1 Introduction

Respiration is a metabolic process that provides energy for the plant’s biochemical process. The metabolic process involves the disintegration of complex organic compounds such as sugars, organic acids, amino acids, and fatty acids resulting in low molecular weight molecules with the subsequent production of energy, ATP, which is, in turn, associated with the liberation of heat. In other words, respiration can be considered as a metabolic process for the oxidative breakage of organic substrates into simple molecules such as CO$_2$ and H$_2$O with the production of energy (FONSECA; OLIVEIRA; BRECHT, 2002). In addition, the respiration rate is also dependent on the storage environment, particularly taking into account its gaseous composition relative humidity and temperature. The decrease in O$_2$ concentration as well as the increase in CO$_2$ concentration lead to a decrease, up to a certain limit, in the respiration rate of fruits and vegetables (SALTVEIT, 2003; ROCCULI et al., 2006).

The basic principle underlying storage under modified and controlled atmospheres is the modification of the gaseous composition to minimize the respiratory rate and other biochemical processes. Low O$_2$ concentration and high CO$_2$ concentration are widely assumed to maintain quality and extend shelf life of fruits and vegetables (IQBAL, 2009).
Minimally processed products have a physiology that differs from intact product since they require different and more controlled handling. Minimally processing results in tissue and cell integrity disruption, with a concomitant increase in the respiration rate and enzymatic and microbial activity can cause a negative impact on quality, affecting color, flavor, and texture (OLIVAS; MATTINSON; BARBOSA-CÁNOVAS, 2007).

Minimally processed carrots have their shelf-life and acceptance limited because of the whiteness developed on the surface during storage. Whiteness may be the result of a partial dehydration of the surface deriving from physical and/or physiological damage (DURANGO; SOARES; ANDRADE, 2006). Coatings of edible material applied as a thin layer can offer a possibility to extend the shelf life of minimally processed carrots by providing a semipermeable barrier for gases and water vapor, and therefore, reducing respiration and water loss (PEREZ-GAGO; SERRA; del RIO, 2006; OLIVAS; MATTINSON; BARBOSA-CÁNOVAS, 2007). Until recently, there has been little research that investigated the effect of the combination of edible coating and temperature for minimally processed carrots to reduce respiration rate and inhibit whiteness.

The objective of the present work was to evaluate the influences of storage temperature on the respiration rate of minimally processed organic carrots stored under a passively modified atmosphere with and without the protection of a gelatin film.

2. Material and methods

2.1 Raw material

The organic carrots (Daucus Carota L. cv. Brasilia) used were purchased from the town of Antônio Carlos - SC, Brazil.

The organic carrots were first subjected to a selection regarding their physical integrity, size, and color, and then they were washed under running water, peeled, and then manually cut into 1 cm³ pieces (cube). The samples were sanitized by immersion into a 50 mg.L⁻¹ sodium hypochlorite solution for 20 minutes.

The samples were divided into two batches, one of which was immersed in a 2% edible gelatin solution for 15 minutes and subsequently air dried for 20 minutes as described by Teixeira et al. (2004). The treated and non-treated batches were then separately centrifuged in a domestic food centrifuge (Walita, Brazil) for 40 seconds, and each batch was then divided into 100 g portions. The samples were manually packed into multilayer Low Density Polyethylene (LDPE) bags with a thickness of 49 μm, length of 16.2 cm, and width of 15.3 cm and sealed using a plastic bag sealer (Sulpack, Brazil).

The temperature in the laboratory was maintained at 15 °C (± 2 °C) during the processing.

2.2 Storage

The samples were stored in temperature controlled chambers (Expelectron Tecnologia Industrial Ltda., model ECB-EX, Brazil) at 1, 5, and 10 °C, for five-day periods. During this period the relative humidity of the atmosphere was from 76 to 80%.

2.3 Determination of the gas concentration inside the packages

The experiments were conducted in triplicate with a total of fifty-four experiments. Three packages were used for each temperature and treatments, and the gas samples were removed three times per each bag for five days. Previous experiments showed that the equilibrium of the gas mixture was achieved on the fifth day.

