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Enhancing Storage and Shelf Life of "Le Conte" Pear Fruits by Using Sodium Bicarbonate and Potassium Sorbate as a Postharvest Treatment

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ABSTRACT

This study was carried out for two successive seasons 2011 and 2012 in order to evaluate the efficiency dipping of Le Conte pear fruits in sodium bicarbonate (SBC) and/or potassium sorbate (KS) either alone or in combination to maintain fruit quality and control postharvest diseases at cold storage or during marketing at room temperature. Pear fruits were kept at 0°C±1 with 90-95% R.H. for 90 days at cold storage and 5 days as marketing at room temperature. The reduction in linear growth and dry weight were correlated to the increase in SBC and/or KS concentrations. The SBC 2.0% +KS 2.0% treatment gave complete inhibition of the linear growth and dry weight while, gave the maximum reduction in disease infection of *Penicillium expansum* and *Botrytis cinerea* (0.0%) in both seasons. Also, application of both SBC+KS showed the best results in reducing loss weight (%) and decay (%), since kept chlorophyll A and carotenoids content to a long time after cold storage and 5 days during marketing. Dipping fruits, in SBC alone, was more effective to progress fruit firmness. Furthermore, dipping fruits with KS alone decreased juice acidity (%) while increasing SSC (%) and total sugar (%) either after cold storage or through marketing.

Key words: Le Conte pear, sodium bicarbonate, potassium sorbate, cold storage, marketing

INTRODUCTION

Pear (*Pyrus communis*) is one of the favorite fruits of temperate zone and considered the third of deciduous fruits, the fourth among all fruits in its global distribution and one of the most important deciduous fruits in Egypt. The total cultivated area for pear fruits were 3741 hectares with total production estimated to 48817 ton (FAO, 2011).

Among the fungal diseases, blue mold caused by *P. expansum*, grey mold caused by *B. cinerea*, mucor rot caused by *Mucor piriformis* are common on pear fruits (Mari *et al.*, 2004). Patulin, a toxic secondary metabolite, is produced by certain species of *Aspergillus*, *Byssochiamys* and *Penicilium*. *P. expansum* is the fungus most commonly associated with patulin contamination of apple juice and apple products. Patulin has been reported to have mutagenic, carcinogenic and teratogenic properties but this is still a matter of debate (Dombrink-Kurtzman and McGovern, 2007). However, there is a need to provide more biocompatible fungicides which are safe in the environment, non-toxic to humans and animals and are rapidly biodegradable. The anti-fungal effects of salt compounds and their usefulness in plant disease control have been documented.

Bicarbonate salts are one of several alternative control options that have recently received attention. Bicarbonate salts are GRAS substances frequently utilized in integrated biological control programs and considered one of several alternative control options that have recently received attention (Janisiewicz and Korsten, 2002). These biocompatible chemicals are particularly interesting because they have fungicidal properties combined with a very low mammalian and environmental toxicity profile. Bicarbonates might have several modes of action against fungi, including buffering and action to raise the pH level and the osmotic pressure of cells at the leaf surface, both factors leading to detrimental conditions for fungal spore. Sodium Bicarbonate (SBC) (NaHCO₃, baking soda) is common food additives permitted by the United States Food and Drug Administration with no restrictions. It has also been used to reduce postharvest decay, mainly on citrus fruits. Thus, the effect of Sodium bicarbonate in decreasing fruit decay incidence may lead to delay respiration rate and improve fruit quality (Palou *et al.*, 2002). The effect of the SBC treatment is to delay spore germination since, it has only fungistatic effect. It can be a useful tool to manage citrus postharvest decays due to its considerable antimicrobial activity, it is inexpensive, readily available and can be used with a minimal risk of injury to the fruit (Usall *et al.*, 2008).

Potassium Sorbate (KS), a common food preservative, was evaluated to control postharvest decay of citrus fruit. Significant advantages of KS over the commonly used sodium bicarbonate, which similarly improved fungicide performance, are the relatively low salt concentration of KS, the absence of sodium and its lower pH, so disposal of used KS solutions would raise fewer regulatory issues (Smilanick *et al.*, 2008). Sorbates are the best characterized of all food antimicrobials as to their spectrum of action. They inhibit certain bacteria and food-related yeasts and mold species. Sorbates are common food preservatives for many applications and its spectrum of activity includes *B. cinerea*. The mode of action of sorbate could be through the alteration of the morphological structure of the cell, genetic changes, cell membrane alterations, inhibition of cell transport processes and inhibition of enzymes involved in metabolism of transport functions (Sofos and Busta, 1993). Potassium sorbate inhibited respiration of fruits and protected fruit skin from decay, thus delaying ripening and improve quality by slowing down the metabolism activity and physiological disorders (Lin *et al.*, 2008).

Also, Karabulut *et al.* (2005) reported a single application of potassium sorbate as applied to harvested grapes to partially control subsequent gray mold during cold storage. Therefore, potassium sorbate could influence both grape quality and postharvest decay and be a commercially feasible treatment. It has a low order of toxicity to workers and the environment, it is inexpensive, readily available and exempt from residue tolerances and the risk of resistance in the pathogen population is probably low.

The objective of this study was to evaluate use of natural alternatives as postharvest treatments such as SBC and KS alone or in combination to maintain Le Conte pear fruits quality, storability and prolong the marketing as long as possible by inhibition fungal diseases.

MATERIALS AND METHODS

Mature Le Conte pear fruits were picked on 25 and 29 July 2011 and 2012 seasons, respectively from trees about ten years old grown in clay loam soil and spaced at 5 m apart growing in a commercial field at Aga region, Egypt. Le Conte pear fruits were harvested at approximately 135-147 days from full bloom, when the average of fruit firmness reached about 14-15 lb inch⁻² according to Swindeman (2002) and when it become soluble, solids in fruits juice reached about 13-14%.

Fruits were harvested from trees expected common horticultural practices, undamaged and free from any obvious pathogen infection, then transported to the laboratory. All fruits were washed

with tap water to remove the dust and foreign materials, then air-dried and a quick sorting was done to exclude any defect in fruits. At the beginning of the experiment, samples of 15 fruits were taken to determine the initial fruits properties and then received the following treatments:

- Dipping fruits in 2% SBC (NaHCO₃) for 5 min
- Dipping fruits in 2% KS ($C_{\theta}H_{7}KO_{2}$) for 5 min
- Dipping fruits in 2%SBC +2% KS for 5 min
- Control (dipping fruits in tap water) for 5 min

For storage study, fruits of all treatments were sorted to remove any infection and damage, then fruits were stored in perforated plastic bags (each contain 5 fruits). All bags with fruits were weighted and put in ventilated carton box. All boxes were stored 90 days at 0°C±1 with 90-95% relative humidity, the fruits were taken 30 days intervals to determine changes in quality during cold storage.

