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Prediction and validation of transpiration model for chickpea sprouts (*Cicer arietinum* L.) in closed system under modified atmosphere

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Abstract
Transpiration is an important physiological process that affects the quality attributes such as package weight, color, appearance and texture of chickpea sprouts, A loss in weight of only 5% may cause fresh produce to lose freshness and appear wilted and it is an important parameter to be considered while designing appropriate packaging system. To measure the transpiration rate an experimental setup was developed to monitor the weight loss of chickpea sprouts at different atmospheric temperatures (5, 10 and 15 °C) and relative humidity conditions. Weight loss of fresh packed chickpea sprouts was measured and transpiration rates were calculated for each treatment. Transpiration rate of sprouts varied from 1.17-2.15 under all the combinations of temperature and humidity tested. A predictive model for transpiration rate behavior of chickpea sprouts was developed to understand the moisture loss pattern at MA storage temperature (10 °C) and varied relative humidity. The developed model was tested and validated at 10 °C and observed in good agreement between actual and simulated transpiration rate values.

Keywords: Diffusion, moisture loss, packaging, storage, transpiration rate, model

Introduction

The moisture accumulation under modified atmospheric packaging (MAP) depends upon transpiration rate (Czarnowski and Starwecki, 1991) [4]. It is influenced by several factors such as surface area, respiration rate, temperature, and humidity and air movement. Other than these factors, crop biological variables like physical properties of the skin, air film resistance, respiration heat generation, temperature distribution inside the produce etc (Baryeh, 2001) [1]. Have also been analyzed as affecting the transpiration rate (Sastry and Buffington, 1983) [14]. These biological variables are complex and difficult to measure, but the transpiration could be easily accessed by the measuring of weight loss of produce and the difference between partial pressure of water vapor at the produce surface and the environment (Lenz, 1966) [9].

During post-harvest handling and storage, chickpea sprouts lose moisture by the transpiration process (Chau *et al*., 1987) [3]. Deterioration, such as shriveling or impaired flavor, may result if moisture loss is high (Gaffney *et al.*, 1985) [6]. In order to minimize losses due to transpiration, and thereby increase both market quality and shelf life, sprouts must be stored in a controlled temperature and humidity environment such as modified atmospheric packaging MAP (Singh *et al.*, 2011) [17]. In addition to proper storage conditions, moisture-proof films can be used during commodity packaging to significantly reduce transpiration and extend storage life (Ben-Yehoshua. 1987) [2]. Metabolic activity in fresh sprouts continues soon after harvest. The energy required to sustain this activity comes from the respiration process (Mannapperuma *et al.*, 1991) [11]. Respiration involves the oxidation of sugars to produce carbon dioxide, water and heat. The storage life of sprouts is influenced by its respiratory activity (Das *et al*., 2008) [5]. By storing sprouts under controlled atmospheric conditions the respiration is reduced and senescence is delayed, thus extending storage life (Halachmy and Mannheim., 1991) [7]. Proper control of the oxygen and carbon dioxide concentrations surrounding a commodity is also effective in reducing the rate of respiration. Properly designed and operated storage system (MAP) facilities will extend the storage life of commodities by providing a suitable atmospheric condition, which reduces moisture loss and decreases respiratory activity. A thorough knowledge of the transpiration and respiration processes will allow both the designer and producer to achieve optimum storage conditions (Toshitaka and Daisuke., 2004) [19].

Chickpea sprouts are packed in perforated PP films. Sprouts dehydration is prevented due to higher humidity inside the packages but this humidity is too high to deter the microbial growth. High humidity, created due to the high transpiration rate of sprouts, causes condensation inside the package as clearly seen underneath the film. Predicting water loss rate, therefore, is helpful for estimating the shelf life of fresh chickpea sprouts, designing its storage and packaging conditions. The objectives of this study were to measure the transpiration rate of sprout samples and develop a simple mathematical model for predicting transpiration rate at modified atmospheric (MA) storage at 10 ºC temperatures at different RH levels and to validate the developed model.

