

**Original Research Article**

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**Effect of Time and Temperature on Respiration Rate of  
Pomegranate arils (cv. 'Bhagwa')**

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The effect of time and temperature on the respiration rate (RR) of fresh-cut produce is critical towards the development of a suitable modified atmosphere packaging (MAP) system. 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 ( $\text{RO}_2$  and  $\text{RCO}_2$ ) of two pomegranate cultivars (cv. 'Bhagwa' and 'Ganesh') fresh arils.  $\text{RO}_2$  and  $\text{RCO}_2$  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.  $\text{RO}_2$  and  $\text{RCO}_2$  were 3-4 folds significantly higher with increased temperature from 5 to 15°C, reducing storage temperature of arils from 15 to 5°C decreased  $\text{RO}_2$  and  $\text{RCO}_2$  by about 67 and 70%, respectively. Temperature had the greatest influence on RR and the interaction of time and temperature also significantly affected  $\text{RO}_2$  and  $\text{RCO}_2$ . The dependence of RR on temperature and time was accurately described with a combination of an Arrhenius-type and power equation model for  $\text{RO}_2$  and  $\text{RCO}_2$  of fresh pomegranate arils and fruits.

**Introduction**

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 *et al.*, 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). 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  $\text{CO}_2$  will build up beyond acceptable levels, leading to anaerobic respiration and ethanol accumulation inside the fresh produce package. This results in the development of off-flavours, odours and decay (Caleb *et al.*, 2012). 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' and 'Ganesh'), thereby provide valuable information on the design of MAP for pomegranate fruit.

### Produce and sample preparation

#### Produce and sample preparation

Fully ripe pomegranate (*Punica granatum* L.) fruit cvs. 'Bhagwa' and 'Ganesh' 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°C until the next day, when they were peeled manually in a clean cold room at 5°C by carefully removing the arils to avoid damage. Samples of arils were weighed ( $\approx 150$  g each sample), and each sample was placed inside a glass jar of about 500 ml, and equilibrated at the desired storage temperature (5, 10 or 15°C) 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

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

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

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°C, 10°C, and 15°C at 75 % RH in an Environmental chamber (Remi Laboratory Instruments, India; Model: CHM-10) and time was recorded (Fig. 1). The O<sub>2</sub> and CO<sub>2</sub> 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 gas analyser. To ensure a hermetic seal, Vaseline was incorporated into the gap between lid and jar for all the glass jars.

The gas composition within the glass jars was monitored over time with an O<sub>2</sub>/CO<sub>2</sub> 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<sub>2</sub> and RCO<sub>2</sub> were determined by fitting experimentally obtained data on yO<sub>2</sub> and yCO<sub>2</sub> with Eqs. (1) and (2), respectively,

where,

$R_{O_2}$ and $R_{CO_2}$	- Respiration rate, in terms of $O_2$ and $CO_2$ evolved respectively, $m^3/kg/h$
$V$	- Free volume inside the package
$y_{O_2}^{ti}$ and $y_{O_2}^{tf}$	- volumetric concentration of $O_2$ at initial and final time respectively, %
$y_{CO_2}^{ti}$ and $y_{CO_2}^{tf}$	- 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

Where  $y_{O_2}$  and  $y_{CO_2}$  are, respectively,  $O_2$  concentration (%) at the initial time  $ti$  (hours, h) (time, zero) and at time  $t$  (h) and  $y_{CO_2}$  and  $y_{CO_2}$  are, respectively,  $CO_2$  concentration (%) at the initial time  $ti(h)$  (or time zero) and at time  $t$  (h).  $RO_2$  and  $RCO_2$  are RR in  $mL/kg$  hand  $W$  is the total weight of the product (kg).  $Vf$  is the free volume inside the glass jar (mL), which is the total volume of the glass jar minus the volume occupied by the sample.

Additionally, in order to characterise the effect of time on respiration 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, 10 and 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 analysed 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 a 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 figure 1.  $RO_2$  and  $RCO_2$  were within the range of  $4.58 \pm 0.34 - 15.21 \pm 1.16$   $mL/kg\ h$  and  $5.72 \pm 0.28 - 18.74 \pm 1.62$   $mL/kg\ h$ , respectively, for whole fruit, and in the range of  $2.52 \pm 0.20 - 8.36 \pm 0.60$   $mL/kg\ h$  and  $2.72 \pm 0.12 - 10.12 \pm 0.26$   $mL/kg\ h$ , 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 and, 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; Tano *et al.*, 2007). For instance, Torrieri *et al.*, (2010) reported a decrease in RR by 88 and 84% for  $\text{RO}_2$  and  $\text{RCO}_2$ , 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 to other types of fresh produce such as broccoli may be attributed to the non-climacteric nature of pomegranate fruit and differences in temperature regimes tested.