For shelf life study, after 90 days of cold storage, fruits were held 5 days at room temperature conditions as shelf life at 28°C±2 with 65-70% relative humidity to determine the following parameters:

Isolation and identification: *P. expansum* and *B. cinerea* were isolated from naturally infected Le Conte pear fruits after storage of 90 days. These isolates were the most aggressive one in our collection and produced the largest lesions on inoculated fruits. These fungi were purified and maintained on Potato Dextrose Agar (PDA) and stored at 4°C, with periodic transfers through Le Conte pear fruits to maintain its aggressiveness. Le Conte pear fruits were ready for examination under a stereoscopic binocular microscope (6-50 X) for the presence of fungi and to study their habit characters. When necessary, the compound microscope was used for confirming the identification after having examined the morphology of conidia and conidiophores. Fungi, presented on infested seeds, were identified by means of comparison with the description sheets of Commonwealth Mycological Institute (CMI) Kew, Surrey, England, Danish Government Institute of Seed Pathology (DGISP) publications as well as publication of Moubasher *et al.* (1977), Ellis (1971) and Singh *et al.* (1991).

Effect of SBC and KS on growth of fungi isolated from Le Conte pear fruits and disease infection percentage:

- Linear growth: Sodium bicarbonate and potassium sorbate were tested *in vitro* on the linear growth of the pathogenic fungi. Different concentrations were added to 10 mL of sterilized PDA before solidification and then poured in sterile petri-dishes. After solidification, the plates were inoculated with fungal disc (5 mm) in the center of the plate and incubated at 27±1°C. Three plates for each particular treatment for each fungus were used as replicates; three plates were prepared to serve as control for each fungus. Linear growth was observed daily and diameter of fungal colonies were recorded when plates of any treatment were filled with the fungal growth.
- Dry weight: One hundred milliliter of liquid PD medium in 250 mL Erlenmeyer flasks were amended with different concentrations of the tested compounds after autoclaving. Each flask was inoculated using two discs of 0.6 mm in diameter of fungal culture, then incubated at 20°C±2 for 7 days. Control flasks contain no concentrations of these compounds. Three

replicates were used for each concentration. At the end of incubation period, the mycelium was filtered off and washed several times with distilled water, then dried in an oven at 80°C for 48 h till constant weight (El-Morsy, 1993).

• **Disease infection:** It was determined according to the following equation:

Disease infection (%) = $\frac{\text{No.of nutural infected fruits}}{\text{No.of total fruits}} \times 100$

Effect of sodium bicarbonate and potassium sorbate on Le Cont pear fruits quality

• Loss in fruit weight: It was determined according to the following equation:

 $Loss in fruit weight (\%) = \frac{Initial weight - Weight at sampling date}{Initial fruit weight} \times 100$

• **Decay:** It was determined according to the following equation:

$$Decay(\%) = \frac{Weight of decayed finits}{Initial fruit weight} \times 100$$

- Fruit firmness: It was measured by using a hand Effegi-Penetrometers supplemented as Ib inch⁻² (Harker *et al.*, 1996).
- Soluble Solids Content (SSC%): Soluble solids content in fruit juice was measured using a Carl-Zeiss hand refractometer according to AOAC (2005).
- **Titratable Acidity (TA%):** It was determined in 10 mL of fruit juice as a percentage of malic acid according to AOAC (2005).
- **Total sugar (%):** The extract was prepared by taking 0.5 g of fresh pulp and extracting with 80% ethanol. The total sugar was estimated according to Ranganna (1979).
- Total carotenoid and chlorophyll content: A weight of 0.5 g fresh skin fruits was ground by 10 mL methanol then determined by spectrophotometer according to the methods of Ranganna (1979).

Statistical analysis: Data of both seasons of the study was analyzed using Analysis of Variance (ANOVA) technique. Differences among treatment means were statistically compared using Duncan's multiple range tests at a level of 0.05 using the CoStat v6.4 program.

RESULTS AND DISCUSSION

Effect of SBC and/or KS on linear growth (cm), dry weight (g) of fungi isolated from Le Conte pear fruits and disease severity (%) as postharvest treatments: Table 1 indicated the effect of treatments by dipping with sodium bicarbonate (SBC) and/or potassium sorbate (KS) on linear growth and dry weight of *P. expansum* and *B. cinerea* isolated from Le Conte pear fruits. It was also noticed that the reduction in linear growth and dry weight were correlated to the increase in sodium bicarbonate (SBC) and/or potassium sorbate (KS) concentrations. Sodium bicarbonate at 2.0% +potassium sorbate at 2.0% treatment was complete inhibition of the linear growth and dry weight of *P. expansum* and *B. cinerea*. This result is in agreement with the finding of Latifa *et al.* (2011) on citrus who reported complete inhibition of mycelia growth of *P. itelicum* which was generally associated with complete inhibition of sporulation by organic acids and salts.

	P. expansum		B. cinerea		
Treatments	Linear growth (cm)	Dry weight (g)	Linear growth (cm)	Dry weight (g)	
Control (water)	9.00a	1.640a	9.000a	1.440a	
Sodium bicarbonate at 2.0%	2.48c	0.410b	3.370c	0.560ab	
Potassium sorbate at 2.0%	4.72b	0.930ab	5.260b	1.050a	
Sodium bicarbonate at 2.0% +	0.000d	0.000b	0.000d	0.000b	
Potassium sorbate at 2.0%					

Table 1: Effects of SBC and/or KS on linear growth (cm) and dry weight (g) of fungi isolated from Le Conte pear fruits

Means followed by the same letters are not significantly different by Duncan multiple range test at 0.05 levels

The addition of 2% (w/v) sodium bicarbonate (SBC) in the suspensions of antagonistic yeast *Cryptococcus laurentii* or *Trichosporon pullulans* significantly limited spore germination and germ tube elongation of *P. expansum* and *Alternaria alternata* in Potato Dextrose Broth (PDB) medium (Yao *et al.*, 2004).

