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Modelling the respiration rates of pomegranate fruit and arils (cv. 'Bhagwa')

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Abstract
To investigate the effect of temperature (5, 10 and 15 °C) and storage time of 1 to 5 days on the RR (RO_2 and RCO_2) of pomegranate cultivar (cv. 'Bhagwa') fresh arils. A study was conducted to determine the influences of storage temperature (5, 10 and 15 ◦C) and duration on RR of whole pomegranate fruit and arils of two pomegranate cultivars. This requires an adequate mathematical model for prediction of RR as a function of both time and temperature. This study investigated the effect of temperature (5, 10 and 15 °C) and storage time of 1 to 5 days on the RR (RO_2 and RCO_2) of pomegranate cultivar (cv. 'Bhagwa') fresh arils. RO_2 and RCO_2 were within the range of 2.54 to 8.36 ml/kg.h and 2.76 to 10.04 ml/kg.h, respectively for both cultivars. Reducing storage temperature of arils from 15 to 5˚C decreased RO_2 and RCO_2 by about 67 and 70%, respectively. Temperature had the greatest influence on RR and the interaction of time and temperature also significantly affected RO_2 and RCO_2.The dependence of RR on temperature and time was accurately described with a combination of an Arrhenius-type and power equation model for RO_2 and RCO_2 of fresh pomegranate arils and fruits.

Keywords: Pomegranate, modelling, respiration rate, respiration quotient

Introduction
In recent years, considerable effort has been made to ensure the quality and safety of minimally processed fruits until they are consumed. Controlling product temperature during refrigerated storage is of critical importance: an optimum temperature maintains the visual quality of fresh cut fruits and reduces their respiration rate, tissue softening and microbial spoilage (Cantwell and Suslow, 2002). A break in the cold chain can lead to a sharp rise in a fruit's respiration rate, affecting the stationary oxygen and carbon dioxide levels inside the package. Therefore, knowledge of the evolution of food products throughout the refrigerated storage process is essential and can be gained through experimental procedures and numerical study. Although experimental research is needed in order to identify real conditions and problems, it can be costly and time consuming. Numerical study is an alternative tool that can be used to reproduce refrigerated storage conditions in order to study the influence of different factors on food product preservation.

Pomegranate fruits is excellent source of sugars, vitamin c and minerals namely iron, potassium, calcium and bioactive compounds, mainly anthocyanins which exhibit strong chemo-preventive activities such as anti-mutagenicity, anti hypertension, antioxidative potential and reduction of liver injury (Lopez-rubira *et al.*, 2005). It has high antioxidant activity, which is attributed to its large amounts of phenolic compounds and sugar containing polyphenolic tannins and anthocyanins (Cam *et al*., 2009).

Modified atmosphere packaging (MAP) technology extends the shelf-life and maintains quality of fresh-cut produce by lowering the respiration rate and retarding the development of physiological disorders and proliferation of spoilage pathogenic microbes (Artés and others 2000). MAP is the dynamic process of altering gaseous composition within a package to extend storage life and optimize fresh produce quality. It relies on the interaction between the RR of the produce, and the transfer of gases through the packaging material, with no further control exerted over the initial gas composition (Caleb *et al*. 2012) [11]. However, a quantitative description of RR of fresh produce via mathematical modelling is essential for the design of MAP (Fonseca *et al*., 2002). When fruit respiration does not correlate to the permeability properties of packaging film, increase in the concentration of CO2 will build up beyond acceptable levels, leading to anaerobic respiration and ethanolic cumulation inside the fresh produce package. This results in the development of off-flavours, odours and decay (Caleb *et al*. 2012) [9]. Although, some studies have reported information on the RR of arils of selected pomegranate cultivars (Ersan *et al*., 2010), there is no predictive model on the RR of fresh pomegranate arils describing the effect of time and temperature. Therefore, the objectives of

this study were (i) to investigate the effect of temperature and time temperature on RR of whole pomegranate fruit and fresh arils cultivars of 'Bhagwa thereby provide valuable information on the design of MAP for pomegranate fruit.

Materials and Methods

Fully ripe pomegranate (*Punica granatum* L.) fruit cvs. 'Bhagwa' were procured from koyambed fruit market, Chennai to the Food science and Technology Laboratory, College of Food and Dairy Technology. The duration of transportation was about 2 hours. On arrival, fruit were immediately stored at 5 ℃ until the next day, when they were peeled manually in a clean cold room at 5℃ by carefully removing the arils to avoid damage. Samples of arils were weighed (≈ 150 g each sample), and each sample was placed inside a glass jar of about 500 ml, and equilibrated atthe desired storage temperature (5, 10 or 15 ℃) for at least 1 hour prior to experiment.