Materials and Methods

Plant Material

Chickpea seeds (*Cicer arietinum* L.) var. PBG-5 was collected in local farm in autumn at full maturity. The chickpea seeds were brought to the laboratory, carefully inspected, and the seeds were bruised to remove any soil residues. Samples were uniformly treated with potassium hypochlorite at the rate of 20,000 ppm for 15 min for sterilization. The sterilized samples were soaked in clean water for 12 h overnight at room temperature. In the morning, the water is drained and the soaked seeds were rinsed with clean fresh water free from any contamination. The washed seeds (soaked) were then shifted to clean sterile muslin cloth and placed in dark at ambient temperature for sprouting. After 36-48 h the sprouts were harvested for experimentation.

Experimental Setup

The experimental setup consisted of one test glass container located within a large insulated, controlled atmospheric chamber with internal air temperature controlled at test levels by a refrigeration unit. Humidity within the test containers was independently controlled by using saturated salts solutions of sodium chloride, potassium chloride and potassium nitrate giving 75 % RH (Patel *et al.* 1988)[13]. Salt solution was placed at the bottom of the container. This setup was found to maintain a constant relative humidity (RH) throughout the experimental run. An electronic balance was located at the top of the container to record the change in weight of the packed sprouts during the storage period (Fig. 1). The weight of each sample could be measured without opening the test containers. Temperature and RH inside the container was monitored continuously using a data logger (Humidiprobe, Pico Tech, UK).

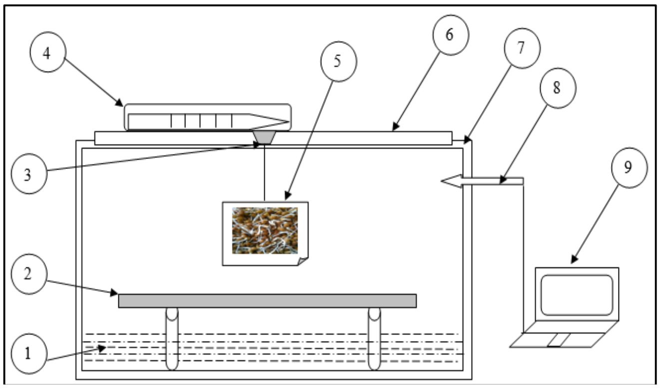

Fig 1: Schematic diagram of the experimental setup for measuring weight loss of chickpea sprouts under closed storage system. (1: Saturated salt solution, 2: Platform, 3: Cork, 4: Electronic balance, 5: Chickpea sprout sample, 6: Lid, 7: Glass container (closed system), 8: Temperature probe, 9: PC).

Transpiration measurement of sprout

The transpiration rate of chickpea sprouts was determined as per the weight loss technique adopted by (Leonardi *et al.*, 2000) [10]. The samples (150±1g) were equilibrated at experimental conditions for two hours before the start of the experiment and hanged independently inside the set-up using cotton string. Caps were placed on the container and sealed with grease. The sealed container was placed inside walk-in type cold room that was maintained within ±1 ºC of the set temperature (Table 1)

Table 1: Temperature, relative humidity and WVP regimes levels for development and validation of predictive model.

Factors	Levels		
Temperature (ºC)	5±1	10±1	15±1
Relative humidity (%)	75±2	85±2	95±2
VP regimes	$WVPD_{seed\text{-}MAair}$	and	$WVPD_{MAair}$

The change in weight of the sample at regular intervals was recorded by electronic balance (Precisa 205A SCS, Dietikon, Switzerland) using the string arrangement. The entire set of experiments for particular temperature was replicated thrice. The fresh and graded chickpea sprouts were taken to keep the size and quality during the experiment. Sprouts transpiration rate, TR (ml/kg-h-kPa) was then calculated from the changes in sprouts weight over time and expressed by dividing the weight loss with respect to its area (Eq.1). The mathematical expression for Transpiration rate (TR) is as bellow:

$$TR = -\frac{1}{A_{Sp}} \times \frac{dm}{dt} \qquad (1)$$

With,

$$A_{Sp} = A_{Seed} + A_{Hypo\cot yl} \qquad (2)$$

Where,

$A_{seed} = \pi\ (Dg)^2$; and $Dg = (L\ W^2)^{1/3}$ calculated from the relationship given by Baryeh (2001). Where, S is the surface area of the sprouts, Dg is geometric mean diameter, linear dimensions of fruits as length (L) and width (W) were measured by using a digital caliper gauge with a sensitivity of 0.01 mm. and;

$A_{hypocotyl} = \pi r^2 + \pi rs$; calculated by assuming hypocotyls surface area identical to standard cone surface area. Where, r is radius of hypocotyls and s is side length of hypocotyls.