Table 2 indicates the effect of treatments by dipping with sodium bicarbonate (SBC) and/or potassium sorbate (KS) on disease infection of Le Conte pear fruits stored for 90 days at room temperature. In both seasons, prolonging the marketing stage resulted in decreased disease infection with the increase in compounds concentrations. Sodium bicarbonate at 2.0% +potassium sorbate at 2.0% treatment gave the maximum reduction in disease infection caused by *P. expansum* and *B. cinerea* (0.0%).

Some fruits, apples among them, are usually stored after harvest. During cold storage losses of economic importance are produced by several decays due to fungal rot. *P. expansum* and *B. cinerea* are well-known postharvest pathogens. They produce blue and gray rots, respectively (Calvo *et al.*, 2007).

This result is in agreement with the finding of Troncoso-Rojas and Tiznado-Hernandez (2007) on fruits and vegetables by chemical alternatives to conventional fungicides for postharvest disease control that should be natural or synthetic compounds with known and minimal toxicological effects on mammals and the environment. The origin of these alternatives includes classifications such as food additives and substances listed as "Generally Regarded As Safe" (GRAS) by the United States Food and Drug Administration, natural compounds obtained from plants, animals or microorganisms including some volatiles and essential oils, phenolic compounds, plant extracts, peptides, alkaloids, lectins, antibiotics, propolis, latex or chitosan and other chemicals such as calcium polysulfide or ammonium molybdate.

Some exogenous substances, such as chitosan, amino acids, carbohydrates, carbonate and bicarbonate salts have been studied to enhance bio-control capability of antagonists against fungal pathogens. Simultaneous application of chemicals and bio-control agents could provide more effective means of control and consistent results than that of one approach alone. The objective of the present study was to evaluate and compare the bio-control efficacy of *Bacillus subtilis* (CFBP 4228) with and without sodium bicarbonate (SBC) against *P. expansum* on apple fruits. The addition of 3% (w/v) SBC in the suspension of *B. subtilis* completely inhibited spore germination of *P. expansum* in potato dextrose broth medium. In combination with *B. subtilis*, SBC exhibited a consistent ability to enhance the biocontrol performance of antagonist against *P. expansum*. Lesion diameter of apple fruits treated with mixture of *B. subtilis* and SBC was

		`		um expa	nsum)	Grey mould (Botrytis cinerea)					
		storage			Market condition (days) 5	I Cold storage (days)				Market condition (days)	Mean
Treatments	0	30	60	90		0	30	60	90	5	treatments
Season 2011											
Control (water)	0.0p	5.5l	19.4e	57.9a	48.70b	0.0p	2.8n	15.40g	30.0c	25.20d	20.5a
Sodium bicarbonate at 2.0 (%)	0.0p	2.9n	8.5j	11.6h	9. 8 0i	0.0p	1.20	4.10m	6.8k	5.71	5.1c
Potassium sorbate at 2.0 (%)	0.0p	3.6m	11.8h	19.0e	16.20f	0.0p	1.80	5.5 l	9.6i	8.10j	7.6b
Sodium bicarbonate at 2.0 (%) +	0.0p	0.0p	0.0p	0.0p	0.00p	0.0p	0.0p	0.00p	0.0p	0.00p	0.0d
Potassium sorbate											
at 2.0 (%)											
		storage								Market condit	
Mean	0		30		60	90	•			5	
Period	0.0e		2.2d		8.1c	16.9a				14.20)b
Fungi 10.75a		a								5.8	lb
	Blue	mould (.	Penicilli	um expa	insum)	Grey	mould	(Botrytis		,	
	Cold s	storage	(days)		Market condition (days)	Cold	storage	e (days)		Market condition (days)	
Treatments	0	30	60	90	5	0	30	60	90	5	Mean treatments
Season 2012					-					-	
Control (water)	p0.0	6.51	23.0e	68.6a	57.7b	0.0q	3.3n	18.2g	35.50	29.90d	24.27a
Sodium bicarbonate at 2.0 (%)	0.0q	3.4n	10.1j	13.7h	11.6i	0.0q	1.4p	4.9m	8.10	x 6.801	6.00c
Potassium sorbate at 2.0 (%)	0.0q	4.3m	14.0h	22.5e	18.9f	0.0q	2.10	6.51	11.40i	9.60j	8.93b
Sodium bicarbonate at 2.0 (%) + Potassium sorbate	0.0q	0.0q	0.0q	0.0q	0.0q	0.0q	0.0q	0.0q	0.000	1 0.00q	0.00d
at 2.0 (%)	Cold s	torage (days)							Market condit	ion (days)
Mean	0		30			90				5	
Period	0.0e		2.63d		9.59c	19.9	98a			16.8	lb
Fungi	12.72a									6.8	

Table 2: Effect of sodium bicarbonate and potassium sorbate on disease infection percentage of Le Cont pear fruits during cold storage at $0\pm1^{\circ}$ C and under market conditions at $20\pm2^{\circ}$ C during 2011 and 2012 seasons

Means followed by the same letters are not significantly different by Duncan multiple range test at $0.05 \ {\rm level}$

significantly reduced, in contrast to inoculation with B. subtilis alone. The results of this study showed that combination of B. subtilis and SCB provided a more effective control on P. expansion than applying the antagonist or SBC alone and can be used as a non chemical alternative treatment against blue mold on apple fruits (Zivkovic et al., 2013).

Bio-control activity of *C. laurentii* or *T. pullulans* against postharvest decay caused by *P. expansum* and *A. alternata* in pear fruits was significantly increased when *C. laurentii* or *T. pullulans* combined with SBC. Combining *C. laurentii* or *T. pullulans* with SBC provided a more effective control on *P. expansum* and *A. alternata* than applying the antagonistic yeast or SBC alone. Effects of *C. laurentii* with and without SBC on controlling *P. expansum* and *A. alternata* were better than those of *T. pullulans*. *C. laurentii* in combination with SBC showed the best control of disease caused by A. alternata in pear fruits (Yao *et al.*, 2004).

