

Effect of a Biocontrol Agent and Modified Atmosphere on Postharvest Control Decay and Quality Retention of Peach during Storage and Marketing Life

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ABSTRACT

The efficacy use of biological control as *Candida saitoana* or *Candida shehatae* yeasts as individual treatments or in combinations with active generated modified atmospheres were investigated to control gray mold and Rhizopus rot on 'Florida prince' peaches caused by *Botrytis cinerea* and *Rhizopus stolonifer*, respectively, under *in vitro* as well as their effects on postharvest quality of peach fruits under *in vivo* condition. The tested modified atmospheres (MA) were 5% O₂ + 5% CO₂ + 90% N₂, 10% O₂ + 5% CO₂ + 85% N₂ or 5% O₂ + 10% CO₂ + 85% N₂. Peach fruits either naturally infected or artificially inoculated with *B. cinerea* or *R. stolonifer* were subjected to such yeast and/or MA treatments, then packed in polypropylene punnets and placed inside low density polyethylene bags. Disease incidence and quality parameters including weight loss, fruit flesh firmness, soluble solids content (SSC), titratable acidity (TA), and vitamin C were determined at the end of cold storage at 0°C and 90-95% relative humidity (RH) for 45 days, followed by 5 days at 25°C and 75% RH as market life period. *In vitro* investigation on PDA medium, individual treatment of modified atmosphere containing 10% O₂ + 5% CO₂ was the most suppressive against gray mold and rhizopus rots on peaches stored for 45 days during both seasons. Yeasts *C. saitoana* and *C. shehatae* were the most suppressive individual treatments against decay during cold storage or market life. The combination of different modified atmospheres with *C. shehatae* increased the suppressive effect against *B. cinerea* on peaches cold stored or market life comparing with such treatment individually. The results *in vivo* showed that peaches treated with the combination of two yeasts (*Candida saitoana* or *Candida shehatae*) and modified atmosphere (5% O₂ + 10% CO₂ + 85% N₂) were the most effective treatments to control *Botrytis cinerea* and *Rhizopus stolonifer* infections and significantly reduced postharvest losses of fruit as delayed decay, loss of weight, firmness, retained titratable acidity and preserved vitamin C, prevented increase soluble solids content and assuring good peaches quality during storage, marketability period and could be used as alternatives to chemicals control.

Key words: Peach, rhizopus rot, gray rot, biological control, modified atmosphere, postharvest decay; yeast; quality

Introduction

Peach (*Prunus persica* (L.) Batsch) is one of the most promising perishable fruit crops either of local consumption or for export. Storing, shipping and marketing of peaches require specific conditions to maintain fruit quality and its marketability. Deterioration of peaches occurs due to decay development incited by several fungal pathogens and due to physicochemical development after harvest toward senescence. The main postharvest losses of peaches are mainly occurred due to fungal infection incited by *Botrytis cinerea* causing gray mold, *Penicillium expansum* causing blue mold, *Rhizopus stolonifer* causing *Rhizopus* rot and *Monilinia fructicola* causing brown rot that can gravely damage, in particular during longer storage periods (Valero and Serrano, 2010). Use of synthetic fungicides is becoming more restricted due to health and environmental concerns, which necessitate developing safer and eco-friendly alternatives to reduce the environmental risk and satisfy national and international consumer demands. Yeasts stand out among microorganisms as potential agents to control plant diseases due to their ability to colonize the fruit surface (Magri *et al.*, 2011). Several studies investigated use of yeast antagonists for biological control of postharvest diseases of stone fruits and consider it one of the most viable alternatives to fungicides (Karabulut, *et al.*, 2002; Spotts *et al.*, 2002, and Zhang *et al.*, 2007).

On the other hand, Modified atmosphere storage containing 5% O₂ + 10% CO₂ was highly effective to control the growth of molds after 15 days storage of sweet cherry fruits (Serradilla *et al.*, 2013). Postharvest treatment using modified atmosphere with low oxygen (O₂) and/or high carbon dioxide (CO₂) concentrations

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lowered down the respiration rate, inhibited ethylene production that induce senescence of fruits and retarded the decrease in titratable acidity (TA) values, maintained fruit flesh firmness, solid soluble content (SSC), vitamin C and delayed fruit deterioration through decreasing fruit injury rates and extended the storage period of many fruits as peach (Pablo and Trujillo, 1997; Arrebol *et al.*, 2010), peach and nectarine (Zoffoli *et al.*, 1998; Akbudak and Eris, 2004), cherry (Tian *et al.*, 2004; Serrano *et al.*, 2005; Khorshidi *et al.*, 2011 and Serradilla *et al.*, 2013), loquat (Amorós *et al.*, 2008), also (Guan and Dou, 2010; Valero and Serrano, 2010 and Díaz-Mula *et al.*, 2011) of plum.

The purpose of the present work to evaluate the efficacy of biological control as use of two yeasts antagonist (*Candida saitoana*) or (*Candida shehatae*) in as stand-alone treatments or various combinations with active modified atmosphere at (5% O₂ + 5% CO₂ + 90% N₂), (10% O₂ + 5% CO₂ + 85% N₂) or (5% O₂ + 10% CO₂ + 85% N₂) *in vitro* as well as *in vivo* to control postharvest rots caused by *B. cinerea* (gray mold) *R. stolonifer* (rhizopus rot) and the effect on quality parameters of peaches during cold storage and market life.

Materials and Methods

Peaches source

'Florida prince' peach fruits were obtained from a private orchard (Maba) in Alexandria road district, Giza Governorate, Egypt. Fruits were harvested at optimal harvest time in the last week of April 2013 and 2014 in the full color stage and average weight of 102 gm. The Physicochemical properties evaluated at harvest time as values: Soluble Solids Content (SSC) [10.5 & 10.2%]; firmness [14.0 & 14.4 (lb/in²)]; titratable acidity (TA) [0.90 & 0.92%] and vitamin C (V.C.) [3.90 & 3.95 mg/100g F.W] in the two seasons, respectively. The fruits were delivered on the same day to the laboratory. Peaches used for experiments were divided into 3 groups prior to treatments. The first group was let for natural infection and the other two groups of peaches were inoculated with *B. cinerea* and *R. stolonifer* as artificial infection.