Experimental setup

Respiration rates measurement using flow through system is technically difficult; since it requires highly accurate analytical equipment (Cameron *et al*., 1989). A closed system is the convenient way of measuring the respiration of fresh produce (Hagger *et al.,* 1992). Hence the respiration rate data was experimentally generated for different temperatures using the closed system method. The respiration rate measurement of pomegranate was done as per the method adopted by Singh (2011). A closed system is used to measure the respiration rate of pomegranate arils. A known weight of mature pomegranate fruit and arils was filled into air tight glass container of known volume. The container was sealed carefully using vacuum grease. A single hole covered with silicon septum was made in container for measurement of gas concentrations. After packaging, container was kept at different temperature i.e. 5 ℃, 10 ℃, and 15 ℃ at 75% RH in an Environmental chamber and time was recorded. The O_2 and CO_2 concentrations in the headspace was measured and recorded after every 0.5 h directly by piercing syringe inside closed glass chamber through septum by a Headspace gasanalyser. To ensure a hermetic seal, Vaseline was incorporated into the gap between lid and jar for all the glass jars.

The gascom position within the glass jars was monitored over time with an O_2/CO_2 gas analyser with an accuracy of 0.5% (Checkmate 3, PBI Dan sensor). Gas samples were taken at an hourly interval from the jar head space through the rubber septum. An additional set of experiments was performed at 8◦C in order to validate the mathematical model. RO_2 and RCO_2 were determine d by fitting experimentally obtained data on yO_2 and yCO_2 with Eqs.(1) and (2), respectively,

$$Ro_2 = \frac{(yo_2^{ti} - yo_2^{tf}) \times V}{m \times (tf - ti)} \quad \text{--- (1)}$$

$$Rco_2 = \frac{(yco_2^{tf} - yco_2^{ti}) \times V}{m \times (tf - ti)} \quad \text{--- (2)}$$

Where

Ro_2 and Rco_2	-	**Respiration rate, in terms of O_2 and CO_2 evolved respectively, m^3/kg/h**
V	-	Free volume inside the package
yo_2^{ti} and yo_2^{tf}	-	volumetric concentration of O_2 at initial and final time respectively,%
yco_2^{ti} and yco_2^{tf}	-	volumetric concentration of CO_2 at initial and final time respectively,%
m	-	Mass of the stored product, kg
ti and tf	-	Initial and final time respectively, h

Additionally, in order to characterise the effect of time onrespiration rate of the arils, periodic gas samples were taken hourly over a period of 5 hours from the hermetic sealed jars, after which the glass jars were opened slightly to minimize rapid moisture loss and also to avoid built-up of sub-atmospheric gases. Following overnight storage time the jars were closed hermetically and gas samples were taken. This cycle was repeated over a 5 day storage period and no spoilage was observed over this period. The gas samples taken during 5 hour measurement period were used to calculate RO_2 and RCO_2 using Eqn. 1 and 2.

Statistical analyses

Response surface methodology (RSM) was used with two factors (time and temperature) each at three levels of temperatures 5, 10and 15 ◦C at 95% confidence interval to assess the effects of time and temperature, and the interaction between time and temperature on the RR data. One-way analysis of variance (ANOVA) at the 95% confidence interval was applied to evaluate the effect of time and temperature on RR and respiratory quotient (RQ). All experiments were carried out in triplicate and data were analyzed using Statistical software (SPSS, 10.0).

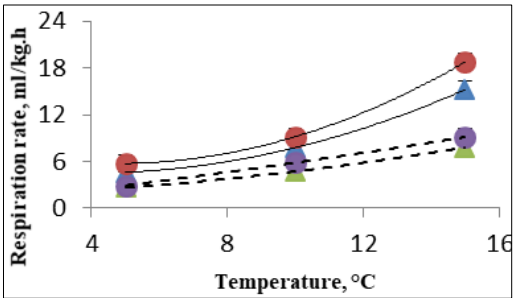
Results and Discussion

Rate of respiration

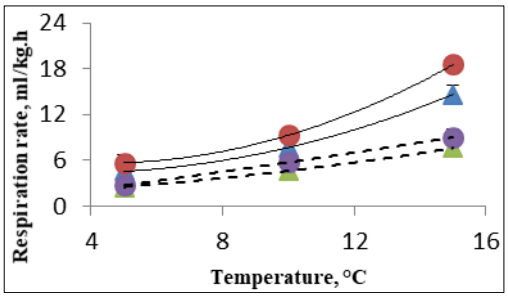
The O_2 concentration decreased and CO_2 increased with time inside the container at all the temperature. The respiration data corresponding to the different temperature indicated that as the temperature increased the respiration progressed at faster rate. The rate of respiration was higher at the start of the experiment and gradually declined as the storage period prolonged, before becoming almost constant.