To reduce the variability in collected data, transpiration rate was measured on 25 sprouts weighing between 2g to 6g and the values were averaged.

Experiments were performed according to factorial design at three levels (Table 1) with 150±1g of sprout samples in each chamber. An additional set of experiments with all the combinations of RH was performed at 10 ºC in order to validate the adopted model.

Statically Analysis

The entire set of experiments was replicated twice. Analysis of variance (ANOVA), with the significance level of $P \leq 0.05$ was carried out using the SPSS 14.0 software for windows. Model development to determine the equations relating the transpiration rate to temperature and humidity was developed and analyzed using GraphPad PRISM® Version 5.00.288 software (GraphPad Software, Inc.).

Surface area of sprout

Transpiration is generally surface dependent phenomenon and influences the rate of transpiration of produce; hence the surface area of chickpea sprouts was determined. For experimentation, twenty five sprouts having different sizes (2g-6g) were selected. The surface area was calculated as per the empirical relationship given by Eq (2) above. The surface area is predicted by a relationship as; Y=0.0036x + 7.3506 with R^2=0.960 (3)

Transpiration Rate (TR_{sprout}) of Chickpea Sprouts

For each of the experimental conditions tested, TR was calculated by fitting the experimental data to Eq. (1). The observation for the determination of transpiration rate of chickpea sprouts is given in Table 1. It was observed that the transpiration rate of chickpea sprouts was 1.17-1.45 at 5 ºC; 1.38-1.47 at 10 ºC and 1.88-2.15 at 15 ºC temperatures and 75, 85 and 95% RH conditions respectively. (Table 2). Similar findings of TR value were reported by Kang and Lee. (1998)[8] for cut onion (transpiration rate: 0.448 g/ (kg h-kPa) at 10 ºC, 82% RH in normal air). Tomato fruits TR: 0.1 mg/ cm^2 –h at the same vapor pressure deficit of 0.45 k Pa as reported by Leonardi *et al*. (2000)[10]. This is because chickpea sprouts do not have a protective skin that leads to higher moisture loss due to exposure of outer skin, micro-pores on skin widened and cracks develop during sprouting. The effect of temperature and humidity on TR of chickpea sprouts was found to be significant at 95% significant level. This analysis showed that, in the range of conditions studied, both temperature and humidity were the influential variables. The effect of temperature and humidity on TR was more pronounced. The interactive effects between temperatures were also significant, with the effect of temperature on TR, increasing with increasing temperature. The results stress the importance of maintaining proper in-pack humidity levels as well as storage temperature in order to extend the shelf life of chickpea sprouts. In-package temperature of 10 ºC and 75% relative humidity was found to be optimum for fresh chickpea sprouts (Singh *et al.*, 2011) [16, 18].

Table 2: Experimental transpiration rate of chickpea sprouts at different temperature and RH combinations during weight loss experiment.

Temperature, ºC	RH, %	R^2	SE	*Transpiration rate, (mg/kg.h-KPa)
5±1	75±2	0.79	0.003	1.45
	85±2	0.83	0.006	1.34
	95±2	0.89	0.018	1.17
10±1	75±2	0.93	0.028	1.47
	85±2	0.89	0.007	1.38
	95±2	0.98	0.046	1.75
15±1	75±2	0.78	0.005	2.15
	85±2	0.85	0.015	1.88
	95±2	0.89	0.016	1.93

* Statistically significant (Temp, RH and Temp XRH) at $P \leq 0.05$ and average of means of three replications.

