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Impact of pack-house chemicals treatments on quality of litchi during low temperature storage

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Abstract
An experiment was conducted to study the impact of chemical treatments on postharvest quality attributes of freshly harvested litchi fruits. Litchi variety "Shahi" fruits were harvested at full maturity (ready-to-eat) from the orchard of National Research Centre for Litchi, Muzaffarpur, Bihar and transported to IARI, New Delhi where treated with sodium hypochlorite (0.2%), sodium hypochlorite (0.2%) + carnauba wax (10%), sodium hypochlorite (0.2%) + KMS (0.5%), sodium hypochlorite (0.2%) + KMS (0.6%), sodium hypochlorite (0.2%) + sodium chlorite (0.05%), sodium hypochlorite (0.2%) + sodium chlorite (0.06%) along with control and packed in punnets for storage study at 2 ºC and 85-90 % RH. The observations related to quality parameters were recorded at 5 days intervals. The results revealed that all treatments reduced pericarp browning. Sodium hypochlorite (0.2%) in combination with KMS (0.5%) treated fruits were found most effective in retaining higher amount of moisture content, total soluble solids, reducing sugars, anthocyanin content and good sensory attributes in the fruits leading to lowest browning index with an extended shelf life up to 25 days. These findings confirmed that treatment with sodium hypochlorite (0.2%) followed by KMS (0.05%) could be used as alternative method to reduce pericarp browning and quality deterioration of litchi fruit during low temperature storage.

Keywords: Litchi, pericarp browning, fruit quality, potassium metabisulfite, sodium chlorite, sodium hypochlorite, carnauba wax

Introduction
Litchi (*Litchi chinensis* Sonn.) is the most renowned edible fruit of soapberry family, Sapindaceae, known for its attractive deep red colour, nutritive value, deliciously flavoured translucent juicy aril and refreshing taste. Litchi is an excellent source of vitamin C with high content in minerals like potassium, phosphorous, magnesium and calcium. It may be eaten fresh, frozen, canned in syrup or dried to produce "litchi nuts". It is cultivated in China, India, Thailand, Taiwan and South Africa. India is the second largest producer of litchi in the world after China. Major producing states are Bihar (40% of total production), West Bengal, Jharkhand, Assam, Punjab and Uttaranchal.

The fruits are harvested at ripe stage which is judged by the development of red colour on the fruit pericarp and flattening of tubercles. Within 2-3 days after harvesting of the fruits, the red colour of the pericarp turn brown, this drastically reduces the commercial value of the fruits. The rapid enzymatic degradation of anthocyanin pigments is believed to be the main causes of browning of pericarp (Jiang *et al*., 2000) [6]. The pericarp browning initially occurs on protuberances and then extends over the entire surface of pericarp but mainly in epicarp and upper layers of mesocarp. Water loss or dehydration causes rapid loss of membrane integrity which leads to interactions of substrate with various enzymes such as peroxidase (POD), polyphenol oxidase (PPO), anthocyanase and phenylalanine ammonia lyase (PAL). The major browning takes place upon bringing the enzymes (e.g. PPO) in close contact with the proposed substrate (e.g. –epicatechin) to initiate browning reaction (Sun *et al*., 2006) [18]. It is worth mention that the substrates of PPO are not completely characterized. Peroxidase activity coupled with ascorbic acid oxidation also enhances anthocyanin degradation. All these enzymatic reactions ultimately lead to the formation of polymeric brown pigments involving colored o-quinones as browning precursors (Wang *et al*., 2010) [19]. Fruit moisture loss during storage and holding typically reduces visual appeal, marketable/saleable value and sensory qualities also. The main reasons for pericarp browning are desiccation, mechanical injury

and microbial or pathogenic infection. These all, by one and another way responsible for rise in pH, reduced membrane fluidity, increased membrane permeability, loss of compartmentation between enzymes and their substrates and thereby, may aid enzymatic browning of pericarp.