Fungal cultures

Pure cultures of *Botrytis cinerea* and *Rhizopus stolonifer* isolates obtained from diseases peaches by single spore technique and proved their pathogenicity on healthy peaches were used for *in vitro* investigations as well as artificial inoculation of fruit of storage experiments. Fungal isolates were maintained on potato dextrose agar medium (PDA).

Inoculation of Fruit with B. cinerea and R. stolonifer

The two groups of peaches were sterilized with 70% ethanol and wounded by puncturing the peel of each fruit on the equator with a template of 4 sterilized steel rods (2-mm deep by 0.5 mm diameter) in circle area of 5 mm diameter. One group of the punctured fruit was inoculated with *B. cinerea* and the last group was inoculated with *R. stolonifer*. The inoculation was carried out by spraying the surface of fruits with fungal spore suspensions of 10⁵ spores/ml of 7 days old cultures. Inoculated fruit were kept at room temperature and allowed to air dry for 24 hours, before postharvest treatments were applied, then treated with two yeasts as biological control.

Yeast tested for biological control of infected peach fruit rots caused by B. cinerea and R. stolonifer.

Isolates of *Candida saitoana* and *Candida shehatae* maintained on silica gel crystals in postharvest diseases laboratory for long-term storage were tested as biological agents to control postharvest diseases of peaches. The crystals were placed on PDA for 72 h under 12 h light and 12 h dark cycles. Yeast cells were swapped and washed with sterilized water containing Tween 20, then centrifuged at 3000 rpm for 15 min. Washing yeast cells and centrifugation was repeated three times. Yeast suspensions prepared with sterilized water containing one drop of Tween 20 were used at 10⁵ colony-forming units (CFU) ml⁻¹. Peaches allocated for biological control investigation were sprayed with yeast spore suspension. Fruits were then dried for 1 h in a laminar flow hood and stored under air or Modified atmosphere or treated with various combinations. Identification of selected yeast isolates showing antagonistic capabilities were identified by at the unit of Identification of the microorganisms, Plant Pathology Research Institute, ARC, using the Biology system.

Modified Atmosphere treatment:

Naturally infected or artificially inoculated fruits with *B. cinerea* or *R. stolonifer* were placed in polypropylene containers (punnets) perforated with 28 holes (0.8 mm- diameter). Each punnet contained about 5 fruits and placed in non-perforated low-density polyethylene bags (30µm thickness). Each bag contains one punnet. The polyethylene bags containing peaches were subjected to gas-flushing by Gas mixer enough to change the internal atmosphere to achieve gas mixtures of (5% O₂ + 5%

CO₂+90%N₂), (10% O₂ + 5% CO₂+85%N₂) and (5% O₂ + 10% CO₂+85%N₂) comparing with the control as normal air containing 21% O₂ + 0.03% CO₂ +78.97 % N₂. Gas mixtures concentrations were measured and adjusted using O₂/CO₂ gas analyzer (Dualtrak model 902D).

Postharvest Treatments of peach fruits with yeasts as biological Control and Modified Atmosphere in vivo:

Fruits under sub each group (naturally infected fruits (N.I), inoculated fruits with *Botrytis cinerea* (B.C) and inoculated fruits with *Rhizopus stolonifer*(R.S) were subjected to different individual or combination treatments of modified atmosphere and spray with two yeasts *Candida saitoana* and *Candida shehatae* as follows:

- 1- Modified Atmosphere (MA₁) at (5% O₂ + 5% CO₂+90%N₂).
- 2- Modified Atmosphere (MA₂) at (10% O₂ + 5% CO₂+85%N₂).
- 3- Modified Atmosphere (MA₃) at (5% O₂ + 10% CO₂+85%N₂).
- 4- *Candida saitoana*.
- 5- *Candida shehatae*.
- 6- *Candida saitoana* + modified atmosphere (MA₁) at 5% O₂ + 5% CO₂ + 90% N₂.
- 7- *Candida saitoana* + modified Atmosphere (MA₂) at 10% O₂ + 5% CO₂ + 85% N₂.
- 8- *Candida saitoana* + modified Atmosphere (MA₃) at 5% O₂ + 10% CO₂ + 85% N₂.
- 9- *Candida shehatae* + modified atmosphere (MA₁) at 5% O₂ + 5% CO₂ + 90% N₂.
- 10- *Candida shehatae* + modified Atmosphere (MA₂) at 10% O₂ + 5% CO₂ + 85% N₂.
- 11- *Candida shehatae* + modified Atmosphere (MA₃) at 5% O₂ + 10% CO₂ + 85% N₂.
- 12- Control BC, artificially inoculated peaches with *B. cinerea*.
- 13- Control RS, artificially inoculated peaches with *R. stolonifer*.
- 14- Control, naturally inoculated untreated peaches.

Storage conditions:

Each treatment contained three performed cartoon boxes ,one box to determine disease incidence, the second to determine weight loss and the third for fruits analysis, each box contained of (10 bags) was replicated three times, and the experiment was repeated twice (2013 and 2014 seasons). Peach fruits were subjected randomly to one of the above treatments and stored at 0°C and 90% RH for 45 days in laboratory of refrigeration Agriculture Development Systems (ADS) project in the Faculty of Agriculture, Cairo University.