Model development

The model developed for chickpea sprouts (Fig 2) were based on Ficks' law. The moisture diffusion through the chickpea hull (C_h) and hypocotyls (C_{hypo}) is implicit to be driven by their respective conductance. Since, the two conductances' are in parallel. So, the total boundary conductance along the diffusion path (C_b) is described by the relationship as below:

$$C_b = C_h + C_s \qquad (4)$$

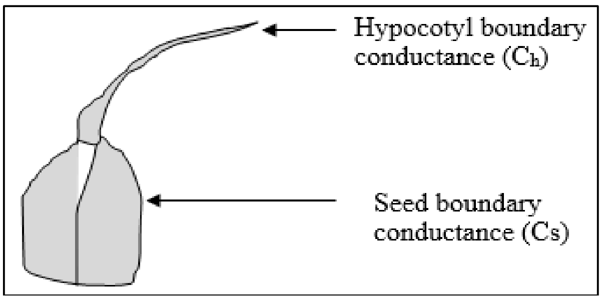

Fig 2: Total boundary conductance (*Cs*) offered by chickpea sprout (Seed + Hypocotyl)

By applying Fick's law of diffusion, the transpiration rate TR_{sprout}, can be expressed as the product of total boundary conductance (*Cs*) offered by chickpea sprout and the water vapour pressure deficit WVPD (KPa) between the internal sites of evaporation from sprouted chickpea (seed + hypocotyl) (e_s) and the modified atmospheric air (e_a) at 10 ºC temperature.

Thus in this case,

$$TR_{sprout} = (\rho\ Cp / \lambda\ \gamma) \times Cs \times WVPD_{sprout\text{-}MAair} \qquad (5)$$

and,

$$WVPD_{sprout\text{-}MAP\ air} = (e_s) - (e_a) \qquad (6)$$

Where,

ρ = Density of MA air, (g/m^3)

Cp = Specific heat capacity, (J/gK)

λ = Latent heat of vaporization, J/g

γ = Psychometric constant, (kPa/K)

With following three assumptions:

1. The concentration of water vapor inside the cotyledons and sprout is at saturation at the sprouted chickpea temperature (Ts) at 10 ºC. Then, e_s = e* (Ts), where e* (Ts), is at saturated water vapor pressure at (Ts).
2. That, the sprouted chickpea sample temperature is equal to modified atmospheric air temperature i.e 10 ºC under such condition; $WVPD_{seed\text{-}MAair}$ will be equal to $WVPD_{MAair.}$
3. Under MA condition, the radiative load incoming on seed sprouts are very low and considered negligible.

Thus by considering above assumptions, the final expression for transpiration rate of chickpea sprouts become:

$$TR_{sprout} = (\rho\ Cp / \lambda\ \gamma) \times km \times WVPD_{MAair} \qquad (7)$$

Where,
km = Transpiration co-efficient and is equal to total boundary conductance (*Cs*).

Adopted Model: In the present investigation**,** conductance model was tested for predicting sprout transpiration TR_{sprout}, at $WVPD_{seed\text{-}MAair}$ (Eq.5) The total mean value for the boundary conductance $(Cs)_{mean}$ and was represented by the slope of the regression line and TR_{sprout} is a linear function of $WVPD_{MAair}$ (Eq. 7) to get mean value for transpiration co-efficient km_{mean} represented by the slope of the regression line.

Conductance model (CM): For identical TR_{sprout} measurement, the corresponding value of the total conductance C_t was calculated, as well as the conductance imparted by hull (C_h) and hypocotyls (C_{hypo}) by means of Eq. (4).

Results and discussion

Transpiration rate by conductance model at $WVPD_{seed\text{-}MAair}$.

At 10 ºC [recommended temperature for storage; Singh *et al*., (2012)] [16] temperature and RH combinations, the corresponding values of transpiration rate, conductance of sprout were measured by using mathematical empirical relationship based on conductance model (Eq. 4) and were examined. It was observed that the transpiration rate of sprout was dependent on increasing with increase in conductance and temperature at increasing relative humidity. The reason for the change could be the change in $WVPD_{seed\text{-}MAair}$ to a lesser extent to change in convection conditions around the sprout seed and hypocotyl.

Under modified atmospheric condition at 10 ºC temperature and different RH combinations, the best suitable correlation was observed when comparing the chickpea sprout to water vapor pressure deficit. Fig 1 represents the relationship between TR_{sprout} and RH at both the water vapor deficit of seed- MA air and water vapor deficit MA air for two seed parts (seed and hypocotyl). The values of boundary conductance $(Cs)_{mean}$ and *km* = (Transpiration co-efficient) obtained by linear regression for different RH combinations are presented in Table 3 & 4. In all situations, all most similar co-relation was observed between TR_{sprout} and $WVPD_{seed\text{-}MAair}$ and among TR_{sprout} and $WVPD_{MAair}$. In both the cases of seed, TR_{sprout} was highly correlated to $WVPD_{seed\text{-}MAair}$ for different RH combinations (R^2=0.89-0.98), while there was slight less correlation with $WVPD_{MAair}$ (R^2=0.64-0.87). The trends can be logically explained by the least variations in the seed and MA air temperature during modified atmospheric storage (Fig 2).