Effect of temperature on the respiration rate

The influence of temperature on the O_2 consumption (RO_2) and CO_2 production (RCO_2) of both whole pomegranate fruit and fresh arils for the two cultivars was significant, as shown in Fig. 1. $RO_2$and RCO_2 were within the range of 4.58±0.34–15.21±1.16 mL/kg h and 5.72±0.28–18.7 ±1.62 mL/kg h, respectively, for whole fruit, and in the range of 2.52±0.20–8.36±0.60 mL/kg h and 2.72±0.12–10.12±0.26 mL/kg h, respectively, for fresh arils. Reducing temperature from 15 to 5 ◦C decreased RO_2 and RCO_2 by about 68 and 67% for whole fruit 67 and 70% for fresh arils; respectively. This significant reduction in fruit respiration rate at lower storage temperature corroborates the findings reported for other types of fresh produce (Nie *et al*., 2005). For instance, Terrier *et al*. (2010) reported a decrease in RR by 88and 84% for RO_2 and RCO_2, respectively, when the storage temperature of minimally processed broccoli was reduced from 20 to 3 ◦C. The slightly lower percentage reduction in respiration rates of both whole fruit and fresh arils found in the present study compared toother types of fresh produce such as broccoli may be attributed to the non-climacteric nature of pomegranate fruit and differences in temperature regimes tested.



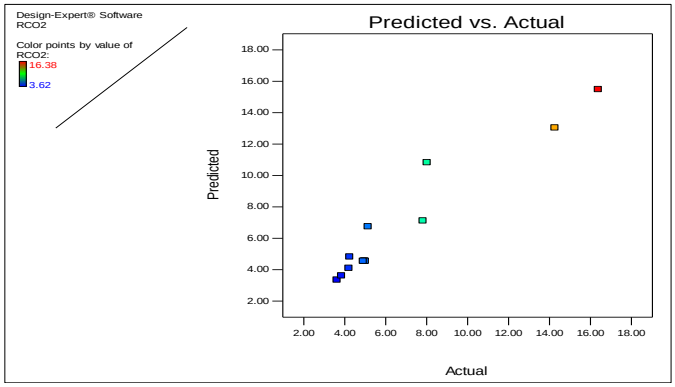
(a)



(b)

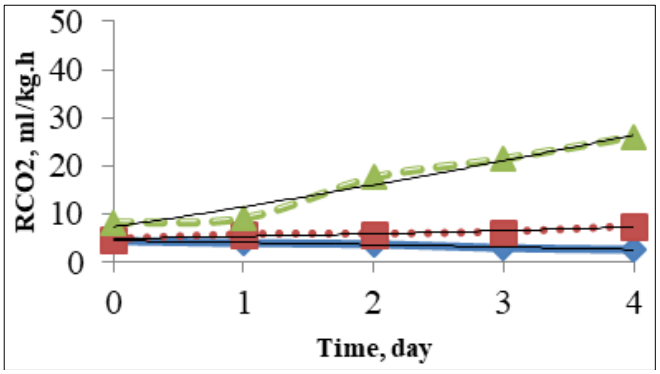
Fig 1: (a, b) Effect of storage temperature on respiration rate of pomegranate fruit and arils of two Indian cultivars: (a) Bhagwa. Continuous and dotted lines represent the respiration rate of pomegranate whole fruit and arils, respectively. Circle and triangle represents the O_2 consumption rate and CO_2 production rate, respectively

There was no significant difference in RR of the two cultivars ('Bhagwa' at all experimental temperatures ($p > 0.05$) studied. However, irrespective of cultivar, the RR of whole fruit was significantly higher than those of fresh arils, as shown in Fig. 1.

