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Determination of the respiration rate parameters of cherry tomatoes and their joint confidence regions using closed systems

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ABSTRACT

Measuring the respiration rate of fresh produce is essential to design modified atmosphere packaging systems to extend their shelf life. A simple and common way of determining the rates of consumption of oxygen and production of carbon dioxide is to measure their variation in a closed system. In this work the respiration rate of cherry tomatoes was measured in a closed system at 5, 10, 15 and 20 °C. The results could be explained by a constant and temperature independent respiratory quotient (1.20 ± 0.01) and a Michaelis-Menten (M-M) model. The analysis of the joint confidence region showed that the two M-M parameters have a very high collinearity, where data of one single temperature set can provide misleading values. The results showed that parameter K_M could be considered independent of temperature but only has statistical significance at higher temperatures, while V_M varied with temperature with a stronger curvature than an Arrhenius model would suggest, with a parabolic functionality giving excellent results, albeit with no linear term, thus having the same number of parameters as an Arrhenius equation. This was due to a small increase in the rate between 5 and 10 °C. The data analysis clearly showed the importance of assessing the joint confidence region to ensure robust respiration rate model parameters.

1. Introduction

The demand for fresh, healthy and convenient products has been rising significantly, leading to dynamic growth in sales and new market opportunities in the fresh produce sector. However, fruits and vegetables are highly perishable commodities, which generally have short life span, resulting in significant losses at point of sale and in households. Shelf life is directly related to respiration rate, the phenomenon of the produce metabolism whereby the cells obtain their basic energy. Respiration involves a series of enzymatic-catalysed oxidation–reduction reactions where the organic substrates found in the cells are oxidized into carbon dioxide and water, with the production of energy (Fonseca et al., 2002). Respiration in harvested vegetable tissues leads to a loss of the stored substrate reserved in the commodity, which hastens senescence as these reserves are consumed. The slower the respiration rate, the slower the evolution of the cells towards senescence and the general deterioration of the product. Likewise, other deteriorative processes, including growth of foreign organisms (moulds, etc.) are also generally retarded. Consequently, accurate measurement of produce respiration rate (RR) and its modelling are fundamental tools to develop shelf life extension solutions.

Temperature is well known to be the most important factor influencing respiration rates and so temperature control provides a great benefit to extend the product postharvest life (Chau and Talasila, 1994). Biological reactions generally increase 2- to 3-fold for every 10 °C rise in temperature. Therefore, in order to assess the value of a carefully controlled (low) temperature distribution chain, and the loss incurred by abuse, it is very important to have accurate forms of predicting the influence of temperature on the respiration rate.

Gas composition should also influence respiration rate. It should be noted that aerobic respiration is the primary interest, as when oxygen concentration is too low the fermentative activity would be excessively detrimental. This equates to a series of oxidative processes and if at atmospheric conditions the rate is controlled by the quantity of endogenous enzyme, the respiration rate could be initially constant, but inevitably, as oxygen decreases the rate must decrease at some point. However, it is possible in some cases that the effect of low oxygen in decreasing the respiration rate is not

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detected until the oxygen concentration is already low enough in that commodity for fermentative processes to occur, in which case gas exchange is no longer due exclusively to aerobic respiration (Peppelenbos et al., 1996). Some produce may therefore show initially constant respiration rates - which may depend on temperature, as the effect of temperature on the different kinetic steps may be different and therefore some temperatures may show constant rates in the oxygen concentration range of interest, whereas others might not (Fonseca et al., 1999; Platenius, 1942). The opposite case occurs with produce where the respiration rate decreases readily as oxygen falls from the atmospheric 21%, such as tomatoes (e.g. Lee et al., 1996).

Hence, storage temperature and composition of the atmosphere in contact with the product are major influencing factors, which can be controlled to maximise shelf life. The influence of temperature and of gas concentrations has been studied extensively (e.g. Lencki, 2004; Rodriguez-Aguilera and Oliveira, 2009; Finnegan et al., 2013). Various mathematical models have been developed to assess the respiration rate of fresh fruits and vegetables as a function of oxygen (O_2) and carbon dioxide (CO_2) concentrations (Fonseca et al., 2002; Bhande et al., 2008; Kaur et al., 2011; Barrios et al., 2014; Kandasamy et al., 2016). Enzyme kinetic models have been applied for a wide variety of commodities (Charles et al., 2005; Jaime et al., 2001; Mahajan and Goswami, 2001; Torrieri et al., 2009). However, due to the specificity of the model parameters, the problem resides in identifying and quantifying the same for each fresh product (Bhande et al., 2008). Respiration rates vary for a same commodity between varieties, and significant changes may even occur from batch to batch of the same variety, and depend on time since harvest.