The gas sampling were intactly taken out of the package by using a syringe (1 μL). A rubber septum was attached to the outside of the bags for gas collection. The concentrations of CO₂ and O₂ inside the bags were determined by gas chromatography using a CG 35 model gas chromatograph with a thermal conductivity detector, a molecular sieve, and a Porapak Q column with helium as the stripping gas at a flow rate of 30 mL/minute. The detector's temperature was 133 °C, the column's was 53 °C, and the current was 240 mA. The retention time for CO₂ was two minutes and the one for O₂, 1 minute. The standard gas samples were removed at a pressure of 1 atm.

2.4 Determination of the respiration rate

The experimental data of O₂ and CO₂ concentration versus time was fitted to Equations 1 and 2 using a nonlinear regression function in Matlab® software (Mathworks Inc., USA).

\[
[O_2] = 21 - A_1 \left[ 1 - e^{-(B_1 + C_1)t} \right] 
\]

\[
[CO_2] = A_2 \left[ 1 - e^{-(B_2 + C_2)t} \right] 
\]

where \(A_1, A_2, B_1, B_2, C_1, C_2, D_1, \) and \(D_2\) are the fitting coefficients.

The respiration rates of O₂ consumption and CO₂ evolution were obtained from Equations 3 and 4. A computer program was developed using the software MATLAB® (Mathworks Inc., USA) to determine the respiration rates using the model proposed by Lee, Song and Yam (1996), according to Equations 3 and 4:

\[
r_{O_2} = \frac{1}{100} \frac{d[O_2]}{dt} = \frac{SP_{O_2}[0.21 - (O_2/100)p]}{Lm} 
\]

\[
r_{CO_2} = \frac{1}{100} \frac{d[CO_2]}{dt} = \frac{SP_{CO_2}[(CO_2/100 - 0.00)p]}{Lm} 
\]

where \(r_{O_2}\) and \(r_{CO_2}\) are the respiratory rates for O₂ consumption and CO₂ formation, respectively, both expressed as mL.kg⁻¹/hour; [O₂] and [CO₂] are the concentrations of O₂ and CO₂, respectively, expressed as %; \(L\) is the thickness of the film in m; \(S\) is the area of the bag in m²; \(P_{O_2}\) and \(P_{CO_2}\) are the values for the permeability of the film with respect to O₂ and CO₂, respectively, in mL.m⁻².hour.atm⁻¹; \(t\) is the time in hour; \(V\) is the free volume inside the bag in mL; and \(m\) is the mass of product in the bag in kg.

2.5 Influence of temperature on the respiration rate

The Arrhenius equation (Equation 5) was used to model the influence of temperature on the respiration rate.

\[
r = r_0 \exp \left( \frac{-E_a}{RT} \right) 
\]

where \(r\) represents the respiratory rate in mL.kg⁻¹/hour, \(r_0\) is the pre-exponential constant in mL.kg⁻¹/hour, \(E_a\) is the activation energy, \(R\) is the gas constant, and \(T\) is the absolute temperature in Kelvin.

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energy in J.mol$^{-1}$, $R$ is the universal ideal gas constant in J.mol$^{-1}$.K$^{-1}$, and $T$ is the temperature in K.

2.6 Kinetic parameters

The parameters for the enzyme kinetics ($V_m$, $k_{m_{O_2}}$ and $k_{m_{CO_2}}$) were estimated by using the respiratory rate values as a function of the $O_2$ and $CO_2$ concentrations. The models employed are represented in Equations 6, 7, and 8 for three different dominant respiration type: competitive, uncompetitive or non-competitive, respectively (PEPELENBOS; LEVEN, 1996).

\[
 r_{O_2} = \frac{V_m O_2}{[O_2]+K_{m_{O_2}}(1+\frac{[CO_2]}{K_{m_{CO_2}}})} \quad (6)
\]

\[
 r_{O_2} = \frac{V_m O_2}{K_{m_{O_2}} + [O_2](1+\frac{[CO_2]}{K_{m_{CO_2}}})} \quad (7)
\]

\[
 r_{O_2} = \frac{V_m O_2}{(K_{m_{O_2}} + [O_2])(1+\frac{[CO_2]}{K_{m_{CO_2}}})} \quad (8)
\]

where $K_{m_{CO_2}}$ is the Michaelis-Menten constant for the competitive inhibition of $CO_2$ on $O_2$ consumption; $K_{m_{CO_2}}$ is the Michaelis-Menten constant for the uncompetitive inhibition of $CO_2$ on $O_2$ consumption; and $K_{m_{CO_2}}$ is the Michaelis-Menten constant for the non-competitive inhibition of $CO_2$ on $O_2$ consumption. These three constants are given in % of CO2. $V_m O_2$ is the maximum respiratory rate for $O_2$ consumption, given in mL.kg$^{-1}$/hour and $K_{m_{O_2}}$ is the Michaelis-Menten constant for $O_2$ consumption, expressed in % of $O_2$.