Effect of yeast and modified atmosphere on growth of Botrytis cinerea and Rhizopus stolonifer in vitro:

The linear growth (mm) of *B. cinerea* and *R. stolonifer* as affected by yeast and modified atmosphere combinations was determined after incubation period, when growth of any culture covers the surface of the medium. Inhibition percentages of culture diameter caused by the tested treatments were calculated as follows:

$$\text{Inhibition (\%)} = \frac{\text{Average linear growth of control} - \text{average linear growth of treatment}}{\text{Average linear growth of control}} \times 100$$

Disease incidence assessment:

Disease incidence (%) of such treatment was determined according to Zeng *et al.* (2006). Peach fruit was counted decayed when the visible rot zone on fruit surface around the wounded puncture was more than 0.5 mm-wide. Efficiency of the tested treatments to control the postharvest diseases was calculated as follows:

$$\text{Efficiency (\%)} = \frac{\text{Mean disease incidence of control fruit} - \text{mean treatment disease incidence}}{\text{Mean control disease incidence}} \times 100$$

Fruit quality parameters of stored peach:

Physicochemical properties of the peaches of such treatments were estimated at the end of cold storage at 0°C for 45 days as well as marketability period as follows:

Weight loss%:

Fruits were periodically weighed and the loss in mass weight was recorded for each replicate. Data were calculated as percentage.

Fruit Firmness (lb/in²):

Fruit Firmness was measured on the two opposite sides of mandarin fruit samples by using a hand Magness Taylor pressure tester.

Soluble Solids Content (SSC) %:

Flesh of peach fruits was ground in an electric juice extractor for freshly prepared juice. Soluble solids content was measured using Digital refractometer PR32 (0.32% Atago Paleta ATago. CO. LTD. Japan).

Titrateable Acidity (TA) %:

Total acidity (expressed as malic acid %) was determined by titrating 5-ml juice with 0.1N sodium hydroxide using phenolphthalein as indicator (A.O.A.C., 2000).

Vitamin C (Ascorbic acid) mg/ 100 g F.W:

Vitamin C content was measured using 2, 5-6 dichlorophenol indophenols' method described by (A.O.A.C., 2000).

Marketing life:

At the end of storage 45 days at 0°C and 90% RH, fruits from every treatment of naturally infected fruits were held in ambient room at 25°C and 75 % relative humidity (RH) for 5 days to simulate handling and market conditions as quality measurements physicochemical properties.

Experimental design and statistical analysis:

The results of the parameters (The linear growth, Efficiency and Disease Severity) were analyzed using the software CoStat version 6.400- CoHort Software. The mean of all treatments were compared by the least significant difference (L.S.D.) at 5% level of probability according to (Gary, 2010). All results of physico-chemical parameters were performed in triplicate using completely randomized factorial design and Marketing life using completely randomized design. Data were analyzed with the Analysis of variance (ANOVA) procedure of MSTAT-C program. When significant differences were detected, treatment means were compared by LSD range test at the 5% level of probability in the two investigated seasons (Snedecor and Cochran, 1980).

Results

Effect of modified atmosphere and/or yeasts on linear growth of *Botrytis cinerea* and *Rhizopus stolonifer* in vitro:

Growth of *Botrytis cinerea* and *Rhizopus stolonifer* was strongly affected *in vitro* by modified CO₂ and O₂ concentrations in ambient atmosphere during incubation period at 25°C as shown in Table (1). It was clear that all tested concentrations of CO₂, O₂ and N₂ other than normal air reduced linear growth of *B. cinerea* and *R. stolonifer*. However, at a gas mixture of 10% O₂, 5% CO₂ and 85% N₂ was the most suppressive modified atmosphere on growth of *B. cinerea* and *R. stolonifer* comparing with normal air treatment (control).

Table 1: Effect of modified atmosphere and/or yeasts on linear growth of *Botrytis cinerea* and *Rhizopus stolonifer* in vitro

Treatments	Botrytis cinerea		Rhizopus stolonifer	
	Linear growth (mm)	Inhibition (%)	Linear growth (mm)	Inhibition (%)
MA1	22.67	74.81	42.33	52.96
MA2	18.67	79.25	25.67	71.48
MA3	29.33	67.41	57.67	35.92
<i>Candida saitoana</i>	0.00	100.00	90.00	0.00
<i>Candida shehatae</i>	0.00	100.00	90.00	0.00
<i>C. saitoana</i> +MA1	21.67	75.92	33.67	62.59
<i>C. saitoana</i> +MA2	0.00	100.00	0.00	100.0
<i>C. saitoana</i> +MA3	0.00	100.00	0.00	100.0
<i>C. shehatae</i> +MA1	10.00	88.88	12.67	85.92
<i>C. shehatae</i> +MA2	0.00	100.00	0.00	100.0
<i>C. shehatae</i> +MA3	0.00	100.00	0.00	100.0
Control	90.00	0.00	90.00	0.00
LSD at 5%	2.32		2.50	

MA = Modified atmosphere & MA₁ = 5 O₂ + 5 CO₂ + 90 N₂ & MA₂ = 10 O₂ + 5 CO₂ + 85 N₂ & MA₃ = 5 O₂ + 10 CO₂ + 85 N₂

The atmosphere containing concentrations of 5% of each CO₂ and O₂ reduced the growth of both fungi by more than 50%. Elevating the CO₂ concentration to 10% caused low suppression to the fungal growth especially for *R. stolonifer*, on contrary to increasing O₂ to 10% which highly suppressed the fungal growth, particularly *R. stolonifer*. On the other hand, both *Candida saitoana* and *C. shehatae* suppressed completely the growth of *B. cinerea in vitro*, while no suppressive effect was obtained on *R. stolonifer*. The combination of MA and *C. saitoana* did not show synergistic suppression on *B. cinerea* or *R. stolonifer* growth than when tested individually, while the combination between MA and *C. shehatae* caused synergistic effect to suppress the fungal growth of both *B. cinerea* and *R. stolonifer*. Moreover, *C. shehatae* alone did not cause any inhibition of *R. stolonifer*, while complete suppression was observed when tested under MA at 10% O₂ or 10% CO₂. Even at lower gas concentrations as 5% of both O₂ and CO₂, higher suppressive effect was obtained than either MA or yeast individually.