Numerous approaches have been tried since the time of commercialization of this fruit but each approaches has it's certain limitations either relating to tedious and costly method of applications or short duration effect or human health concern. Also, these approaches could not prove to be satisfactory in retaining the desirable color of litchi. According to review literature, the combinatorial application of chemicals alone or with edible coatings by dip or spray method may maintain the quality of fruit for a longer time which will thus help in extending the marketability of litchi for a prolonged period. Accordingly, some of the chemicals such as sodium hypochlorite were used as a treatment due to its acidic nature as well as disinfectant and fungicidal property (Cerioni *et al*., 2009) [4] and coating treatments like carnauba-based wax, which are known to maintain the quality of stored fruit crops by suppressing moisture loss, improving the strength of peel tissue and retaining volatile components and controlling ripening by modifying CO_2 and O_2 concentrations inside the fruit (Baldwin *et al.*, 1996) [3] were tried. The potassium metabisulphite (KMS) as a source of SO_2 which acts as antimicrobial and antioxidant have been used since it is reported to inhibit browning in several fruits (Milne and Johnson, 1994) [14]. Sodium chlorite (SC) is an oxidising and sanitising agent which is able to generate chlorine dioxide (ClO_2) in an acidic environment which was reported to reduce enzymatic browning of fruits and vegetables (Liu *et al.*, 2006) [18]. In our present study, it has been evaluated the possibility of using these chemicals and edible coating as combinational application for inhibiting the pericarp browning of litchi fruits during low temperature storage.

Materials and Methods

Litchi fruits (cv. Shahi) were harvested from the orchard of National Research Centre on Litchi, Muzaffarpur, Bihar when 90-95% of peel color was red color. After sorting and grading, the fruits were transported to Division of Food Science and Post Harvest Technology laboratory, IARI, New Delhi in CFB boxes within 28 hours of harvest and again sorting was done to remove the spoiled fruits. Then the fruits were treated with T1: sodium hypochlorite (0.2%), T2: sodium hypochlorite (0.2%) + carnauba wax (10%), T3: sodium hypochlorite (0.2%) + KMS (0.5%), T4: sodium hypochlorite (0.2%) + KMS (0.6%), T5: sodium hypochlorite (0.2%) + sodium chlorite (0.05%), T6: sodium hypochlorite (0.2%) + sodium chlorite (0.06%), T7: control; by dipping the fruits in solution for 5 minutes followed by next chemical/edible coating as treatment. The fruits were dried for 10 minutes prior to application of next treatment. The treated fruits were then packed in CFB boxes and stored at 2 ºC and 85-90 % relative humidity. The fruit samples were analyzed for physio- chemical attributes for every 5 days interval till the fruits became unacceptable.

Weight loss

Weight loss was determined by weighing the fruits at different intervals which were calculated as the difference between the initial weight and the final weight at the time of measurement and expressed as the percentage of initial fruit weight.

Browning Index

Browning of litchi pericarp was accessed visually by measuring the total browned area on the pericarp by using following scale: 0 = no browning (excellent quality); 1 = slight browning; 2 = <1/4 browning (marketable quality); 3 = 1/4–1/2 browning; 4 = 1/2–1/3 browning and 5 = >1/3 browning (poor quality). The browning index was calculated as ∑(browning scale x percentage of corresponding fruits within each class).

Total anthocyanin content

Total anthocyanin in the fruit pericarp was determined by the pH-differential method using two buffers: potassium chloride buffer (pH- 1.0) and sodium acetate buffer (pH- 4.5). First, the pigments were extracted from 2 g fruit pericarp by slicing and crushing using 80% ethanol and done centrifugation at 10,000 rpm for 10 min where obtained supernatants were diluted in pH 1.0 and pH 4.5 buffers for taking the observations at 520 and 700 nm in a spectrophotometer. Finally, the anthocyanin content was calculated in terms of cyanidin-3-glucoside equivalent which was expressed as mg/100g of fresh pericarp weight using molar absorption coefficient of 26,900 L/mol/cm and a molecular weight of 449.2 g/mol.

Total soluble solid and reducing sugars

Total soluble solid was analyzed by putting few drops of the sample slurry over the prism of the hand refractometer (Fisher 0-50, Japan) and the reading was recorded in ºB. Reducing sugars were estimated by titration against boiling Fehling solution A and B using methylene blue as an indicator till brick red colour appears as the end point.

Sensory evaluation

The sensory quality evaluation of the litchi fruits was done by a semi trained panel of 7 judges and aged 25-45 years from both of the genders, using a 9 point Hedonic scale (Amerine *et al.* 1965) [1].