Effect of sodium bicarbonate (SBC) and/or potassium sorbate (KS) on characteristics of Le Conte pear fruits under cold storage and through marketing at room temperature: Loss in fruit weight percentage: The loss in weight of Le Conte pear fruits at cold storage and 5 days during shelf life are presented in Table 3. The data reveal that, the loss in fruit weight increased as storage period advanced under cold storage or at room temperature. Thus, all the applied treatments reduced the loss in fruit weight than the control. Since, the loss percentage in fruit weight of the untreated fruits were 3.54 and 3.76% after 90 days of cold storage and these were 5.10 and 4.91% after 5 days as marketing in both seasons, respectively.

The lowest significant values of weight loss percentage were recorded by the combined applications of SBC+KS ranged 2.29 and 2.14% after 90 days of cold storage and these were 4.08 and 3.80% after 5 days as marketing in the two seasons, respectively.

In contrast, potassium sorbate and sodium bicarbonate are food additives which have inhibitory action against fruit postharvest pathogens and are more fungistatic than fungicidal and not very persistent (Palou *et al.*, 2002). Control of citrus green mold and sour rot by potassium sorbate was similar to that of sodium bicarbonate when they were used at similar concentrations. Also, combination resulted in an additive increase in their effectiveness, particularly when fruits were immersed in these solutions.

	Cold storag	Cold storage (days)						
Treatments	0	30	60	90	5			
Season 2011								
Control (water)	0.0j	1.99f	2.46c	3.54a	5.10a			
Sodium bicarbonate at 2.0%	0.0j	1.66h	2.03f	2.53c	4.26c			
Potassium sorbate at 2.0%	O.Oj	1.86g	2.41c	2.76b	4.79b			
Sodium bicarbonate at 2.0% +	O.Oj	1.32i	2.16e	2.29d	4.08c			
Potassium sorbate at 2.0%								
Mean	0.00d	1.70c	2.26b	2.78a	-			
Season 2012								
Control	0.0k	2.13g	2.83b	3.76a	4.91a			
Sodium bicarbonate at 2.0%	0.0k	1.58i	2.30f	2.43e	3.93c			
Potassium sorbate at 2.0%	0.0k	1.98h	2.56d	2.69c	4.54b			
Sodium bicarbonate at 2.0% +	0.0k	1.19j	2.11g	2.14g	3.80d			
Potassium sorbate at 2.0%								
Mean	0.00d	1.72c	2.45b	2.75a	-			

Table 3: Effect of sodium bicarbonate and potassium sorbate on weight loss percentage of Le Cont pear fruits during cold storage and under market conditions during 2011 and 2012 seasons

Means followed by the same letters are not significantly different by Duncan multiple range test at 0.05 level

	Cold storage	Market condition (days)			
Treatments	0	30	60	90	5
Season 2011					
Control	0.0h	0.0h	4.91d	7.00a	33.42a
Sodium bicarbonate at 2.0%	0.0h	$0.0\mathbf{h}$	3. 8 1f	5.19c	19.00c
Potassium sorbate at 2.0%	0.0h	0.0h	4.46e	6.63b	23.17b
Sodium bicarbonate at 2.0% +	$0.0\mathbf{h}$	0.0h	2.90g	3.85f	13.56d
Potassium sorbate at 2.0%					
Mean	0.0c	0.0c	4.02b	5.66a	-
Season 2012					
Control	0.0g	0.0g	4.39d	7.30a	31.95a
Sodium bicarbonate at 2.0%	0.0g	0.0g	3.29e	5.00c	24.26c
Potassium sorbate at 2.0%	0.0g	0.0g	3.97d	6.11b	28.84b
Sodium bicarbonate at 2.0% +	0.0g	0.0g	2.44f	3.07e	17.14d
Potassium sorbate at 2.0%					
Mean	0.00c	0.00c	3.52b	5.37a	-

Table 4: Effect of sodium bicarbonate and potassium sorbate on decay percentage of Le Cont pear fruits during cold storage and under market conditions during 2011 and 2012 seasons

Means followed by the same letters are not significantly different by Duncan multiple range test at 0.05 level

Decay percentage: It is clear from Table 4 that all dipping treatments did not present any decayed fruits till 30 days of cold storage in both seasons. Since, all treatments significantly reduced the percentage of decayed fruits than the untreated ones either after 90 days of cold storage or 5 days during shelf life at room temperature in both seasons. Thus, the percentage of decayed fruits for the control were 7.0 and 7.30% after 90 days of cold storage but it reached about 33.42 and 31.95% through marketing in both seasons. Yet, dipping Le Conte pear fruits in combined applications of sodium bicarbonate and potassium sorbateat 2% significantly reduced decay percentage during storage period after 90 days of cold storage (3.85 and 3.07%) but it was 13.56 and 17.14% during marketing in both seasons, respectively.

Gabler and Smilanick (2001) demonstrated the potential of carbonate and bicarbonate salts for the control of post-harvest gray mold on grapes.

The mode of action of sorbate could be through the alteration of the morphological structure of the cell, genetic changes, cell membrane alterations, inhibition of cell transport processes and inhibition of enzymes involved in metabolism of transport functions. However, inhibition of microorganisms by sorbates varies, depending on species and strain differences, extent of contamination, type and composition of the substrate, concentration and pH of sorbate, water activity, presence of other additives, temperature of storage and type of packaging.

Firmness lb inch⁻²: Fruit firmness testing is currently the main method used to determine pear maturity. Table 5 describes clearly that, fruit firmness was reduced as storage period advanced under cold storage or through shelf life at room temperature. The data also confirm that all treatments used significantly reduced changes in fruit firmness than the control at cold storage or during shelf life at room temperature through the two seasons. However, the reduction in fruit firmness was higher during shelf life at room temperature than under cold storage. Thus, fruit firmness for the control were 8.26 and 8.30 lb inch⁻² after 90 days of cold storage but it reached

	Cold storage	Market condition (days)			
Treatments	0	30	60	90	5
Season 2011					
Control	14.00a	12.46cd	10.30g	8.26i	4.56b
Sodium bicarbonate at 2.0%	14.00a	13.36ab	11.66ef	10.16g	5.30a
Potassium sorbate at 2.0%	14.00a	13.53ab	11.86de	10.10g	5.53a
Sodium bicarbonate at 2.0% +	14.00a	13.13bc	11.03f	9.10h	4.90ab
Potassium sorbate at 2.0%					
Mean	14.00a	13.12b	11.21c	9.40d	-
Season 2012					
Control	13.66a	12.20b	10.20d	8.30e	4.16b
Sodium bicarbonate at 2.0%	13.66a	13.06a	11.46c	10.20d	5.26a
Potassium sorbate at 2.0%	13.66a	13.03a	11.20c	9.60d	5.30a
Sodium bicarbonate at 2.0% +	13.66a	13.03a	11.13c	9.40d	5.00a
Potassium sorbate at 2.0%					
Mean	13.66a	12. 8 3b	10.99c	9.37d	-