Use of modified atmosphere and/or yeasts to control gray mold and Rhizopus rot of peaches:

Keeping peaches at 0°C under modified atmospheres of high concentrations of CO₂ up to 10% markedly minimized infection of fruits with *B. cinerea* and *R. stolonifer* for 45 days followed by 5 days market life at 25°C for 5 days in seasons 2013 (Tables 2) and 2014 (Tables 3). First season data revealed that *B. cinerea* caused gray mold of about 50% of artificially inoculated peaches after cold storage and increased to 61% after the market life, while less disease incidence (37%) was determined after the cold storage, which reached 53% after the market life in the second season. On the other hand, *R. stolonifer* caused rhizopus rot 13% of artificially inoculated peaches after the cold storage, and 21% of inoculated fruits after the market life in the first season, while it was 9% and 18% rot of peaches after cold storage and market life, respectively, in the second season. In naturally infected peaches, disease incidence was about 9% when fruit were cold stored during both seasons, which was increased up to 13% and 15% after market life in both seasons, respectively.

The tested modified atmospheres showed high control of postharvest diseases comparing with the control. However, investigating the disease incidence of peaches stored under modified atmosphere revealed that MA containing 10% O₂: 5% CO₂: 85% N₂ was the most suppressive treatment, where it did not allow any fungus to cause postharvest diseases on naturally infected peaches as well as almost totally suppressed the infection with both *B. cinerea* and *R. stolonifer* on artificially inoculated fruits during the cold storage for 45 days and after market life in both seasons. However, efficacy of this treatment of MA was more than 80% for almost naturally infected or artificially inoculated peaches during both seasons, even after the market life. It is worth to mention that elevating CO₂ concentration up to 10% with reducing O₂ concentration to 5% showed less suppressive effect than when used at lower CO₂, i.e. at 5% and 10% O₂. On the other hand, increasing CO₂ concentration from 5% to 10% in presence of same concentration of O₂ at 5% caused more inhibition for postharvest diseases of peaches.

Yeasts *C. saitoana* and *C. shehatae* tested as biocontrol agents against gray mold and rhizopus rot of peaches showed high suppressive effect along the cold storage. In the first season as shown in Table (2), no decay was found on naturally infected peaches treated with such yeast when the fruits were stored for either 45 days at 0°C or during market life at 25°C for 5 days. In the second season (Table 3), yeasts showed efficacy to suppress decay development by more than 85% on naturally infected peaches. Simultaneously, two seasons investigations showed that development of gray mold and rhizopus rot on artificially inoculated fruits was highly suppressed by both tested yeasts during the market life with more than 89% efficacy. However, no superiority was emphasized for yeast on another to suppress the fungal decay on either naturally infected or artificially inoculated peaches along the market life test for 5 days.

Evaluating the efficacy of individual treatments, either modified atmospheres or yeasts showed that the modified atmosphere containing 10% O₂ + 5% CO₂ was the most effective treatment to suppress gray mold and *Rhizopus* rot on peaches cold stored for 45 days during both seasons, followed by treatment with *C. saitoana* yeast in the first season and *C. shehatae* for the second one (Tables 2 and 3). The market life investigation revealed that both tested yeasts were the most effective treatment to control decay on peaches incited by *B. cinerea* and *R. stolonifer*. On the other hand, modified atmosphere containing 5% O₂ + 5% CO₂ was the least suppressive treatment to control decay on peaches during such cold storage as well as market life periods.

The combination between modified atmosphere and *C. saitoana* did not show increase in suppressive effect against *B. cinerea* or *R. stolonifer* than each individual treatment when treated peaches were cold stored for 45 days during both seasons (Tables 2 and 3). However, increment of *R. stolonifer* infection was detected when the peaches were treated with *C. saitoana* and stored under modified atmosphere in the first season contrarily to the second one. During the market life, the

combination of modified atmosphere with *C. saitoana* resulted in higher suppressive effect against *B. cinerea* than when modified atmosphere adopted alone, but less than when the yeast was individually applied. On the other hand, modified atmosphere and *C. saitoana* combination showed negative effect on controlling *R. stolonifer* during the market life, except when the yeast was applied in combination with 5% O₂ + 5% CO₂ as positive suppressive effect was obtained.

The combination of different modified atmospheres with *C. shehatae* increased the suppressive effect against *B. cinerea* on peaches cold stored for 45 days or maintained at 25°C for 5 days as market life comparing with such treatment individually, but a little bit less than the individual yeast application for longer period of storage as shown in Tables (2 and 3). The opposite was true regarding the suppression of *R. stolonifer* during 45 day cold storage and 5 day market life, where negative effect obtained by the combination comparing with the treatments individually, except for the second season for the shorter storage period.

Table 2: Effect of modified atmosphere and/or yeasts on gray mold and *Rhizopus* rot of peaches after cold storage at 0°C for 45 days followed by 5 days at 25°C as market life, season 2013.