Statistical Analysis

The experiment was conducted in a factorial completely randomized design with three replications, each replication having 50 fruits. The data were analyzed using the WASP 2.0 (Web Agri Stat Package) and the results were compared from ANOVA by calculating the critical difference (CD) at 5% level of significance.

Results and Discussion

Pericarp browning is the main factor influencing post-harvest quality and storage life of litchi fruits. The PLW of the fruits increased with the increasing storage period. The lower PLW in KMS (0.5%) treated fruits may be due to SO_2 liberated by KMS which possibly had maintained cell integrity and permeability of tissues, thereby hindering the loss of moisture from the fruit surface. Similar results were obtained in litchi fruits by Khan *et al.*, 2012 [8]. Furthermore, higher PLW in control fruits may be due to higher metabolic activity than treated fruits.

Table 1: Effect of treatments on PLW (%) of litchi fruits during storage (2 ºC and 85-90 % RH)

Treatments	Storage (days)														
	0	2	4	6	8	10	12	14	16	18	20	22	24	26	Mean
NaOCl (0.2 %)	0^{o}	1.63^{n}	4.92^{ji}	6.58^{ycxbaz}	7.42^{vuw}	8.25^{rst}	$9.09^{kmpqnol}$	9.6^{kmijhl}	10.22^{gfh}	10.7^{4ef}	11.34^{ed}	11.87^{cd}	12.29^{cb}	12.6^{cb}	8.25^{b}
CW (10 %)	0^{o}	1.98^{n}	$3.6l^{m}$	5.50^{ghefi}	5.96^{cefd}	6.42^{cbadz}	$6.88^{yvxbawz}$	7.17^{yvxuwz}	7.85^{sut}	8.25^{rst}	8.71^{rpqo}	$9.21^{kmpjnol}$	9.71^{kgijhl}	9.91^{gijh}	6.51^{c}
KMS (0.5 %)	0^{o}	1.76^{n}	$3.47l^{m}$	5.21^{ghjfi}	5.71^{ghefd}	6.32^{cbd}	7.20^{yvxuw}	7.41^{vuw}	7.88^{sut}	8.33^{rsqt}	8.73^{rpqno}	$9.17^{kmpjnol}$	$9.50^{kmijnhl}$	9.74^{kgijhl}	6.46^{c}
KMS (0.6 %)	0^{o}	1.54^{n}	3.09^{m}	4.72^{jk}	5.37^{ghfi}	5.75^{ghefd}	6.23^{cebd}	6.83^{yxbawz}	7.32^{vxuw}	7.61^{vut}	8.37^{rsqt}	8.77^{rpqno}	$9.38^{kmijnol}$	9.93^{gijh}	6.06^{e}
$NaClO_2$ (0.05 %)	0^{o}	1.81^{n}	3.44^{lm}	4.74^{ji}	5.17^{ghji}	5.73^{ghefd}	6.40^{cbad}	$6.91^{yvxbawz}$	7.32^{vxuw}	7.75^{sut}	8.24^{rst}	8.82^{rpqno}	$9.35^{kmijnol}$	9.82^{kgijh}	6.10^{e}
$NaClO_2$ (0.06 %)	0^{o}	1.38^{n}	3.0^{m}	3.96^{lk}	5.15^{ghji}	5.85^{gcefd}	6.43^{ycbadz}	$7.16^{yvxuawz}$	7.84^{sut}	8.46^{rspq}	9.04^{mpqnol}	9.60^{kmijhl}	10.0^{gifh}	10.40^{gf}	6.30^{d}
Control	0^{o}	2.86^{m}	5.0^{hji}	5.85^{gcefd}	6.35^{cbd}	7.62^{vut}	8.91^{rmpqno}	9.72^{kgijhl}	10.41^{gf}	11.23^{ed}	11.96^{cd}	12.75^{b}	13.68^{a}	14.4^{a}	8.96^{a}
Mean	0^{n}	1.85^{m}	3.78^{l}	5.22^{k}	5.87^{j}	6.56^{i}	7.30^{h}	7.83^{g}	8.40^{f}	8.91^{e}	9.48^{d}	10.02^{c}	10.55^{b}	10.97^{a}	

* Means with same superscript are not significantly different.
NaOCl = Sodium hypochlorite, CW = Carnauba Wax, KMS = Potassium metabisulphite, $NaClO_2$ = Sodium chlorite

Total soluble solids content increased in the initial upto 10 days of storage but thereafter it decreased as the storage period extended, in all the fruits which might be due to dehydration and conversion of starch and polysaccharides into simple sugars. Decrease in TSS might be due to biochemical activities like utilisation of reducing sugars and other organic metabolites (Marboh *et al*., 2012) [12]. However, lower TSS in control fruits might be due to loss of sugars due to high rate of respiration (Jha *et al*., 1990) [5].