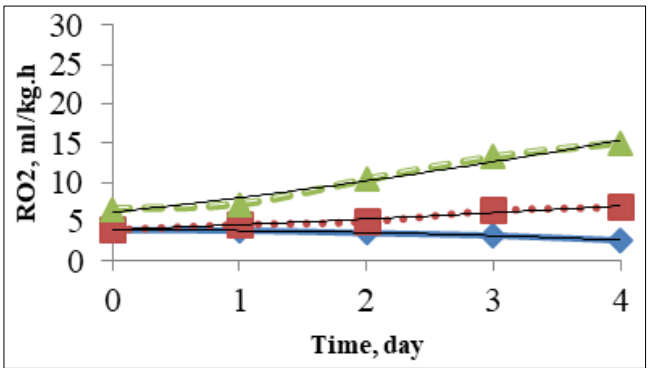


(c). Relationship between experimental and predict respiration rate values of pomegranate whole fruits and arils

The RR of whole fruit was two to three folds higher, in comparison to those of the fresh arils across all experimental temperatures. Contrary to other fresh-cut fruit in which membranes and cells are damaged, resulting in increased tissue metabolic processes such as enzymatic browning, increased rate of water loss andrespiration rates due to the increased surface area in contact with atmospheric oxygen (Zagory, 1998; Iqbal *et al*., 2009; Torrieri *et al*.,2009), pomegranate arils have a protective membrane which prevents direct tissue or cellular interaction of its succulent portion with atmospheric conditions after the husk is carefully removed.



(a)



(b)

Fig 2: (a, b) Changes in respiration rate of arils with time at different temperatures: (a) and (b): RCO2 and RO2 of arils ("Bhagwa')

The observed effect of temperature on RR of arils as shown in Fig. 2, is similar to those reported by Gil *et al*. (1996) [24], who reported respiration rates of 1.94, 1.30, and 0.53 mL CO_2/kg h for pomegranate arils (cv. 'Mollar') stored at 8, 4, and 1◦C, respectively. However, the difference between the responses of the two cultivars in this study at 15 ◦C highlights the possible influence of physiological differences between cultivar responses to storage condition (Al-Mughrabi *et al*., 1995). Furthermore, the spike observed in RR at 15 ˚C (Fig. 2), suggests the possible influence of ethylene. Devlieghere *et al*. (2003) found a linear relationship when RR at a specific temperature was plotted against the ethylene production rate for different O_2 and CO_2 concentrations for climacteric and non-climacteric fruit.

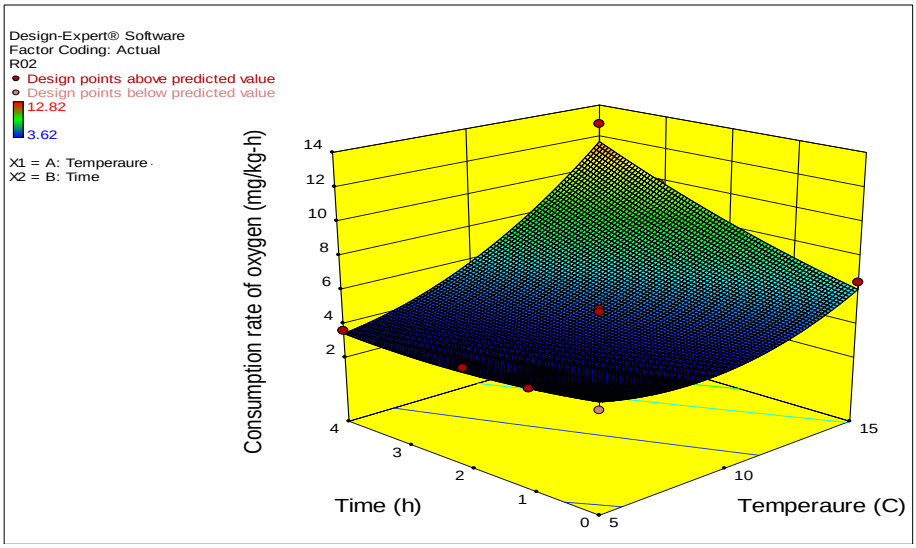


Fig 3: A fitted surface plot showing the effect of temperature and time on RO2 (mL/kg h) for pomegranate arils ('Bhagwa')