Table 5: Effect of sodium bicarbonate and potassium sorbate on fruit firmness Ib inch⁻² of Le Cont pear fruits during cold storage and under market conditions during 2011 and 2012 seasons

Means followed by the same letters are not significantly different by Duncan's multiple range test at 0.05 level

about 4.56 and 4.16 lb inch⁻² through marketing in both seasons. Furthermore, treated fruits with sodium bicarbonate at 2.0% maintained fruit firmness (10.16 and 10.20 lb inch⁻²) after 90 days of cold storage.

The inhibitory effect of bicarbonate salts on postharvest pathogens is probably due to the reduction of fungal cell turgor pressure that results in collapse and shrinkage of hyphae and spores and consequent inability of fungi. Loss of firmness is one of the main factors limiting quality and the postharvest shelf life of fruits. Abdel Wahab and Rashid (2012) reported that, coating Navel orange fruits with potassium sorbate in wax or sorbate alone may protect fruit skin from decay and thus delaying dehydration and changes loss in the firmness and improve quality.

Soluble Solids Content (SSC%): Concerning to the effect on SSC, data from Table 6 showed that soluble solids content in fruit juice of Le Conte pear was gradually increased as storage period prolonged either after cold storage or during shelf life at room temperature. Since, all treatments gave somewhat higher values of SSC in fruit juice than the untreated fruit which ranged 14.0 and 14.50% after 90 days of cold storage and it were 13.56 and 14.30% after 5 days as marketing in both seasons under the study. The data also disclose that, potassium sorbate application at 2.0% gave a, somewhat, increment in SSC values in fruit juice averaged 14.40 and 14.93% after 90 days of cold storage and it was 14.0 and 14.50% after 5 days as marketing in both seasons, respectively.

Furthermore, the percent of SSC in fruit juice was gradually increased as storage periods advanced, either a cold storage or during marketing in both seasons. Pear is a climacteric fruit that tend to have increased SSC until maximum is reached at the fully ripe stage, followed by a decreasing trend when the fruits reaches full senescence (Xiao *et al.*, 2011). Evaluation of the use of sodium foliar applications of potassium sorbate or other sources of potassium to grapes were reported to accelerate accumulation of soluble solids, reduce berry size and increase titratable acidity (Strydum and Loubser, 2008).

	Cold storag	Market condition (days)			
Treatments	0	30	60	90	5
Season 2011					
Control	12.90d	13.30bd	13. 8 3ad	14.00ac	13.56a
Sodium bicarbonate at 2.0%	12.90d	13.50ad	14.23ab	14.26a	13.70a
Potassium sorbate at 2.0%	12.90d	13.76ad	13.90ac	14.40a	14.00a
Sodium bicarbonate at 2.0% +	12.90d	13.20cd	14.00ac	14.10a	13. 8 3a
Potassium sorbate at 2.0%					
Mean	12.90c	13.44b	13.99a	14.19a	-
Season 2012					
Control	13.46g	13.90eg	14.20cf	14.50ad	14.30a
Sodium bicarbonate at 2.0%	13.46g	13.80eg	14.30be	14.80ab	14.43a
Potassium sorbate at 2.0%	13.46g	13.73fg	14.10df	14.93a	14.50a
Sodium bicarbonate at 2.0% +	13.46g	13.80eg	14.30be	14.70ac	14.33a
Potassium sorbate at 2.0%					
Mean	13.46d	13.80c	14.22b	14.73a	-

Table 6: Effect of sodium bicarbonate and potassium sorbate on SSC% of Le Cont pear fruits during cold storage and under market conditions during 2011 and 2012 seasons

Means followed by the same letters are not significantly different by Duncan multiple range test at 0.05 level

Table 7: Effect of sodium bicarbonate and potassium sorbate on titratable acidity percentage of Le Cont pear fruits during cold storage and under market conditions during 2011 and 2012 seasons

	Cold storag	e (days)			Market condition (days)
Treatments	0	30	60	90	5
Season 2011					
Control	0.263a	0.250bc	0.238ef	0.227h	0.224ab
Sodium bicarbonate at 2.0%	0.263a	0.246cd	0.240ef	0.235fg	0.229a
Potassium sorbate at 2.0%	0.263a	0.249c	0.233g	0.227h	0.221b
Sodium bicarbonate at 2.0% +	0.263a	0.254b	0.242de	0.239ef	0.228a
Potassium sorbate at 2.0%					
Mean	0.263a	0.250b	0.238c	0.232d	-
Season 2012					
Control	0.261a	0.253c	0.241d	0.235e	0.230a
Sodium bicarbonate at 2.0%	0.261a	$0.254 \mathrm{bc}$	0.244d	0.241d	0.228a
Potassium sorbate at 2.0%	0.261a	0.259ab	0.241d	0.233e	0.228a
Sodium bicarbonate at 2.0% +	0.261a	0.253c	0.245d	0.241d	0.230a
Potassium sorbate at 2.0%					
Mean	0.261a	0.254b	0.241c	0.237d	-

Means followed by the same letters are not significantly different by Duncan multiple range test at 0.05 level

Titratable Acidity (TA): From Table 7, it's clear that the content of total acidity in fruit juice was decreased as a storage period advanced from harvest till 90 day at cold storage or during shelf life at room temperature which may be attributed to the use of acids as substrate for respiration. The values of total acidity in fruit juice were almost lower during shelf life than those obtained at cold storage.

Moreover, the application of KS produced a lower acidity in fruit juice, since it ranged 0.227 and 0.233% after 90 days of cold storage and it was 0.221 and 0.228% after 5 days as marketing

in the two seasons, respectively but the reduction was unpronounced. Moreover, there are no clear effect that are obtained between the applied treatments and the control at cold storage or during storage period in both seasons.