A decline in reducing sugars content was observed in fruits which might be due to utilization of sugars for respiration. The data also revealed that fruits treated with CW retained higher reducing sugars (8.6%) on the last day which might be due to modified atmospheric condition in CW treated fruits which might have reduced the respiration rate.

Table 2: Effect of treatments on TSS (ºB) of litchi fruits during storage (2 ºC and 85-90 % RH)

Treatments	Storage (days)						
	0	5	10	15	20	25	Mean
NaOCl (0.2 %)	17.5^{i}	18.0^{fgeh}	18.5^{bdac}	18.3^{fbdec}	18.2^{fgdec}	17.9^{fgih}	18.06^{c}
CW (10 %)	17.5^{i}	17.8^{ghi}	18.4^{bdec}	18.2^{fgdec}	18.0^{fgeh}	17.8^{gih}	18.05^{c}
KMS (0.5 %)	17.8^{gih}	18.1^{fgde}	18.5^{bdac}	18.4^{bdec}	18.1^{fgde}	18.06^{fge}	18.16^{bc}
KMS (0.6 %)	17.6^{ih}	18.0^{fgeh}	18.5^{bdac}	18.3^{fbdec}	18.0^{fgeh}	18.0^{fgeh}	18.07^{c}
$NaClO_2$ (0.05 %)	17.8^{gih}	18.2^{fgdh}	18.6^{bac}	18.5^{bdac}	18.1^{fgde}	18.1^{fgde}	18.21^{ba}
$NaClO_2$ (0.06 %)	18.0^{fgeh}	18.3^{fbdec}	18.7^{ba}	18.5^{bdac}	18.0^{fgeh}	18.1^{fgde}	18.27^{ba}
Control	18.1^{fgde}	18.5^{bdac}	18.9^{a}	18.3^{fbdec}	18.2^{fgdec}	17.9^{fgih}	18.33^{a}
Mean	17.75^{e}	18.13^{c}	18.58^{a}	18.35^{b}	18.19^{c}	17.97^{d}	

* Means with same superscript are not significantly different.

Table 3: Effect of treatments on reducing sugars (%) of litchi fruits during storage (2 ºC and 85-90 % RH)

Treatments	Storage (days)						
	0	5	10	15	20	25	Mean
NaOCl (0.2 %)	$9.4^{ebdagcf}$	$9.3^{ebidhagcf}$	$9.0^{ejidhlgf}$	8.6^{jmoln}	8.4^{pmoqn}	8.0^{pq}	8.7^{b}
CW (10 %)	9.6^{bdac}	$9.3^{ebidhagcf}$	$9.2^{ejbidhagcf}$	$8.9^{ejmihlgnf}$	8.7^{jmioln}	8.6^{moln}	9.0^{a}
KMS (0.5 %)	9.8^{a}	9.5^{bdac}	$9.3^{ebidhagcf}$	$9.2^{ejbidhagcf}$	$8.8^{jmihlgn}$	8.5^{pmoln}	9.2^{a}
KMS (0.6 %)	9.7^{ba}	9.5^{ebdac}	$9.3^{ebidhagcf}$	$9.1^{ejbidhgcf}$	$8.7^{jmiohln}$	8.5^{pmoln}	9.1^{a}
$NaClO_2$ (0.05 %)	$9.3^{ebidhagcf}$	$8.9^{ejidhlgf}$	8.7^{jmioln}	8.5^{pmoln}	8.2^{poqn}	8.2^{poqn}	8.6^{b}
$NaClO_2$ (0.06 %)	$9.4^{ebdhagcf}$	$9.0^{ejidhlgf}$	$8.8^{jmihlgnf}$	8.6^{jmoln}	8.4^{pmoqn}	8.2^{poqn}	8.7^{b}
Control	9.5^{ebdacf}	$9.2^{ejbidhagcf}$	$9.0^{ejidhlgf}$	8.3^{poqn}	8.2^{poq}	7.8^{q}	8.6^{b}
Mean	9.5^{a}	9.2^{b}	9.0^{c}	8.7^{d}	8.4^{e}	8.2^{f}	

* Means with same superscript are not significantly different.