Treatments	Disease incidence (%) after 45 days-cold storage						Disease incidence (%) aftermarket life					
	Natural infection		A.I. <i>B. cinerea</i>		A.I. <i>R. stolonifer</i>		Natural infection		A.I. <i>B. cinerea</i>		A.I. <i>R. stolonifer</i>	
	D.I. %	E. %	D.I. %	E. %	D.I. %	E. %	D.I. %	E. %	D.I. %	E. %	D.I. %	E. %
MA1	3.3	66.6	6.7	86.7	3.3	74.4	5.3	60.0	15.0	75.4	4.7	77.8
MA2	0.0	100	0.0	100	0.0	100	0.0	100	9.0	85.2	2.7	87.3
MA3	0.0	100	3.3	93.3	3.7	71.8	2.0	85.0	16.7	72.6	5.7	73.0
<i>Candida saitoana</i>	0.0	100	2.0	96.0	0.7	94.9	0.0	100	4.3	93.0	2.3	89.0
<i>Candida shehatae</i>	0.0	100	3.3	93.3	1.1	91.6	0.0	100	5.0	91.8	1.7	92.1
<i>C. saitoana</i> +MA1	0.0	100	7.7	84.7	5.0	61.5	0.0	100	12.7	79.2	6.0	71.4
<i>C. saitoana</i> +MA2	0.0	100	1.3	97.3	0.0	100	0.0	100	5.3	91.3	0.7	96.9
<i>C. saitoana</i> +MA3	0.0	100	2.7	94.7	5.0	61.5	3.0	77.5	5.7	90.7	7.3	65.1
<i>C. shehatae</i> +MA1	1.7	82.1	4.7	90.7	4.0	69.2	2.3	82.5	8.0	86.9	7.7	63.5
<i>C. shehatae</i> +MA2	0.0	100	0.0	100	3.3	74.4	0.0	100	5.7	90.7	6.3	69.9
<i>C. shehatae</i> +MA3	0.0	100	3.3	93.3	5.0	61.5	1.7	87.5	6.0	90.2	7.7	63.5
Control	9.3	0.0	50.0	0.0	13.0	0.0	13.3	0.0	61.0	0.0	21.0	0.0
LSD at 5%	2.4		0.5		3.4		5.0		5.4		5.2	

A.I. = artificially inoculation with: *B.C* (*Botrytis cinerea*) or *RS* (*Rhizopus stolonifer*)

D.I. = Disease incidence & E. = Efficiency

MA = Modified atmosphere & MA₁ = 5 O₂ + 5 CO₂ + 90 N₂ & MA₂ = 10 O₂ + 5 CO₂ + 85 N₂ & MA₃ = 5 O₂ + 10 CO₂ + 85 N₂

Table 3: Effect of modified atmosphere and/or yeasts on gray mold and *Rhizopus* soft rot of peaches after cold storage at 0°C for 45 days followed by 5 days at 25°C as market life, season 2014.

Treatments	Disease incidence (%) after 45 days-cold storage						Disease incidence (%) after market life					
	Natural infection		A.I. <i>B. cinerea</i>		A.I. <i>R. stolonifer</i>		Natural infection		A.I. <i>B. cinerea</i>		A.I. <i>R. stolonifer</i>	
	D.I. %	E. %	D.I. %	E. %	D.I. %	E. %	D.I. %	E. %	D.I. %	E. %	D.I. %	E. %
MA1	1.0	88.9	4.3	88.6	1.0	89.3	2.0	86.9	6.3	88.1	3.0	83.6
MA2	0.0	100	1.3	96.6	0.0	100	0.3	98.0	4.3	91.9	2.0	89.1
MA3	0.7	92.2	5.0	86.7	1.3	86.0	2.3	85.0	9.0	83.0	6.7	63.4
<i>Candida saitoana</i>	0.7	92.2	2.3	93.9	0.0	100	2.0	86.9	2.7	94.9	1.3	92.9
<i>Candida shehatae</i>	0.0	100	1.3	96.6	0.0	100	2.3	85.0	2.3	95.7	1.7	90.7
<i>C. saitoana</i> +MA1	0.3	96.7	6.3	83.3	0.7	92.5	0.7	95.4	4.7	91.1	3.7	79.8
<i>C. saitoana</i> +MA2	0.0	100	1.7	95.5	0.0	100	0.0	100	5.0	90.6	2.0	89.1
<i>C. saitoana</i> +MA3	0.0	100	3.3	91.3	0.7	92.5	0.0	100	6.3	88.1	3.3	82.0
<i>C. shehatae</i> +MA1	1.3	85.6	3.7	90.2	0.7	92.5	0.0	100	6.3	88.1	2.7	85.2
<i>C. shehatae</i> +MA2	0.3	96.7	1.0	97.4	0.0	100	1.0	93.5	5.7	89.3	2.0	89.1
<i>C. shehatae</i> +MA3	0.7	92.2	1.7	95.5	0.7	92.5	1.7	88.9	9.0	83.0	4.0	78.1
Control	9.0	0.0	37.7	0.0	9.3	0.0	15.3	0.0	53.0	0.0	18.3	0.0
LSD at 5%	1.9		2.6		1.7		2.6		3.3		1.8	

A.I. = artificially inoculation with: *B.C* (*Botrytis cinerea*) or *R.S* (*Rhizopus stolonifer*)

D.I. = Disease incidence & E. = Efficiency

MA = Modified atmosphere & MA₁ = 5 O₂ + 5 CO₂ + 90 N₂ & MA₂ = 10 O₂ + 5 CO₂ + 85 N₂ & MA₃ = 5 O₂ + 10 CO₂ + 85 N₂

Influence Biological control and Modified Atmosphere treatments on physicochemical parameters *in vivo*:

Weight loss percent:

At the end of cold storage period (45 days) at 0°C, all tested MA and yeasts either individually or in combination significantly decreased peaches weight losses % comparing to control fruit (Table 4). MA treatments were achieved higher reduction in weight loss than yeast individual treatments. The MA at containing 5% O₂ and 10% CO₂ showed the least weight loss. Also, *C. saitoana* yeast treatment reduced weight loss more than *C. shehatae* yeast. The combination of MA and yeasts achieved better reduction in weight loss than the individual treatments, and the least weight loss determined was in peaches treated with *Candida saitoana* or *Candida. shehatae* yeasts in combination with MA at 5% O₂ and 10% CO₂ compared to control treatment that show maximum weight loss percent. Weight loss percent of naturally infected peach fruits significantly decrease more than inculcated fruits with *B. cinerea* and *R. stolonifer* compared to control treatment. Artificially inoculated peaches with *R. stolonifer* caused more weight loss than caused by *B. cinerea*. The interaction between treatments and inoculation significantly caused a decrease in weight loss in treated fruits with MA (5% O₂+10% CO₂+85% N₂) and *Candida saitoana* yeast of naturally infected fruit and in artificially inoculated fruits with either gray rot or rhizopus rot compared to control treatment.