Abdel Wahab and Rashid (2012) reported that, coating Navel orange fruits with sorbate alone or potassium sorbate in wax inhibited the decline of TA significantly. Therefore, these treatments inhibited respiration of fruits and subsequent decline production of acids and protect fruit skin from decay, thus delaying ripening and improve quality.

Total sugar: Concerning to the effect on total sugar, Table 8 showed that total sugar content in fruit juice of Le Conte pear was gradually increased as storage period prolonged either after cold storage or during shelf life at room temperature.

Since, all treatments produced higher significant values of total sugar in pear fruits than the untreated fruits which ranged 9.84 and 10.11% after 90 days of cold storage and it was 10.66 and 11.37% after 5 days as marketing in both seasons under the study.

The data also disclose that, potassium sorbate application at 2.0% produced higher values of total sugars compare to the applied treatments averaged 11.84 and 12.18% after 90 days of cold storage and it was 12.88 and 13.17% after 5 days as marketing in both seasons, respectively. Sugars are the most important constituent of fruit product and are essential factor for the flavor of the food product and also act as a natural food preservative. Potassium sorbate protected fruit skin from decay, inhibited respiration of fruits during the storage period and subsequent decline production of acids by slowing down the metabolism activity and physiological disorders (Lin *et al.*, 2008). So, these results illustrate the reason for increasing total sugars.

Chlorophyll A content: Table 9 presented that chlorophyll A in pear fruits decreased with storage period advanced during cold storage or under room temperature. Since, most treatments applied maintained the content of chlorophyll A in the skin of pear fruits than the control either

	Cold storag	ge (days)	Market condition (days		
Treatments	0	30	60	90	5
Season 2011					
Control	8.35i	8.74h	9.20fg	9. 84 d	10.66d
Sodium bicarbonate at 2.0%	8.35i	8.70h	9.33ef	10.48c	11.40c
Potassium sorbate at 2.0%	8.35i	9.19fg	9.94d	11.84a	12.88a
Sodium bicarbonate at 2.0% +	8.35i	9.01g	9.51e	11.20b	12.00b
Potassium sorbate at 2.0%					
Mean	8.35d	8.91c	9.49b	10. 84 a	-
Season 2012					
Control	8.13j	8.73i	9.43g	10.11e	11.37c
Sodium bicarbonate at 2.0%	8.13j	9.15h	9.69f	10.87c	11.90b
Potassium sorbate at 2.0%	8.13j	9.60f	10.37d	12.18a	13.17a
Sodium bicarbonate at 2.0% +	8.13j	9.41g	10.06e	11.96b	12.87a
Potassium sorbate at 2.0%					
Mean	8.13d	9.22c	9.88b	11.28a	-

Table 8: Effect of sodium bicarbonate and potassium sorbate on total sugar percentage of Le Cont pear fruits during cold storage and under market conditions during 2011 and 2012 seasons

Means followed by the same letters are not significantly different by Duncan multiple range test at 0.05 level

	Cold storag	ge (days)	Market condition (days)		
Treatments	0	30	60	90	5
Season 2011					
Control	0.623a	0.533i	0.524j	0.507k	0.486c
Sodium bicarbonate at 2.0%	0.623a	0.591de	0.575g	0.563h	0.541b
Potassium sorbate at 2.0%	0.623a	0.594d	0.581f	0.561h	0.552ab
Sodium bicarbonate at 2.0% +	0.623a	0.617b	0.603c	0.587e	0.562a
Potassium sorbate at 2.0%					
Mean	0.623a	0.584b	0.570c	0.554d	-
Season 2012					
Control	0.610a	0.524g	0.513h	0.506h	0.472c
Sodium bicarbonate at 2.0%	0.610a	0.583cd	0.561ef	0.558ef	0.532b
Potassium sorbate at 2.0%	0.610a	0.580d	0.563e	0.554 f	0.545a
Sodium bicarbonate at 2.0% +					
Potassium sorbate at 2.0%	0.610a	0.595b	0.590 bc	0.579d	0.551a
Mean	0.610a	0.570b	0.557c	0.549d	-

Table 9: Effect of sodium bicarbonate and potassium sorbate on chlorophyll A (mg/100 g fresh weight) of Le Cont pear fruits during cold storage and under market conditions during 2011 and 2012 seasons

Means followed by the same letters are not significantly different by Duncan multiple range test at 0.05 level

at cold storage (0.507 and 0.506 mg/100 g fresh weight) or during shelf life (0.486 and 0.472 mg/100 g fresh weight). Whereas, the values of chlorophyll A during shelf life were almost lower than those obtained at cold storage during the both seasons of study.

Moreover, SBC+KS at 2% kept the content of chlorophyll A than all treatments or the control after 90 days of cold storage and 5 days during shelf life in both seasons. The values in these treatment reached about 0.587 and 0.579 mg/100 g fresh weight after 90 days of cold storage, respectively during both seasons. While, after 5 days during shelf life in the same period, the values averaged as 0.562 and 0.551 mg/100 g fresh weight in both seasons, respectively.

Chlorophyll is present in unripe fruits, which at the beginning of their development contain chloroplast in high amount in peel than in pulp. During ripening, the chloroplast are gradually disorganized as the chloroplast broken down, although, this phenomenon is almost ubiquitous occurring in the great majority of fruits, there are some exceptions, fruits which retain chlorophyll at the ripe stage, like apple, pear and goose berry cultivars (Gross, 1987).

Chlorophyll B content: Table 10 showed that chlorophyll B content decreased as storage period advanced from harvest till 90 days under cold storage and after 5 days during marketing at room temperature. Yet, the reduction of chlorophyll B during shelf life was lower than those obtained at cold storage during the both seasons of study.

In this respect, all treatments used, kept the content of chlorophyll B than the control. Yet, potassium sorbate at 2.0% maintained higher values of chlorophyll B than the other treatments used during cold storage and through shelf life. Since the values reached about 0.454 and 0.465 mg/100 g fresh weight after 90 days of cold storage while reached 0.448 and 0.458 mg/100 g fresh weight after 5 days during shelf life of both seasons. Furthermore, the untreated fruits produced a less value of chlorophyll B in both seasons than all treatments used either after 90 days of cold storage averaged about 0.400 and 0.398 mg/100 g fresh weight or after 5 days during shelf life presented 0.386 and 0.384 mg/100 g fresh weight.