2.7 Respiratory quotient

The ratio of the volume of $CO_2$ produced to the volume of $O_2$ consumed in the post-harvest respiratory process, known as the Respiratory Quotient (RQ), was calculated from Equation 9, according to Fonseca, Oliveira e Brecht (2002).

\[
 RQ = \frac{0.727r_{CO_2}}{r_{O_2}} \quad (9)
\]

2.8 Statistical analysis

The experiments at 1, 5, and 10 °C for the samples with and without the film application were carried out in triplicate, and the analyses for each experiment were carried out three times. The results were evaluated by means of the analysis of variance (ANOVA) using the software Statistica® 6.0 (Statsoft Inc., USA). The factors that presented significant difference (p ≤ 0.05) were submitted to the Tukey test. The variables evaluated were the following: influences of the storage period, temperature, and film application.

3 Results and discussion

3.1 Determination of the gas composition

Figures 1-6 show the profiles of gas compositions of the minimally processed organic carrots, stored in Low Density Polyethylene (LDPE) bags, with and without the film, at 1, 5, and 10 °C. As expected, the results showed that in all cases the behavior followed the same tendency during the analyzed period with a reduction in the oxygen content and an increase in the carbon dioxide content.

For the carrot samples without the film, stored at 1 °C, the $O_2$ content decreased from 21% (1st day) to 4.32% (last day analyzed), and the $CO_2$ content increased from 0.04 to 8.12% (Figure 1). For the samples with the film, the $O_2$ content decreased from 21 to 5.59%, while the $CO_2$ content increased from 0.04 to 7.92% (Figure 2). Thus, the profiles obtained were very similar for the samples with and without the film, with very close final values for the $O_2$ and $CO_2$ contents. Equations 1 and 2 fitted well the experimental data concentration with coefficient

Figure 1. Behavior $O_2$ and $CO_2$ concentrations as a function of time for minimally processed organic carrots without the film and stored at 1 °C.

Figure 2. Behavior of the $O_2$ and $CO_2$ concentrations as a function of time for minimally processed organic carrots with the film and stored at 1 °C.
and 2 to O₂ and CO₂ concentrations, respectively) fitted well the experimental concentration data, both for the samples without the film (R² = 0.8985) and with the film (R² = 0.9352).

As shown in Figure 5, for the carrot samples without the film and stored at 10 °C, the O₂ concentration decreased from 21 to 2.43%, and the CO₂ concentration increased from 0.04 to 16.32%, while for the samples with the film, the O₂ content decreased from 21 to 2.25%, and the CO₂ content increased from 0.04 to 16.27%, as shown in Figure 6. Equations 1 and 2 fitted well the experimental data of the samples stored at, obtaining R² = 0.9327 for the samples without the film and R² = 0.9273 for those with the film.

As shown in Figure 3, for the carrot samples without the film and stored at 5 °C, the O₂ concentration decreased from 21 to 2.66% and the CO₂ concentration increased from 0.04 to 14.10%, while for the samples with the film, the O₂ content decreased from 21 to 2.59%, and the CO₂ content increased from 0.04 to 13.75%, as shown in Figure 4. The profiles obtained were similar for the samples with and without the film, and the differences between the final values for the concentrations of O₂ and CO₂ were not significant (p ≥ 0.05). The model (Equations 1 and 2) of determination R² of 0.9954 for the sample without film and R² of 0.9864 for the sample with film, according to Figures 1 and 2, respectively.

Figure 3. Behavior of the O₂ and CO₂ concentrations as a function of time for minimally processed organic carrots without the film and stored at 5 °C.

Figure 4. Behavior of the O₂ and CO₂ concentrations as a function of time for minimally processed organic carrots with the film and stored at 5 °C.

Figure 5. Behavior of the O₂ and CO₂ concentrations as a function of time for minimally processed organic carrots without the film and stored at 10 °C.