There was no significant difference in RR of the two cultivars ('Bhagwa' and 'Ganesh') 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 figure 1.

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 and respiration 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.

#### **Effect of time and temperature on the respiration rate**

Changes in respiration rate for *Pomegranate arils* during storage at different temperatures (5, 10 and 15°C) are summarized in figure 2.

The influence of both time and the interaction between temperature and time on the  $\text{RO}_2$  and  $\text{RCO}_2$  of fresh arils were significant ( $p < 0.05$ ). These effects were adequately described by the fitted surface plot, which are summarized in figures 3 and 4, respectively.

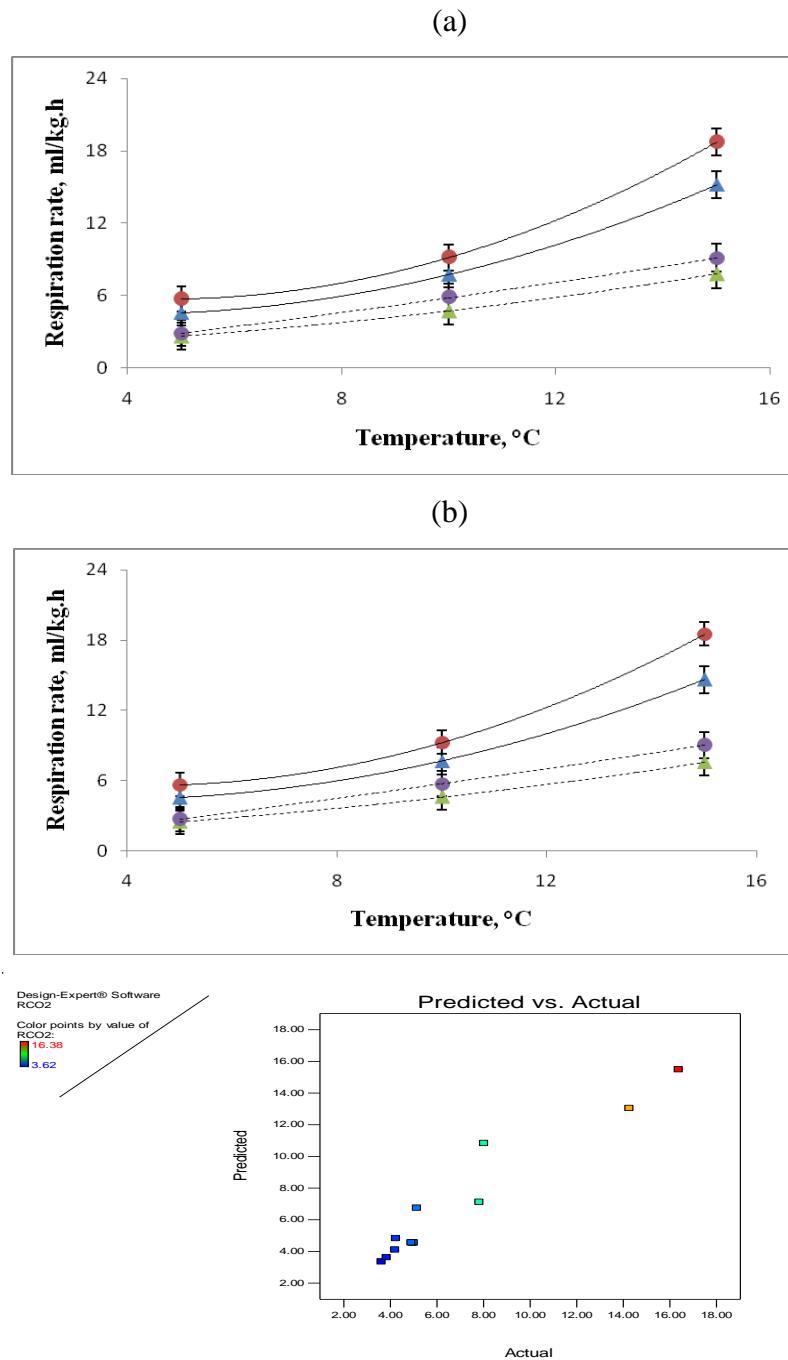
The observed effect of temperature on RR of arils as shown in figure 2, is similar to those reported by Gil *et al.* (1996), who reported respiration rates of 1.94, 1.30, and 0.53 mL  $\text{CO}_2/\text{kg h}$  for *Pomegranate arils* (cv. 'Mollar') stored at 8, 4, and 1°C, respectively. However, the 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  $\text{O}_2$  and  $\text{CO}_2$  concentrations for climacteric and non-climacteric fruit.