	Cold storag	e (days)	Market condition (days)		
Treatments	0	30	60	90	5
Season 2011					
Control	0.535a	0.533a	0.414e	0.400f	0.3 8 6b
Sodium bicarbonate at 2.0%	0.535a	0.535a	0.466bc	0.451d	0.448a
Potassium sorbate at 2.0%	0.535a	0.535a	0.477b	0.454cd	0.448a
Sodium bicarbonate at 2.0% +	0.535a	0.535a	0.469b	0.447d	0.447a
Potassium sorbate at 2.0%					
Mean	0.535a	0.534a	0.456b	0.438c	-
Season 2012					
Control	0.514a	0.422i	0.409j	0.398k	0.3 84 c
Sodium bicarbonate at 2.0%	0.514a	0.483de	0.476ef	$0.456 \mathrm{h}$	0.443b
Potassium sorbate at 2.0%	0.514a	0.504b	0.489cd	$0.465~{ m g}$	0.458a
Sodium bicarbonate at 2.0% +	0.514a	0.493c	0.475 f	0.459gh	0.440b
Potassium sorbate at 2.0%					
Mean	0.514a	0.475b	0.462c	0.444d	-

Table 10: Effect of sodium bicarbonate and potassium sorbate on chlorophyll B (mg/100 g fresh weight) of Le Cont pear fruits during cold storage under market conditions during 2011 and 2012 seasons

Means followed by the same letters are not significantly different by Duncan multiple range test at 0.05 level

The disappearance of chlorophyll A and B during the maturation of pears was found to be a reaction of the first order. In the process, chlorophyll A decreased more rapidly than chlorophyll B. Changes in color, from green to red, are consequence of chlorophyll degradation and accumulation of large amount of carotenoids within the plastids as the chloroplast present in the mature green fruit are transformed into chromoplasts (Sampaio *et al.*, 2007).

Carotenoids content: It is clear from Table 11 that, carotenoids content in pear fruits increased slowly with storage period advanced from harvest till 90 days at cold storage and during shelf life at room temperature. Yet, the values of total carotenoid during shelf life were higher than those obtained at cold storage during the both seasons of study. Data also reveal that all treatments used gave less values of total carotenoids than the control under cold storage or at room temperature.

SBC+KS at 2% gave a, somewhat, decrement in total carotenoids since its averaged about 0.876 and 0.869 mg/100 g fresh weight after 90 days of cold storage while reached 0.889 and 0.881 mg/100 g fresh weight after 5 days during shelf life of both seasons. Furthermore, the untreated fruits gave a higher value of total carotenoids reached about 0.903 and 0.895 mg/100 g fresh weight after 90 days of cold storage while reached 0.918 and 0.904 mg/100 g fresh weight after 5 days during shelf life of both seasons. It was observed that the behavior of chlorophyll and carotenoide pigments during storage were continuous decrease in chlorophyll and a continuous increase in the carotenoide content of the fruit.

The loss of the green color of chlorophyll in the fruit is a result of the degradation of its structure caused by the change in pH, presence of the oxidation system and the activity of chlorophyllase enzyme (Sampaio *et al.*, 2007). Since, Simon (1997) found that carotenoid are protecting the chlorophyll from photo-oxidation and are accessory, light harvesting pigments and photoreceptors.

	Cold storage	e (days)			Market condition (days)
Treatments	0	30	60	90	б
Season 2011					
Control	0. 8 31c	0.858b	0.866b	0.903a	0.918a
Sodium bicarbonate at 2.0%	0. 8 31c	0. 8 63b	0.873b	0. 8 99a	0.904a
Potassium sorbate at 2.0%	0. 8 31c	0.869b	0.869b	0. 8 96a	0.908a
Sodium bicarbonate at 2.0% +	0.831c	0.873b	0.873b	0. 8 76b	0. 88 9a
Potassium sorbate at 2.0%					
Mean	0.831c	0.865b	0.870b	0. 8 93a	-
Season 2012					
Control	0. 8 25e	0.850d	0. 8 41de	0. 8 95a	0.904a
Sodium bicarbonate at 2.0%	0. 8 25e	0.852cd	0.872b	0. 8 90a	0.900a
Potassium sorbate at 2.0%	0.825e	0.850d	0.881ab	0. 8 93a	0. 8 99a
Sodium bicarbonate at 2.0% +	0.825e	0.869bc	0.880ab	0.869bc	0. 88 1a
Potassium sorbate at 2.0%					
Mean	0. 8 25d	0.855c	0.868b	0.886a	-

Table 11: Effect of sodium bicarbonate and potassium sorbate on carotenoid (mg/100 g fresh weight) of Le Cont pear fruits during cold and under market conditions during 2011 and 2012 seasons

Means followed by the same letters are not significantly different by Duncan multiple range test at 0.05 level

CONCLUSION

Based on the results of this study, it can be concluded that postharvest dipping with sodium bicarbonate (SBC) and/or potassium sorbate (KS) at 2% alone or in combination had more pronounced effect on quality of Le Cont pear fruits than the control either at cold storage or through marketing at room temperature. Dipping of a mixture containing SBC and KS gave the best results for reducing disease infection of *Penicillium expansum* and *Botrytis cinerea*, fruit loss weight and decay percentage keeping the quantity of chlorophyll A and carotenoids content after cold storage and 5 days during marketing at room temperature compared with the other treatments used or the control.

REFERANCES

AOAC, 2005. Official Methods of Analysis. 16th Edn., AOAC, Washington, DC., USA.

- Abdel Wahab, S.M. and I.S.A. Rashid, 2012. Safe postharvest treatments for controlling *Penicillium molds* and its impact maintaining Navel orange fruit quality. Am. Eurasian J. Agric. Environ. Sci., 12: 973-982.
- Calvo, J., V. Calvente, M. Edith de Orellano, D. Benuzzi and M.I.S. de Tosetti, 2007. Biological control of postharvest spoilage caused by *Penicillium expansum* and Botrytis *cinerea* in apple by using the bacterium *Rahnella aquatilis*. Int. J. Food Microbiol., 113: 251-257.
- Dombrink-Kurtzman, M.A. and A.E. McGovern, 2007. Species-specific identification of *Penicillium* linked to patulin contamination. J. Food Prot., 70: 2646-2650.
- El-Morsy, T.H.A., 1993. Ecological and physiological studies on fungi present in water and its relation to pollutants in Dakahlia province. M.Sc. Thesis, Botany Department, Faculty of Science, Mansoura University, Egypt.
- Ellis, M.B., 1971. Dematiaceous Hyphomycetes. 1st Edn., Commonwealth Mycological Institute, Kew, Surrey, UK., Pages: 608.