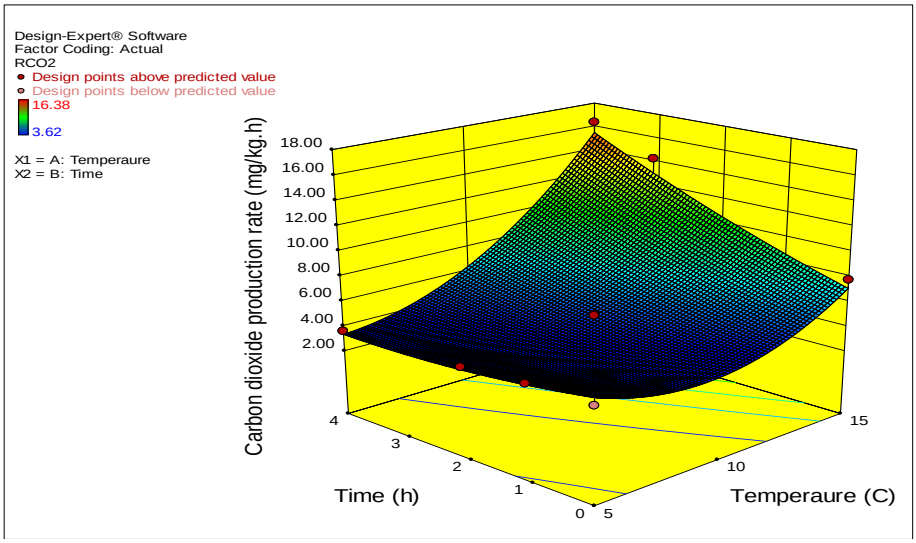


Fig 4: A fitted surface plot showing the effect of temperature and time on RCO_2 (mL/kg h) for pomegranate arils ('Bhagwa')

In terms of relevance to MAP design, the pattern of RR of pomegranate arils in relation to storage temperature and times shown in Fig. 3. Can serve as guiding tool towards other MAP parameters such as package volume to packed arils volume, typeof packaging material, barrier properties and temperature sensitivity of packaging material (Fonseca *et al*., 2002). For instance at15 ◦C, if the permeability property of a packaging film does not correlate with the respiration rate observed. This can lead to excessive accumulation of CO_2, resulting in cell membrane damage and physiological injuries to the product (Caleb *et al*., 2013) [12].

Furthermore, at 5 ◦C storage temperature, the respiration rate was at its lowest and appeared to be relatively constant over time. Thus, if an inappropriate ratio of package volume to packed arils volume or packaging material is used the gas equilibrium level at steady-state required inside the package for passive-MAP will take a longer time to establish. MAP has been reported to strongly reduce water loss and chilling injuries without incidence of decay in pomegranate fruit (Artés *et al*., 2000), and to maintain arils pigments (anthocyanin's) better in comparison to samples packed without MAP (Gil *et al*., 1996) [24].

RQ of pomegranate arils ranged between 1.08 ± 0.06 and 1.64 ±0.08 for cv. 'Bhagwa'. The RQ value of arils estimated by linear regression of RCO_2 vs. RO_2 was 0.98 ± 1.14 (R^2 adj = 98%) at 95% significant level. These values compares favorably with normal RQ limits (0.7 to 1.3) for aerobic respiration (Kader *et al*., 1989), with the exception of pomegranate arils (cv. 'Bhagwa') at 15 ℃. However, experimental evidence suggests that the significant ($p < 0.05$) influence of time and temperature on the observed high RQ for pomegranate arils (cv. 'Bhagwa') occurred under aerobic conditions, similar to the findings reported by Wang *et al*. (2009) for guava fruit.

Based on the experiments, it was concluded that the steady-state respiration rates were found to be decreasing with storage time. Temperature had the most significant impact on the RR of arils of both pomegranate cultivar (cv. 'Bhagwa') and the RR were 3-4 folds significantly higher with increased temperature from 5 to 15 ℃. The influence temperature and time also had a significant influence on the RR of fresh arils. This highlights the importance of maintaining optimal cold-storage condition for fresh produce along the supply chain. The applicability of two different models for the prediction of respiration rate was verified for pomegranate fruit using the experimental data generated at different temperatures. The respiration rates obtained through the models exhibited a trend, which was in close agreement with the experimentally determined respiration rates. The RQ was dependent on both temperature and time as the RQ value increased with rising

temperature from 5 to 15 ℃ towards the end of the storage time. An Arrhenius type equation accurately predicted the effect of temperature on RR of fresh pomegranate arils. The power function equation combined with Arrhenius-type equation adequately predicted the influence of time and temperature on RR of fresh pomegranate arils for both cultivars. These models would be useful towards the design of appropriate modified atmosphere package for freshly processed pomegranate arils.

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