In terms of relevance to MAP design, the pattern of RR of *Pomegranate arils* in relation to storage temperature and time as shown in figure 3. Can serve as guiding tool towards other MAP parameters such as package volume to packed arils volume, type of packaging material, barrier properties and temperature sensitivity of packaging material (Fonseca *et al.*, 2002).

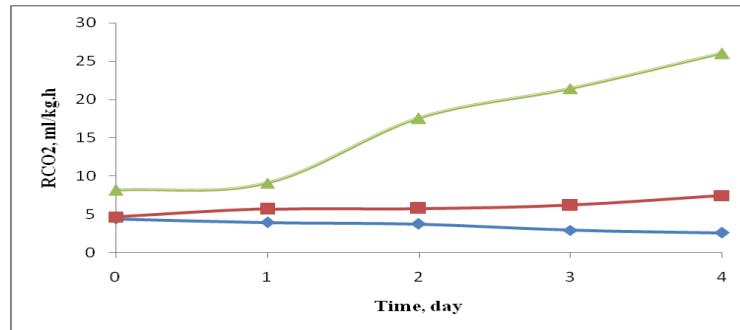
For instance at 15°C, if the permeability property of a packaging film does not correlate with the respiration rate observed. This can lead to excessive accumulation of  $\text{CO}_2$ , resulting in cell membrane damage and physiological injuries to the product (Caleb *et al.*, 2013).

**Fig.1** Effect of storage temperature on respiration rate of pomegranate fruit and arils of two Indian cultivars: (a) Bhagwa and (b) Ganesh. Continuous and dotted lines represent the respiration rate of pomegranate whole fruit and arils, respectively. Circle and triangle represents the O<sub>2</sub> consumption rate and CO<sub>2</sub> production rate, respectively. (c). Relationship between experimental and predict respiration rate values of pomegranate whole fruits and arils

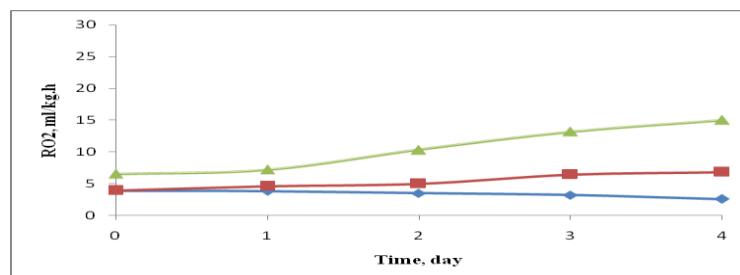


**Fig.2** Changes in respiration rate of arils with time at different temperatures: (a) and (b): RCO<sub>2</sub> and RO<sub>2</sub> of arils ("Bhagwa"); (c) and (d): RCO<sub>2</sub> and RO<sub>2</sub> of arils (cv. 'Ganesh') with (♦) representing 5 °C, (□) for 10 °C and (Δ) for 15 °C

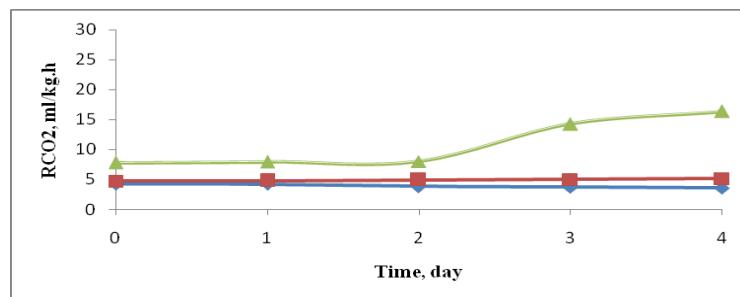
(a)



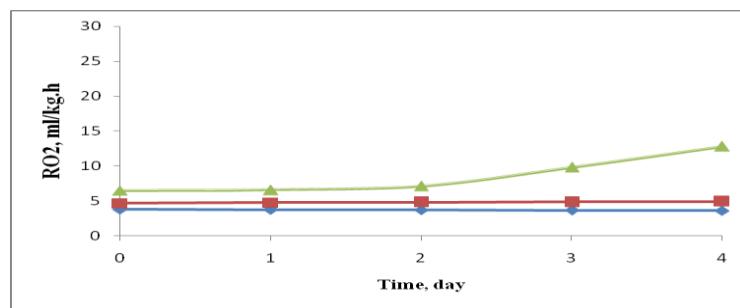
(b)