Table 4: Effect of yeasts, modified atmosphere and their interaction of natural and inoculated peach fruits with *Botrytis cinerea* and *Rhizopus stolonifer* at end of cold storage on Weight loss percentage in two seasons.

Treatments (A)	Season 2013				Season 2014			
	Infection (B)				Infection (B)			
	N.I	B.C	R.S	Mean	N.I	B.C	R.S	Mean
MA1	1.20	1.90	1.95	1.68	1.60	2.00	2.20	1.93
MA2	1.50	2.00	2.10	1.86	1.90	2.20	2.40	2.16
MA3	0.76	0.97	1.20	0.97	0.78	1.10	1.30	1.06
<i>Candida saitoana</i>	1.95	2.14	2.37	2.15	2.17	2.44	2.55	2.38
<i>Candida shehatae</i>	1.99	2.48	2.54	2.33	2.21	2.73	2.83	2.59
<i>C. saitoana</i> +MA1	0.80	1.30	1.50	1.20	0.82	1.20	1.40	1.14
<i>C. saitoana</i> +MA2	0.79	1.00	1.65	1.14	0.84	1.50	1.70	1.34
<i>C. saitoana</i> +MA3	0.60	0.70	0.75	0.68	0.70	0.76	0.80	0.75
<i>C. shehatae</i> +MA1	0.82	1.70	1.80	1.44	0.94	1.90	2.00	1.61
<i>C. shehatae</i> +MA2	0.84	1.85	1.90	1.53	0.86	1.90	2.10	1.62
<i>C. shehatae</i> +MA3	0.70	0.80	0.90	0.80	0.74	0.83	0.92	0.83
Control	5.60	7.10	8.01	6.90	6.30	7.50	8.20	7.33
Mean	1.36	1.93	2.17	1.82	1.54	2.08	2.30	1.97
LSD at 0.05 probability level:	A = 0.01 B = 0.01 AXB = 0.02				A = 0.01 B = 0.02 AXB = 0.03			

N.I=naturally infection fruit & B.C or R.S = artificially inoculated fruit with *Botrytis cinerea* or *Rhizopus stolonifer*

MA = Modified atmosphere & MA₁ = 5 O₂ + 5 CO₂ + 90 N₂ & MA₂ = 10 O₂ + 5 CO₂ + 85 N₂ & MA₃ = 5 O₂ + 10 CO₂ + 85 N₂

Firmness:

Results in Table (5) for both seasons showed that, the softening process was significantly delayed by the use of MA (5%O₂+10%CO₂). Also, the highest firmness values were obtained from *C. saitoana* yeast followed by *C. shehatae* yeast application. The combination MA (5% O₂ and 10% CO₂) and *C. saitoana* yeast was the best treatment to show firmer peaches comparing with individual or combined treatments. The highest firmness was achieved by naturally infected fruit compared with that diseased fruits with gray mold or Rhizopus rot. Artificial inoculation of control peaches with *R. stolonifer* caused less firmness percentage than that inoculated with *B. cinerea*. As for the interaction between individual treatments with modified atmosphere or two yeasts or combination with biological control agents of the two yeasts and infection fruits, data referred that, MA containing 5% O₂ and 10% CO₂ and yeasts *Candida saitoana* or *Candida shehatae* significantly maintained on firmness parameter and it was the most effective on inoculated fruits with *B. cinerea* and *R. stolonifer* for keeping fruit firmness at end of storage period compared with control fruits of natural infection or artificial inoculation control during both seasons of study.

Soluble Solids Content (SSC %):

As shown in Table 6 that Soluble Solids Content % of the control fruits highly increased at the end of storage period. The highest SSC percentage was observed in control NI treatment at the end of storage period comparing with peaches treated with yeasts and/or MA. The increases of SSC % was

significantly lower in peaches stored under MA (5%O₂+10%CO₂) condition. Also, SSC accumulation was lower in incubated peaches with *Candida saitoana* yeast followed by *Candida shehatae* yeast. Keeping peaches during cold storage under MA containing 5% O₂ and 10% CO₂ combined with *C. saitoana* yeast lowered significantly SSC % followed by that MA combined with *C. shehatae* yeast. Also, naturally infected peaches maintained significantly lower levels of SSC% comparing with artificially inoculated ones. Artificial inoculation of control peaches with *R. stolonifer* caused more SSC percentage than that inoculated with *B. cinerea*. Interaction results between treatments and naturally infected or artificial infected fruits show that, the changes in SSC content were much low in MA (5%O₂+10%CO₂+85% N₂) and two yeasts *Candida saitoana* or *Candida shehatae* on naturally infected peaches or on artificially inoculation with *B. cinerea* (gray mold) or *R. stolonifer* (rhizopus mold) .

Table 5: Effect of yeasts, modified atmosphere and their interaction of natural and inoculated peach fruits with *Botrytis cinerea* and *Rhizopus stolonifer* at end of storage on Firmness in two seasons.

Treatments(A)	Season 2013				Season 2014			
	Infection (B)				Infection (B)			
	N.I	B.C	R.S	Mean	N.I	B.C	R.S	Mean
MA1	7.40	6.96	6.80	7.05	7.20	7.00	6.80	7.00
MA2	7.20	7.00	7.10	7.10	7.00	6.80	6.60	6.80
MA3	8.60	8.20	8.00	8.26	8.20	8.00	7.80	8.00
<i>Candida saitoana</i>	7.10	6.70	6.50	6.76	6.90	6.50	6.30	6.56
<i>Candida shehatae</i>	7.00	6.50	6.20	6.56	6.70	6.30	6.10	6.36
<i>C. saitoana</i> +MA1	8.00	7.60	7.40	7.66	8.00	7.40	7.20	7.53
<i>C. saitoana</i> +MA2	7.80	7.40	7.20	7.46	7.60	7.20	7.00	7.26
<i>C. saitoana</i> +MA3	9.20	8.00	7.80	8.33	8.80	8.40	8.20	8.46
<i>C. shehatae</i> +MA1	8.00	7.60	7.40	7.66	7.80	7.40	7.20	7.46
<i>C. shehatae</i> +MA2	7.60	7.40	7.20	7.40	7.40	7.20	7.00	7.20
<i>C. shehatae</i> +MA3	8.80	8.20	8.00	8.33	8.50	8.00	7.60	8.03
Control	6.20	5.80	5.40	5.80	6.00	5.30	5.10	5.46
Mean	7.88	7.416	7.23	7.51	7.65	7.27	7.05	7.32
LSD at 0.05 probability level:	A = 0.01 B = 0.02 AXB = 0.03				A = 0.02 B = 0.03 AXB = 0.05			