- FAO, 2011. Food and Agriculture Organization Production Year Book. Vol. 61, FAO, Washington, DC., USA.
- Gabler, F.M. and J.L. Smilanick, 2001. Postharvest control of table grape gray mold on detached berries with carbonate and bicarbonate salts and disinfectants. Am. J. Enol. Viticult., 52: 12-20.
- Gross, J., 1987. Pigments of Fruit: Food Science and Technology. Academic Press, Oxford, UK., pp: 167-191.
- Harker, F.R., J.H. Maindonald and P.J. Jackson, 1996. Penetrometer measurement of apple and kiwifruit firmness: Operator and instrument differences. J. Am. Soc. Hort. Sci., 121: 927-936.
- Janisiewicz, J.W. and L. Korsten, 2002. Biological control of postharvest diseases of fruits. Annu. Rev. Phytopathol., 40: 411-441.
- Karabulut, O.A., G. Romanazzi, J.L. Smilanick and A. Lichter, 2005. Postharvest ethanol and potassium sorbate treatments of table grapes to control gray mold. Postharvest Biol. Technol., 37: 129-134.
- Latifa, A., T. Idriss, B. Hassan, S.M. Amine, B. El Hassane and A.B.A. Abdellah, 2011. Effects of organic acids and salts on the development of *Penicillium italicum*: The causal agent of citrus blue mold. Plant Pathol. J., 10: 99-107.
- Lin, L., B. Wang, M. Wang, J. Cao, J. Zhang, Y. Wu and W. Jiang, 2008. Effects of a chitosan-based coating with ascorbic acid on post-harvest quality and core browning of Yali pears (*Pyrus bertschneideri* Rehd.). J. Sci. Food Agric., 88: 877-884.
- Mari, M., R. Gregoria and I. Donati, 2004. Postharvest control of *Monilinia laxa* and *Rhizospus stolonifer* in stone fruit by peracetic acid. Postharvest Biol. Technol., 33: 319-325.
- Moubasher, A.H., I.A. El-Kady and S.M. Farghally, 1977. The mycoflora of some Egyptian seeds and their potentialities for production of aflatoxins. Zeszyty Problemowe Postepow Nauk Rolniczych, 189: 141-147.
- Palou, L., J. Usall, J.L. Smilanick, M.J. Aguilar and I. Vinas, 2002. Evaluation of food additives and low-toxicity compounds as alternative chemicals for the control of *Penicillium digitatum* and *Penicillium italicum* on citrus fruit. Pest Manage. Sci., 58: 459-466.
- Ranganna, S., 1979. Manual of Analysis of Fruit and Vegetable Products. 2nd Edn., Tata McGraw-Hill Publ. Co. Ltd., New Delhi, India, Pages: 634.
- Sampaio, S.A., P.S. Bora, H.J. Holschuh and S.D.M. Silva, 2007. Postharvest respiratory activity and changes in some chemical constituents during maturation of yellow mombin (*Spondias mombin*) fruit. Food Sci. Technol. (Campinas), 27: 511-515.
- Simon, P.W., 1997. Plant pigments for color and nutrition. HortScience, 32: 12-13.
- Singh, K., J.C. Frisvad, U. Thrane and S.B. Mathur, 1991. An Illustrated Manual on Identification of Some Seed-Borne Aspregilli, Fusaria, Penicillia and their Mycotoxins. Danish Government Institute for Seed Pathology for Developing Countries, Hellerup, Denmark, ISBN-13: 9788770263177, Pages: 133.
- Smilanick, J.L., M.F. Mansour, F.M. Gabler and D. Sorenson, 2008. Control of citrus postharvest green mold and sour rot by potassium sorbate combined with heat and fungicides. Postharvest Biol. Technol., 47: 226-238.
- Sofos, J.N. and F.F. Busta, 1993. Sorbic Acid and Sorbates. In: Antimicrobials in Foods, Davidson, P.M. and A.L. Branen (Eds.). 2nd Edn., Marcel Dekker, New York, USA, pp: 49-94.
- Strydum, G.J. and J.T. Loubser, 2008. The effect of metalosate calcium and metalosate potassium on sugar accumulation in Waltham Cross (table grapes). Proceedings of the Albion Conference on Plant Nutrition, (ACPN'08), Midway, UT., USA., pp: 141-156.

- Swindeman, A.M., 2002. Fruit packing and storage loss prevention guidelines. Postharvest Information Network, Tree Fruit Research and Extension Center, USA., pp: 1-9. http://postharvest.tfrec.wsu.edu/REP2002D.pdf.
- Troncoso-Rojas, R. and M.E. Tiznado-Hernandez, 2007. Natural Compounds to Control Fungal Postharvest Diseases. In: Recent Advances in Alternative Postharvest Technologies to Control Fungal Diseases in Fruits and Vegetables, Troncoso-Rojas, R., M.E. Tiznado-Hernandez and A. Gonzalez-Leon (Eds.). Transworld Research Network, Trivandrum, Kerala, India, pp: 127-156.
- Usall, J., J. Smilanick, L. Palou, N. Denis-Arrue, N. Teixido, R. Torres and I. Vinas, 2008. Preventive and curative activity of combined treatments of sodium carbonates and *Pantoea agglomerans* CPA-2 to control postharvest green mold of citrus fruit. Postharvest Biol. Technol., 50: 1-7.
- Xiao, Z., Y. Luo, Y. Luo and Q. Wang, 2011. Combined effects of sodium chlorite dip treatment and chitosan coatings on the quality of fresh-cut d'Anjou pears. Postharvest Biol. Technol., 62: 319-326.
- Yao, H., T. Tian and Y. Wang, 2004. Sodium bicarbonate enhances biocontrol efficacy of yeasts on fungal spoilage of pears. Int. J. Food Microbiol., 93: 297-304.
- Zivkovic, S., V. Gavrilovic, S. Stosic, D. Delic and N. Dolovac, 2013. Control of *Penicillium exapansum* by combining *Bacillus subtilis* and sodium bicarbonate. Proceedings of the 4th International Symposium on Agrosym, October 3-6, 2013, Jahorina, pp: 535-539.