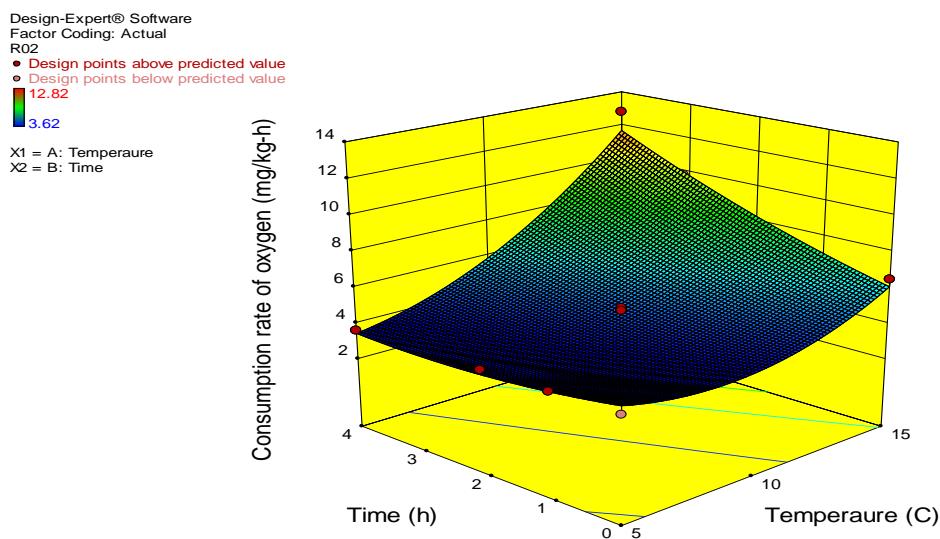
(C)



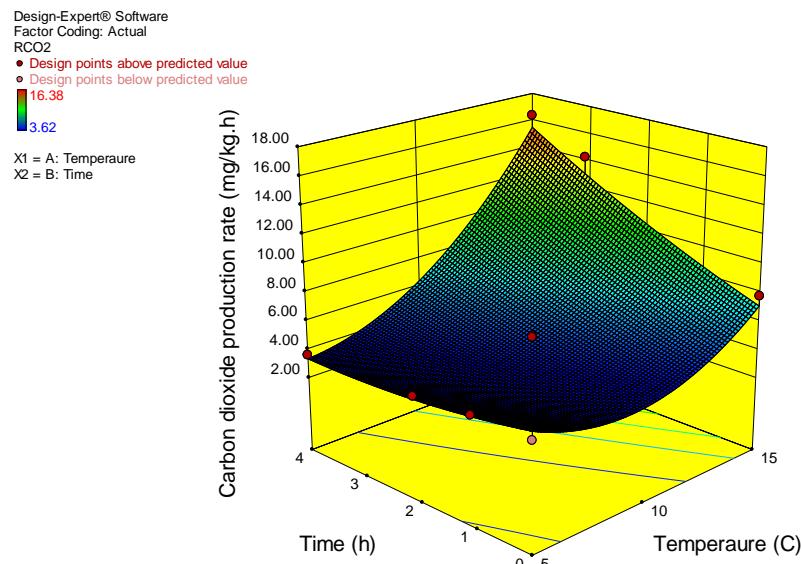
(d)



**Fig.3** A fitted surface plot showing the effect of temperature and time on RO<sub>2</sub> (mL/kg h) for *Pomegranate arils ('Bhagwa')*



**Fig.4** A fitted surface plot showing the effect of temperature and time on RCO<sub>2</sub> (mL/kg h) for *Pomegranate arils ('Bhagwa')*



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 (anthocyanins) better in comparison to samples packed without MAP (Gil *et al.*, 1996).

RQ of *Pomegranate arils* ranged between  $1.08 \pm 0.06$  and  $1.64 \pm 0.08$  for cv. ‘Bhagwa’ and  $1.26 \pm 0.06$  to  $1.36 \pm 0.08$  for cv. ‘Ganesh’. The RQ value of arils estimated by linear regression of  $\text{RCO}_2$  vs.  $\text{RO}_2$  was  $0.98 \pm 1.14$  ( $R^2 \text{ adj} = 98\%$ ) at 95 % significant level. These values compares favourably 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  $15^\circ\text{C}$ . 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.

In conclusion, 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 cultivars (cv. ‘Bhagwa’ and ‘Ganesh’) and the RR were 3-4 folds significantly higher with increased temperature from 5 to  $15^\circ\text{C}$ . The influence of time, and the interaction between 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 RQ was dependent on both temperature and time as the RQ value increased with rising temperature from 5 to  $15^\circ\text{C}$  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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