Figure 6. Behavior of the O₂ and CO₂ concentrations as a function of time for minimally processed organic carrots with the film and stored at 10 °C.
Through the comparison of the results obtained at the three temperatures, it can be observed that at 10 °C the value of the CO₂ concentration is higher and the value of the O₂ concentration is lower on the last day of storage at this temperature. Opposite behavior is observed at 1 °C. These results are expected due to the increase in the metabolic activity as a consequence of the increase in the storage temperature of the samples. There was a significant difference (p ≤ 0.05) between the final concentrations of O₂ and CO₂ for the samples at different temperatures. Regarding the film application treatment, no significant differences (p ≥ 0.05) were found among the samples with and without the film when stored at the same temperature, neither for the CO₂ nor the O₂ concentrations. The model that best fitted the experimental data, however, were those obtained at 1 °C.

Carlin et al. (1990) stated that low density polypropylene and polyethylene films generally resulted in considerable modifications of the atmosphere inside the package when used to store processed carrots reaching levels above 30% for CO₂ and below 5% for O₂. However, in the present study, although considerable modifications in the internal atmosphere were observed, such extremely high values for the CO₂ concentration were not obtained.

According to Francis, Thomas and O’Beirne (1999), fresh and ready-to-eat products are usually stored in semi-permeable packaging since they continue respiring and alter the gas composition inside the package. In this context, the ideal situation would be to attain O₂ levels varying from 2 to 5% and the CO₂ levels from 3 to 10%, which, combined with refrigeration, would reduce the respiratory rate and microbial growth decreasing the alterations in the product characteristics and consequently extending its shelf life. Similar values were attained in the present study for samples with and without the film when stored at 1 °C.

3.2 Respiration rate for minimally processed organic carrots

With regard to the concentration data obtained, the respiration rates were calculated for the samples stored under different conditions using Equations 3 and 4 and a computer program. Figure 7 shows the results obtained for the respiration rate, expressed in mL of CO₂.kg⁻¹/hour.

At 1 °C, the respiratory peaks of the samples with and without the film were 10.44 and 10.82 mL of CO₂.kg⁻¹/hour, respectively, after 72 hours of storage.

For the samples at 5 °C, the maximum respiration rate was 18.13 mL of CO₂.kg⁻¹/hour for samples without the film and 17.75 mL of CO₂.kg⁻¹/hour for those with the film after 50 hours, and for those stored at 10 °C, the maximum respiration rates were 21.50 and 21.75 mL of CO₂.kg⁻¹/hour for samples with and without the film, respectively, also after 50 hours.

As expected, the respiration rates increased with the temperature increase since higher temperatures accelerate the metabolism of fruits and vegetables. Significantly lower values (p ≤ 0.05) were found for the respiration rates of samples stored at 1 °C, and significantly higher (p ≤ 0.05) respiration rates were found for samples stored at 10 °C. According to Iqbal et al. (2008, 2009), temperature has been identified as the principal environmental factor affecting the respiration rate of minimally processed vegetables. The authors studied the effect of the temperature and the type of cuts on the respiration rate of carrots during storage and showed an increase from 3 to 5 times in the respiration rate for all cut-types of carrots with the increased temperature. Therefore, the effect of temperature is much more important for the respiration rate of minimally processed carrots.

The results for the respiration rate obtained in the present work at 1 and 5 ºC are similar to those obtained by Spagnol, Park and Sigrist (2006). These authors studied the behavior of sliced minimally processed carrots of the Nantes cultivar, which were put into glass jars and stored at 1, 5, and 11 ºC. They obtained respiration rates of 12.49, 19.58, and 36.04 mL of CO₂.kg⁻¹/hour, respectively. The authors observed a much higher respiration rate for the samples stored at 11 ºC than those found in the present study at 10 ºC.

Watada, Ko and Minott (1996) presented data for the respiration rate of minimally processed carrots as different from those found in the present study. They found values of 5.5 mL of CO₂.kg⁻¹/hour at 0 ºC, 15.3 mL of CO₂.kg⁻¹/hour at 5 ºC, and 28.6 mL of CO₂.kg⁻¹/hour at 10 ºC. However, they also stated that these values were obtained with carrots which were maintained under controlled atmospheric conditions by keeping the concentration of the atmospheric air.