It was found that there was degradation of anthocyanin pigments in all the treatments as the storage period extended. However, all the treatments delayed degradation of anthocynins in which KMS (0.5%) treated fruits showed highest retention of anthocyanin which might be due to bleaching action of the chemical (KMS as a source of SO_2) caused by nucleophilic ion reactions which might have resulted in the removal of its original colour and subsequent release of positive ions by anthocyanin, generating the red colour under acidic medium as reported Neog and Saikia (2010) [15]. They further elaborated that when sulphite causes reduction of oxygen, the oxidase cannot oxidise polyphenol or can't combine with quinines which cause inactivity of quinines to take part in the reaction. Thus, this hinders the oxidation of pericarp, thereby reducing the pericarp browning. Lower anthocyanin pigments in control fruits might be due to higher water loss and respiratory utilizations of substrates during storage.

Table 4: Effect of treatments on anthocyanin content (mg/100g) of litchi fruits during storage (2 ºC and 85-90 % RH)

Treatments	Storage (days)						
	0	5	10	15	20	25	Mean
NaOCl (0.2 %)	38.5^{a}	30.5^{ifgh}	24.2^{lmn}	20.4^{o}	15.6^{p}	11.1^{q}	23.37^{d}
CW (10 %)	38.6^{a}	34.3^{ecd}	31.1^{fgh}	28.2^{ijk}	24.3^{lm}	21.4^{on}	29.66^{c}
KMS (0.5 %)	38.8^{a}	35^{bcd}	35.6^{bc}	32.8^{efcd}	28.9^{ijh}	25.8^{lk}	32.81^{ba}
KMS (0.6 %)	38.9^{a}	34.5^{bcd}	34.9^{bcd}	31.6^{efgh}	27.5^{jk}	24.3^{lm}	31.95^{b}

$NaClO_2$ (0.05 %)	39.1^{a}	37.2^{ba}	35.4^{bc}	32.9^{efcd}	28.8^{ijh}	25.9^{lk}	33.23^{a}
$NaClO_2$ (0.06 %)	39.0^{a}	37.2^{ba}	35^{bcd}	32.3^{efgd}	27.8^{ijk}	24.1^{lmn}	32.57^{ba}
Control	38.5^{a}	29.5^{ijgh}	21.5^{omn}	14.12^{p}	10.5^{q}	8.2^{q}	20.39^{e}
Mean	38.77^{a}	34.03^{b}	31.09^{c}	27.46^{d}	23.35^{e}	20.11^{f}	

* Means with same superscript are not significantly different.

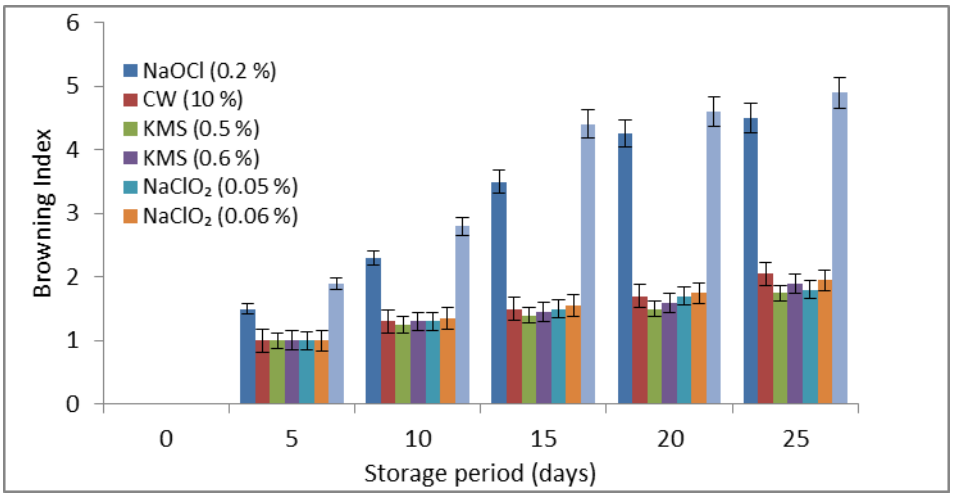

Fig 1: Effect of treatments on browning index of litchi fruits during storage (2 ºC and 85-90 % RH)