N.I=Naturally infection fruit & B.C or R.S = artificially inoculated fruit with *Botrytis cinerea* or *Rhizopus stolonifer*

MA = Modified atmosphere & MA₁ = 5 O₂ + 5 CO₂ + 90 N₂ & MA₂ = 10 O₂ + 5 CO₂ + 85 N₂ & MA₃ = 5 O₂ + 10 CO₂ + 85 N₂

Table 6: Effect of yeasts, modified atmosphere and their interaction of natural and inoculated peach fruits with *Botrytis cinerea* and *Rhizopus stolonifer* at end of cold storage on SSC % in two seasons.

Treatments(A)	Season 2013				Season 2014			
	Infection (B)				Infection (B)			
	N.I	B.C	R.S	Mean	N.I	B.C	R.S	Mean
MA1	12.60	12.87	12.97	12.81	12.80	12.77	13.10	12.89
MA2	12.80	12.97	13.10	12.95	13.00	13.23	13.43	13.22
MA3	11.40	11.60	11.80	11.60	11.80	12.00	12.50	12.10
<i>Candida saitoana</i>	12.88	13.30	13.47	13.21	13.10	13.55	13.73	13.46
<i>Candida shehatae</i>	12.95	13.50	13.65	13.36	13.25	13.77	13.92	13.64
<i>C. saitoana</i> +MA1	11.80	12.03	12.23	12.02	12.00	12.20	12.40	12.20
<i>C. saitoana</i> +MA2	12.00	12.20	12.40	12.20	12.20	12.40	12.57	12.39
<i>C. saitoana</i> +MA3	10.90	11.20	11.57	11.22	11.20	11.40	11.63	11.41
<i>C. shehatae</i> +MA1	12.20	12.40	12.53	12.37	12.40	12.60	12.80	12.60
<i>C. shehatae</i> +MA2	12.40	12.63	12.87	12.63	12.60	12.80	12.90	12.77
<i>C. shehatae</i> +MA3	11.00	11.20	11.40	11.20	11.40	11.60	11.80	11.60
Control	13.80	14.50	14.60	14.30	14.40	14.80	15.00	14.73
Mean	12.09	12.36	12.55	12.33	12.38	12.58	12.81	12.59
LSD at 0.05 probability level:	A = 0.04 B = 0.06 AXB = 0.12				A = 0.05 B = 0.09 AXB = 0.16			

N.I=naturally infection fruit & B.C or RS = artificially inoculated fruit with *Botrytis cinerea* or *Rhizopus stolonifer*

MA = Modified atmosphere & MA₁ = 5 O₂ + 5 CO₂ + 90 N₂ & MA₂ = 10 O₂ + 5 CO₂ + 85 N₂ & MA₃ = 5 O₂ + 10 CO₂ + 85 N₂

Titrateable acidity %:

As shown in (Table 7), the quantity of organic acids expressed as malic acid decreased in peach fruits with ending of fruit maintenance period in cold storage 45days. There were significant differences in the levels of Titrateable acidity (TA) between treated peaches with yeasts and/or MA and control fruit. The main control of NI fruits had the lowest TA % than other control of the artificially inoculated fruits with fungi. TA in control inoculated with *R. stolonifer* peaches was lower than TA in control peaches inoculated with *B. cinerea*. The decreases being significantly lower in peaches stored

under MA (5%O₂+10%CO₂+ 85% N₂) alone. Also, acidity losses were retarded by the use of two yeasts(*Candida saitoana*)or (*Candida shehatae*). The combination of *C. saitoana* yeast+ MA (5% O₂ + 5% CO₂)achieved higher acidity peaches, as it had higher ability in maintaining in the TA content of peach fruits thus at significant higher levels. TA content in natural infected fruits was higher than unhealthy fruits inoculated with gray and rhizopus molds in both considered seasons at the end of the storage period. Artificial inoculation of peaches with *R. stolonifer* caused less TA percentage than that inoculated with *B. cinerea*. As for the interaction, data referred significant in both seasons by using combination of *C. saitoana* yeast+ MA (5% O₂ + 5% CO₂) followed by *C. shehatae* yeast + MA (5% O₂ + 10% CO₂) caused much inhibition to the decline of TA in inoculated fruits with both *B. cinerea* and *R. stolonifer* .

Table 7: Effect of yeasts, modified atmosphere and their interaction of natural and inoculated peach fruits with *Botrytis cinerea* and *Rhizopus stolonifer* at end of cold storage on Acidity % in two seasons.