The values obtained by Izumi et al. (1996) for minimally processed carrots stored at 0, 5 and 10 ºC also differed, in part, from the results obtained in the present study. These authors obtained values of 5.7, 12.1, and 22.1 mL of CO₂.kg⁻¹/hour at 0, 5 and 10 ºC, respectively, for samples which were also maintained under controlled atmospheric conditions by keeping the concentration of the atmospheric air.

Vargas et al. (2009) showed the effect of the chitosan edible film applied on minimally processed carrots with and without the use of a vacuum pulse during immersion. The respiration...
rate presented average values of 4.5 mL of CO₂.kg⁻¹/hour, results that are different from those found in the present study. The authors showed that in the beginning of the storage, the respiration rate of the coated samples was not significantly different from that found in non-coated samples, justifying that the high moisture content promotes a high level of plasticization and affects the permeability to CO₂ and O₂.

The different results presented in the literature for the respiration rate of carrots are probably due to differences in the raw material, as well as in the processing and storage conditions.

### 3.3 Kinetic parameters

Enzyme kinetics, based on Michaelis-Menten, was employed to describe gaseous exchanges between product and package for minimally processed carrots. Table 1 shows the kinetic parameters obtained using a regression analysis of the curves prepared from the experimental data for O₂ consumption and CO₂ production.

The maximum consumption rate (Vmₒ₂) was greater at 10 °C and differed statistically for the different models at 1 °C. At 5 and 10 °C the values for Vmₒ₂ of the uncompetitive model differed significantly from models competitive and non-competitive.

The values for Kmₒ₂ were close for all temperatures and treatments, with and without the film. On the other hand, the values for Kmuₒ₂ of the uncompetitive and non-competitive models were higher than those obtained for the competitive model.

Km values were significantly different (p ≤ 0.05) among the three models used. An analysis of the data, representing the three O₂ concentrations tested, showed that the data obtained by linear regression of the curve were closer to the O₂ concentration obtained experimentally in the case of the uncompetitive model, at the three temperatures. Hence, it is likely that the maximum respiratory rate was slightly influenced under high CO₂ concentrations, and this inhibitor did not react with the enzyme but with the enzyme-substrate complex.

Vegetable products present a series of resistances to gas diffusion between the cell membrane and the atmosphere around the product, but resistance to diffusion affects the result of the model applied. Peppelenbos and Leven (1996) presented two hypotheses that could occur during the respiratory process. The first hypothesis is that the available substrate (O₂) affects the values of Vmₒ₂, Kmₒ₂, and Kmuₒ₂. The second hypothesis is that a concentration gradient occurs in the tissues of the product causing differences in the respiratory rates of the product. In the present study, it was shown that the substrate presented similar behavior for the kinetic parameters of the uncompetitive and non-competitive models suggesting that sometimes the inhibitor did not react with the enzyme but did react with the enzyme-substrate complex, and sometimes it reacted with both of them. According to Peppelenbos & Leven (1996), two types of inhibition can occur simultaneously; although in the present study such behavior could not be confirmed since the influences of the CO₂ on O₂ consumption on the two models were not very clear.

Peppelenbos and Leven (1996) applied the same models to apples, broccoli, and tomatoes and observed discrepancies between the experimental data and the data obtained from the literature, without making any definite conclusions.

### 3.4 Respiratory quotient

Table 2 shows the results obtained for the respiratory quotients for the minimally processed organic carrots stored at 1, 5, and 10 °C.

The results showed that the respiratory quotient increased with the temperature increase. According to the literature, values for RQ below 1 indicate that the main metabolic substrate used in the respiration of the minimally processed organic carrots was formed from lipids (WATADA; KO; MINOTT, 1996).

Since the lipid content of carrots is very low, it was probably the substrate initially used for respiration followed by the consumption of other components such as soluble solids and organic acids.

