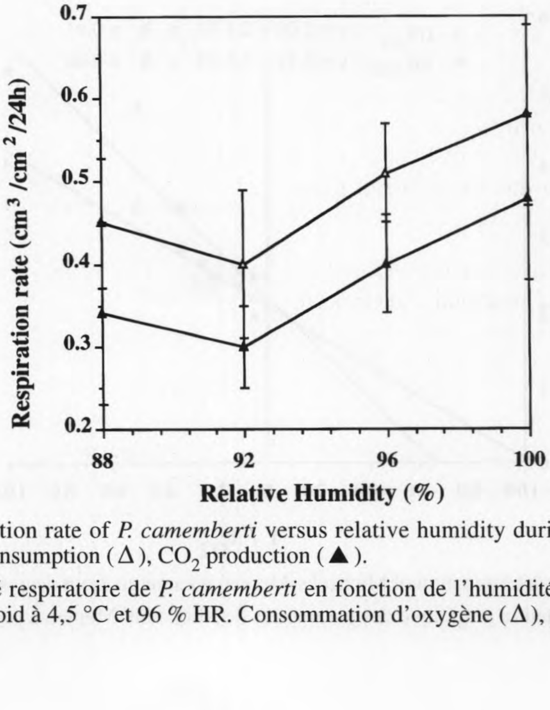

Figure 7. Respiration rate of *P. camemberti* versus relative humidity during cold storage at 4.5 °C. Oxygen consumption (Δ), CO_2 production (▲).

Figure 7. Activité respiratoire de *P. camemberti* en fonction de l'humidité relative durant la conservation au froid à 4,5 °C et 96 % HR. Consommation d'oxygène (Δ), production de CO_2 (▲).

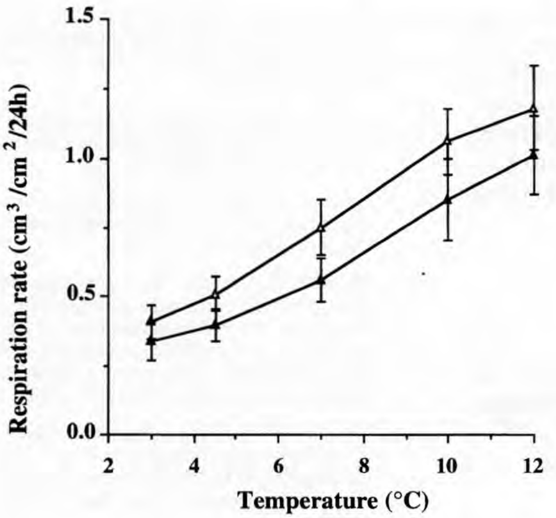

Figure 8. Respiration rate of *P. camemberti* versus temperature. Oxygen consumption (Δ), CO_2 production (▲).

Figure 8. Activité respiratoire de *P. camemberti* en fonction de la température. Consommation d'oxygène (Δ), production de CO_2 (▲).

effect on respiration (0.21 bar). *Figure 6* shows the effect of CO_2 partial pressure on Pc respiration. *Figure 7* and *table II* show the effect of RH from 88% to 100% or wrapping conditions on the respiration rate. In *figures 6* and *7* and *table II*, the variation coefficients in respiration rate are between 15 and 20%. A variance analysis showed no significant difference between the respiration rates at the 95% confidence level. Effect of CO_2, RH and wrapping need further experiments to be understood.

The only industrial problem is certainly on the side of the wrapped cheese were oxygen is less available and where the pressure applied to the cheese is much higher.

3.4. Effect of temperature during cold storage

Respiration increased with temperature in range of 3–12 °C (*figure 8*). The data could be fitted by an Arrhenius relationship:

$$R = R_O \exp\left(\frac{-Ea}{R.T}\right)$$

Good linear relations were found between the logarithm of respiration rate and the inverse of temperature in K ($r^2 = 0.99$ for O_2 consumption rate and 0.98 for CO_2 production rate) which indicated a strong dependence of *Penicillium camemberti* metabolism on temperature. Activation energies (Ea) were 79 KJ/mole for O_2 consumption and 82 KJ/mole for CO_2 production and showed the great influence of temperature on respiration rate.

4. CONCLUSION

Oxygen partial pressure and temperature are the main controlling factors of respiration rates of *Penicillium camemberti* during cold storage. Respiration rate was modeled using an enzymatic reaction model which adequately described the respiration of surface flora in terms of O_2

consumption rate and CO_2 production rate as a function of O_2 partial pressure. The effect of the respiration of *Penicillium camemberti* on changes in this gaseous environment for particular storage conditions, such as modified atmosphere packaging should allow to control and extend cheese preservation.

ACKNOWLEDGMENT

This study was financially supported by the French Ministry of Research and Technology (program 'Aliment Demain')

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Abbreviations: Ea, activation energy (KJ/mol); Km_{O_2} and Km_{CO_2}, Michaelis-Menten constants (mmol/L); P_{CO_2}, carbon dioxide partial pressure (bar); ΔP_{CO_2}, change in carbon dioxide partial pressure (bar); P_{O_2}, oxygen partial pressure (bar); ΔP_{O_2}, change in oxygen partial pressure (bar); R, gas constant (KJ/mol/K); R_O, respiration rate constant in Arrhenius model ($cm^3/d/cm^2$); R_{O_2}, O_2 consumption rate ($cm^3/d/cm^2$); $R_{O_2\text{-max}}$, maximum O_2 consumption rate ($cm^3/d/cm^2$); R_{CO_2}, CO_2 production rate ($cm^3/d/cm^2$); $R_{CO_2\text{-max}}$, maximum CO_2 production rate ($cm^3/d/cm^2$ or $mmol/d/cm^2$); S_f, surface flora of cheese (cm^2); t, time (days); T, temperature (K); V, free container volume (cm^3).