The slope and subsequently the Conductance C_b and *Km*, mean are higher for different RH combinations, for both seed and hypocotyl.

Table 3: Prediction of transpiration rate at 10 ºC by conductance model at $WVPD_{seed\text{-}MAair}$.

Seed part	RH, %	R^2	Slope	σ	Conductance C_b (mm/s)	TR_{sprout}, (mg/kg-h-KPa)
Hypocotyl	75±2	0.89	0.19	0.013	0.036	0.79
	85±2	0.83	0.16	0.011	0.022	0.32
	95±2	0.87	0.13	0.012	0.016	0.26
seed	75±2	0.93	0.11	0.009	0.027	1.82
	85±2	0.89	0.07	0.008	0.019	1.39
	95±2	0.98	0.04	0.010	0.018	1.48
Total	75±2	Transpiration rate of seed (Hypocolyl+seed)				2.61
	85±2					1.71
	95±2					1.74

Table 4: Prediction of transpiration rate by conductance model at $WVPD_{MAair}$.

Seed part	RH, %	R^2	Slope	σ	*Km*, mean (mm/s)	TR_{sprout}, (mg/kg-h-KPa)
Hypocoyl	75±2	0.79	0.13	0.031	0.021	0.22
	85±2	0.81	0.17	0.020	0.012	0.33
	95±2	0.87	0.15	0.032	0.016	0.46
Seed	75±2	0.72	0.08	0.049	0.036	1.57
	85±2	0.71	0.14	0.068	0.025	1.39
	95±2	0.64	0.09	0.080	0.022	0.98
Total	75±2	Transpiration rate of seed (Hypocolyl+seed)				1.79
	85±2					1.72
	95±2					1.44

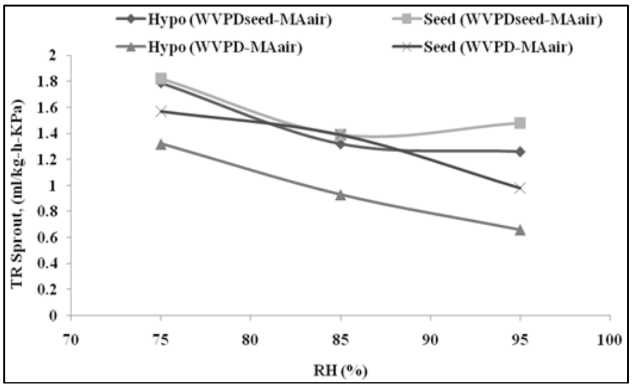

Fig 4: Seed transpiration (TR sprout, ml/kg-h-KPa) at different RH in both the WVPD $_{(seed\text{-}MAair)}$ and WVPD $_{(MA\ air)}$ regimes.

The sprout transpiration TR_{sprout}, linear model at $WVPD_{seed-MAair}$ was found to fit the data almost in similar way than the other represented by the slope of the regression line and TR_{sprout} is a linear function of $WVPD_{MAair}$ especially in case of seed. Similarly the other model based on linear function of $WVPD_{MAair}$ also appears fit to predict the transpiration rate TR_{sprout}. It is also evedent that the model based on linear function of $WVPD_{MAair}$ is able to predict accurately the seed transpiration TR_{sprout} using $WVPD_{MAair}$ as driving variables. The current assumption that the seed sprout temperature is equal to MA air temperature as 10 ºC seems to be acceptable in post harvest conditions and storage and well supported by the study carried out by Leonardi *et al*., (2000) [10] supporting the statement.

The slope of both the regression model based on linear model at $WVPD_{seed-MAair}$ and linear function of $WVPD_{MAair}$ representing boundary conductance $(Cs)_{mean}$ and transpiration co-efficient *(km)* seems to be varied non-significantly with respect to RH and seed parts. These values are in good agreement with experimental calculated values of TR seed.