### Table 1. Kinetic parameters obtained from the experimental data for O₂ consumption versus concentration.

| Treatment | Vmₒ₂ (mL.kg⁻¹/hora) | Kmₒ₂ (%) | Kmuₒ₂ (%) |
|-----------|----------------------|----------|-----------|
| NF 1 °C   | 8.38                 | 1.06     | 0.56      |
| WF 1 °C   | 9.73                 | 0.31     | 0.84      |
| NF 5 °C   | 19.03                | 0.61     | 0.10      |
| WF 5 °C   | 17.19                | 0.76     | 0.10      |
| NF 10 °C  | 18.05                | 0.70     | 0.17      |
| WF 10 °C  | 22.91                | 0.65     | 0.13      |

| Treatment | Vmₒ₂ (mL.kg⁻¹/hora) | Kmₒ₂ (%) | Kmuₒ₂ (%) |
|-----------|----------------------|----------|-----------|
| NF 1 °C   | 10.73                | 2.52     | 56.56     |
| WF 1 °C   | 7.51                 | 4.15     | 43.35     |
| NF 5 °C   | 25.40                | 2.04     | 15.12     |
| WF 5 °C   | 19.09                | 1.07     | 52.74     |
| NF 10 °C  | 18.24                | 1.26     | 60.40     |
| WF 10 °C  | 21.95                | 1.03     | 31.61     |

| Treatment | Vmₒ₂ (mL.kg⁻¹/hora) | Kmₒ₂ (%) | Kmuₒ₂ (%) |
|-----------|----------------------|----------|-----------|
| NF 1 °C   | 12.74                | 2.69     | 21.51     |
| WF 1 °C   | 9.99                 | 3.72     | 20.36     |
| NF 5 °C   | 19.06                | 0.61     | 63.07     |
| WF 5 °C   | 17.19                | 0.76     | 18.86     |
| NF 10 °C  | 19.67                | 1.10     | 33.09     |
| WF 10 °C  | 21.91                | 0.63     | 43.20     |

Vmₒ₂: maximum consumption rate and Km: Michaelis-Menten constant.

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}{$\text{Table 1. Kinetic parameters obtained from the experimental data for O}_2\text{ consumption versus concentration.}$

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Barbosa et al.
Respiration rate of minimally processed organic carrots

Watada, Ko and Minott (1996) reported RQ of 0.6, 0.8, and 0.6 for minimally processed carrots which were cut into pieces and stored at 0, 5, and 10 °C, respectively. Although these values were a bit different from those determined in the present study, they were also below 1 thus indicating the use of the same substrate for respiration.

Iqbal et al. (2009) showed that for the samples of carrots stored at 4, 8, 12, and 20 °C the RQ values went from 1.05 to 1.94 at the 15% CO₂ concentration and the 2% O₂ concentration, values which differed from those determined in the present study. RQ above 1.3 were considered to be indicative of anaerobiosis and off-flavor. According to the authors, the results indicated that the tolerance limits for shredded carrots were of 2% for O₂ and 16% for CO₂.

3.5 Activation energy

Figures 8 and 9 show the curves drawn to determine $E_a$ from the calculation of the angular coefficient of the linearized curve of the Arrhenius equation, for carrots with and without the film, respectively. The activation energy was 50.59 kJ.mol⁻¹ for the samples with the film and 51.88 kJ.mol⁻¹ for those without the film. The results obtained for the activation energy in the two treatments were close and there was no significant ($p \geq 0.05$) effect with the film application.

The values of activation energy obtained in the present study are comparable to the one obtained by Spagnol, Park and Sigrist (2006) for whole carrots, which was 54.60 kJ.mol⁻¹. However, for sliced minimally processed carrots, the same authors reported higher activation energy values, corresponding to 69.82 kJ.mol⁻¹.

Iqbal et al. (2008) presented activation energy values ranging from 36 to 62 kJ.mol⁻¹ for different shapes (types of cuts) of carrots stored at different temperatures. These results are similar to those obtained in this work for sliced carrots.

4 Conclusions

The gas concentration for the minimally processed organic carrots stored at different temperatures reached equilibrium on the fifth day. There was an increase in the CO₂ concentrations and a decrease in the O₂ concentrations, as expected. The model used, based on enzyme kinetics, fitted the experimental data well. The respiration rate was higher at the highest temperatures, 10 and 5 °C, with a maximum respiration rate after 50 hours. The application of a film failed to show significant effect on the respiration rate of the minimally processed carrots. The ratio between the O₂ concentration rate and the O₂ consumption rate described for the kinetic parameters showed similar behavior for uncompetitive and non-competitive inhibition suggesting that two types of inhibition probably occurred. The application of a film failed to significantly affect the activation energy for either treatment.

Acknowledgements

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