Browning of litchi fruits is due to degradation of anthocyanin pigments by the activities of polyphenol oxidase as reported by Jiang *et al*., 2002 [7]. Fruits treated with KMS (0.5%) had lowest browning index which may be due lower water loss from the fruits and also, action of SO_2 which is liberated from KMS causing inhibition of PPO activity leading to no enzymatic browning. In a similar manner, Also, $NaClO_2$ (0.5%) showed significant result which may be due to acidic nature of sodium chlorite would have reduced the pH of the fruits thus intensifying the colour of litchi as well as inhibiting the PPO activity in litchi (Khunpon *et al.*, 2011) [9]. In our study, higher concentrations of KMS (0.6 %) and sodium chlorite (0.06 %) were also used but found less effective which may be due to tissue damage (Lu *et al.*, 2007) [11] causing leakage of substrates leading to oxidation of phenolic compounds resulting in low performance in maintaining the quality parameters of litchi fruit. In contrast, control fruits showed maximum browning index which may be due to maximum water loss because of higher metabolic activity during storage.

It was found that texture, taste, flavour and aroma of the litchi fruits were degraded during storage in spite of treatments which might be due to degradation of quality attributes causing lowering of acceptability. However, fruits treated with $NaClO_2$ (0.05%) showed higher overall sensory attributes on the last day of storage which might be due to better maintenance of texture and quality attributes by the treated fruits. But, in fruits treated with KMS (0.5%), there might be development of off-flavour (sulphur flavour) in the litchi fruits (Schutte *et al.*, 1990) [17] causing lower acceptability than the fruits treated with $NaClO_2$ (0.05%).

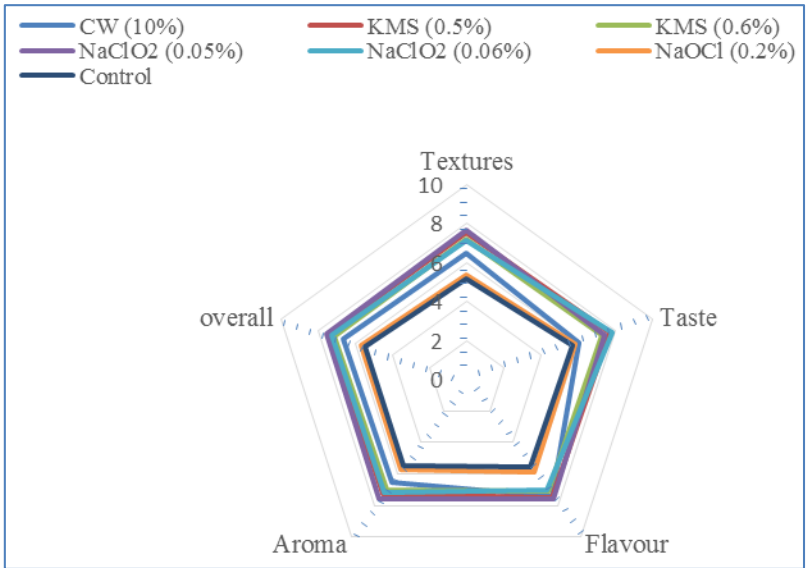

Fig 2: Sensory score of litchi fruits as influenced by different treatments on last day of storage

Conclusion

Combinational treatments of chemicals at low temperature storage were found effective for extending the shelf life of litchi fruits up to 25 days. All the treatments reduced pericarp browning but sodium hypochlorite (0.2%) in combination with KMS (0.5%) treated fruits was more effective in

retaining anthocyanin, delaying loss in weight, total soluble solids and reducing sugars in the fruits leading to lowest browning index when stored at 2 ºC and 85-90 % relative humidity. Also, the treatment showed good overall sensory attributes among all except the fruits treated with $NaClO_2$ (0.05%). Therefore, application of these chemicals which are easily available, can be cost effective and as an alternative method for SO_2 fumigation to maintain the quality of litchi for longer period.

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