The estimated values for seed boundary conductance gave between 0.002 mm/s and 0.010 mm/s, with an average value of 0.017 mm/s by assuming the seed boundary conductance $(C_s) \approx$ hypocotyl boundary conductance (C_h). However, Sastry and Buffington (1979) [15]; Nobal (1975) [12] reported estimated values of 0.06-0.036 mm/s for tomatoes fruit.

Validation of the Model

The model developed was validated, for its predictions of the transpiration rate at 10 ºC with different treatment RH and were compared with the set of experimental data. Fig. 6 and 7 shows the agreement between experimental and predicted transpiration rate of chickpea sprouts sample both for linear model at $WVPD_{seed-MAair}$, and linear model at $WVPD_{MAair}$ thus validating and close approximation and predictive ability of the developed model.

The transpiration rate data TR_{sprout} of conductance model was estimated by fitting Eq. (4) to the experimental data by non-linear regression using Statistical software GraphPad PRISM® Version 5.00.288 software (Graph Pad Software, Inc.). Analysis of variance was performed with the SPSS 14.0 software. The values for weight of chickpea sprouts as predicted by Eq. (4) were in close agreement with those obtained experimentally for linear model at $WVPD_{seed-MAair}$, (R^2=0.87; Fig 6) and linear model at $WVPD_{MAair}$ (R^2 = 0.82; Fig. 7). Transpiration rate was then predicted at each set of temperature and humidity studied using Eq. (4). A good agreement was found between observed and predicted transpiration rates as shown in Fig. 6 & 7.

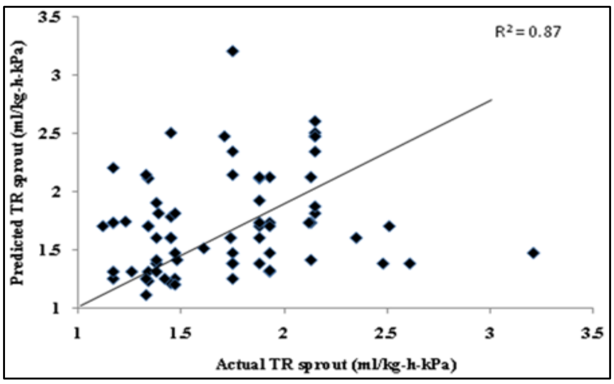

Fig 6: Actual transpiration rate and simulated transpiration rate relationship of chickpea sprouts based on linear model at $WVPD_{seed-MAair}$ with R^2 = 0.87.

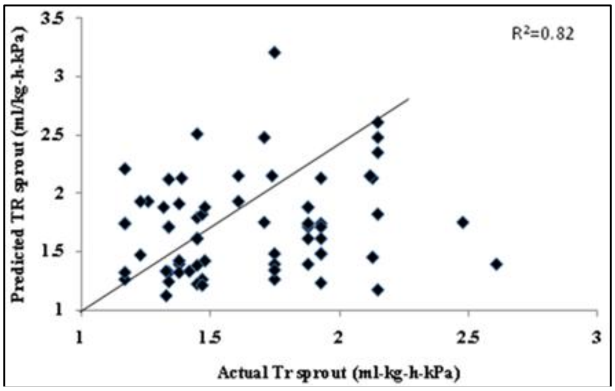

Fig 7: Actual transpiration rate and simulated transpiration rate relationship of chickpea sprouts based on linear model at $WVPD_{MAair}$ with R^2 = 0.82.

Conclusions

The transpiration rate of chickpea sprout sample was significantly affected by temperature at all relative humidity. Increasing temperature of the experimental storage container from 5 ºC to 15 ºC increases the transpiration rate of chickpea sprouts by 54.43%. The developed model for transpiration rate estimation on the basis of Fick's law of diffusion was confirmed to yield near approximation of weight loss of chickpea sprout samples during MA storage at 10 ºC temperature at different combinations of relative humidity. Both the proposed model based on linear function of $WVPD_{MAair}$ and $WVPD_{seed-MAair}$ is able to predict accurately the seed transpiration TR_{sprout} using WVPD as driving variables. The developed model would be helpful to value the moisture loss with temperature, humidity and time, and aid in designing suitable packaging system for fresh horticultural produce.

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