However, collecting a set of data at only one type of condition (one temperature) and fitting a model to it with a cursory view of the statistics to ensure a reasonable goodness of fit can lead to significant bias and errors (Stroberg and Schnell, 2016). The error space needs to be explored properly. Errors bias leads to situations where different sets of data seem to be represented by different models while a single model could actually explain different data sets. This is a particularly significant problem when the model parameters have strong collinearity.

The respiration rate of tomato fruit has been studied by several authors. Henig and Gilbert (1975) suggested a linear decrease of respiration rate with O_2 concentrations from 11.5 to 4%, and a constant rate for higher concentrations for tomatoes. Other best-fitted equations were polynomial functions that require many adjustable coefficients (Gong and Corey, 1994; Yang and Chinnan, 1988) or exponential functions (Cameron et al., 1989). Lee et al. (1991) obtained a good fit of the Michaelis-Menten equation to the data from Cameron et al. (1989). Michaelis-Menten uncompetitive inhibition equation has also been applied to model the O_2 and CO_2 concentration of tomato fruit by Lee et al. (1996) and Peppelenbos & Van't Leven (1996). More recently, Kandasamy et al. (2016) observed that the inhibition constant for CO_2 concentration tended to negative, and the model was modified as a function of O_2 concentration only. While the best models provide a good fit of the set of data analysed, the robustness of the fit has not been considered. In addition, it is necessary to assess also the influence of temperature, consider the error regions and statistical significance of the parameters, and establish a good practice for data analysis ensuring robustness.

The objectives of this study were to: i) analyse the effect of temperature and oxygen concentration on the respiration rate of cherry tomatoes in a closed system in order to obtain an integrated model of the effect of temperature and oxygen concentration under these conditions; ii) analyse the parameter collinearity and confidence regions to ensure that robust parameters are obtained, able to explain different conditions (temperatures, within the grange of interest to food packaging).

2. Materials and methods

2.1. Product

Tomato (*Solanum lycopersicum*) is one of the most popular and widely consumed fresh products in the world, due to its richness in health-related compounds (i.e. vitamins, carotenoids and phenolic compounds). As a climacteric fruit, tomato reaches its respiratory peak during the ripening process. Tomato fruit is a very perishable commodity, and when not handled properly it rapidly deteriorates. Small tomatoes, such as cherry varieties, sell at premium prices, so this is an example of products where an accurate understanding of respiration rates is the basis to minimise significant losses. Fresh cherry tomatoes were sourced from a local supermarket (Lidl, Cork, Ireland) on day of arrival from the distribution centre.

2.2. Experimental procedure

Respiration rate data were experimentally obtained with the closed system method (Fishman et al., 1996; Gong and Corey, 1994; Haggar et al., 1992; Ratti et al., 1996). It is a convenient way of obtaining data speedily over the relevant range of gas concentrations (Hong and Kim, 2001; Iqbal et al., 2009).

It should be noted that when determining the respiration rate from data obtained with a single type of non-flow through system, whether closed or open (i.e. a container with known permeability), there is an exact mathematical confounding between carbon dioxide inhibition parameters due to the unique relationship between carbon dioxide and oxygen concentrations. This makes it impossible to distinguish between uninhibited and competitively inhibited models, and between uncompetitive and non-competitive inhibited models. However, it is also difficult to distinguish with statistical certainty between uninhibited and uncompetitively inhibited because the carbon dioxide inhibition parameter of the latter is only distinguished mathematically in the coefficient of a second order term of a second order polynomial (in oxygen concentration), which is necessarily much smaller than the other terms, the more so the lower the oxygen concentration. Therefore, the error in determining the carbon dioxide inhibition parameter from fitting data from a single system is too excessive, specially if the high collinearity between parameters is taken into account. Even if it was possible to obtain statistical significance, it is not possible to assert with certainty what type of inhibition occurs.

This gives an added advantage to the closed system compared to open, which is that the confounding is expressed by a very simple mathematical relation (see equation (7)) and therefore it is easy to unconfound the two effects with data from another system (for instance, all that is actually needed is the equilibrium atmosphere achieved in a package of known permeability). If using a container of known permeability, although there is a unique relation between carbon dioxide and oxygen concentrations too, this is not expressed by an analytical expression because the mass balances do not have an analytical solution. Thus, it is more appropriate to determine the parameters of an uninhibited model with a closed system (which can be apparent parameters) with high accuracy and robustness, and then either validate that respiration is uninhibited by carbon dioxide and the closed system parameters are true, or then unconfound them. Furthermore, closed system data suffices to identify if the aerobic respiratory quotient is constant, which can greatly simplify the applicable models.