Treatments (A)	Season 2013				Season 2014			
	Infection (B)				Infection (B)			
	N.I	B.C	R.S	Mean	N.I	B.C	R.S	Mean
MA1	0.75	0.71	0.71	0.72	0.72	0.70	0.71	0.71
MA2	0.72	0.70	0.69	0.70	0.70	0.68	0.65	0.67
MA3	0.84	0.80	0.78	0.80	0.80	0.78	0.76	0.78
<i>Candida saitoana</i>	0.73	0.68	0.66	0.69	0.69	0.66	0.63	0.66
<i>Candida shehatae</i>	0.72	0.66	0.63	0.67	0.67	0.64	0.61	0.64
<i>C. saitoana</i> +MA1	0.88	0.74	0.70	0.77	0.80	0.75	0.72	0.77
<i>C. saitoana</i> +MA2	0.80	0.76	0.73	0.76	0.84	0.76	0.74	0.78
<i>C. saitoana</i> +MA3	0.82	0.73	0.80	0.78	0.79	0.80	0.79	0.79
<i>C. shehatae</i> +MA1	0.78	0.74	0.70	0.74	0.75	0.73	0.68	0.72
<i>C. shehatae</i> +MA2	0.76	0.83	0.70	0.76	0.74	0.72	0.69	0.71
<i>C. shehatae</i> + MA3	0.86	0.83	0.80	0.83	0.84	0.82	0.78	0.81
Control	0.70	0.63	0.60	0.64	0.65	0.62	0.59	0.62
Mean	0.79	0.75	0.72	0.75	0.76	0.74	0.71	0.74
LSD at 0.05 probability level:	A =0.01 B =0.01 AXB =0.02				A =0.01 B = 0.02 AXB = 0.03			

N.I=naturally infection fruit & B.C or RS = artificially inoculated fruit with *Botrytis cinerea* or *Rhizopus stolonifer*

MA = Modified atmosphere &MA₁ =5 O₂ + 5 CO₂ + 90 N₂ & MA₂ =10 O₂ + 5 CO₂ + 85 N₂ & MA₃ =5 O₂ + 10 CO₂ + 85 N₂

Vitamin C:

The effects of the postharvest treatments on peaches V.C content were found to be statistically significant .At the end of the 45 days storage period, it is clear that V.C content of control peaches fruits was decreased in both seasons (Table 8). Deterioration in vitamin C content was observed in artificially inoculated peaches with *B. cinerea* and *R. stolonifer* .Less deterioration was determined in fruits kept under MA containing 5% O₂ and 10% CO₂ as individual treatment or in combination with *C. saitoana* or *C. shehatae* yeasts, as these treatments maintained on retention Vit C. at the end of storage period in both seasons. Maximum averages vitamin C content was achieved by naturally infected fruits (non-inoculated fruits) with significant differences from inoculated peaches with both *B. cinerea* and *R. stolonifer*. Artificial inoculation peaches with *R. stolonifer* caused less Vit. C content than that inoculated with *B. cinerea*. The interaction data show that minimum decrease of Vit. C content was observed especially in peaches treated with MA at 5% O₂ + 10 % CO₂ in combination with *C. saitoana* or *C. shehatae* yeasts on naturally fruits as kept significantly on Vit. C compared to decayed peaches with both gray and rhizopus rots.

Marketability:

Evaluation of the marketability period of peaches at 25°C and 75% RH for 5 days after the cold storage on quality parameters of naturally infected fruits (non-inoculated) is presented in Table (9). Results showed significant increase in parameters weight loss % and SSC %, there were significantly decreased in firmness, titratable acidity and Vitamin C in two seasons in all treatments during the 45 days cold storage .The best treatment kept on quality of peach fruits after 5 days from 45 days cold storage at 0°Cas marketing period, was MA at 5% O₂ + 10% CO₂ in combination with *C. saitoana* or *C. shehatae* which showed the best physicochemical properties, as fruits had less weight loss ,high texture and Vitamin C with keeping on levels of SSC % and TA% without high changes indicating higher marketability than other treatments .Meanwhile ,the control peaches did not show remarked deviation after marketability period investigation than the determinations after the cold storage .

Table 8: Effect of yeasts, modified atmosphere and their interaction of natural and inoculated peach fruits with *Botrytis cinerea* and *Rhizopus stolonifer* at end of cold storage on V.C in two seasons.

Treatments (A)	Season 2013				Season 2014			
	Infection (B)				Infection (B)			
	N.I	B.C	R.S	Mean	N.I	B.C	R.S	Mean
MA1	2.11	1.30	1.25	1.55	2.11	1.20	1.23	1.51
MA2	2.10	1.20	1.22	1.51	2.00	1.10	1.41	1.50
MA3	2.90	1.90	1.70	2.17	2.60	1.80	1.80	2.07
<i>Candida saitoana</i>	2.08	1.17	1.15	1.46	2.00	1.17	1.13	1.43
<i>Candida shehatae</i>	2.04	1.14	1.12	1.43	1.95	1.13	1.10	1.39
<i>C. saitoana</i> +MA1	2.50	1.50	1.40	1.80	2.40	1.15	1.15	1.57
<i>C. saitoana</i> +MA2	2.29	1.30	1.20	1.59	2.20	1.08	1.08	1.45
<i>C. saitoana</i> +MA3	3.60	2.50	2.30	2.80	3.00	1.60	1.60	2.07
<i>C. shehatae</i> +MA1	2.20	1.60	1.50	1.77	1.80	1.56	1.20	1.52
<i>C. shehatae</i> +MA2	2.15	1.40	1.30	1.62	2.33	1.53	1.10	1.65
<i>C. shehatae</i> +MA3	3.40	2.30	2.10	2.60	2.58	1.93	2.20	2.24
Control	1.90	1.10	1.09	1.36	1.70	1.10	1.69	1.50
Mean	2.51	1.61	1.51	1.87	2.27	1.40	1.45	1.71
LSD at 0.05 probability level:	A = 0.01 B = 0.02 AXB = 0.04				A = 0.01 B = 0.03 AXB = 0.05			

N.I=naturally infection fruit & B.C or RS = artificially inoculated fruit with *Botrytis cinerea* or *Rhizopus stolonifer*

MA = Modified atmosphere & MA₁ = 5 O₂ + 5 CO₂ + 90 N₂ & MA₂ = 10 O₂ + 5 CO₂ + 85 N₂ & MA₃ = 5 O₂ + 10 CO₂ + 85 N₂

Table 9: Effect of yeasts, modified atmosphere of natural peach fruits at end of storage on market life in two seasons.

Treatments (A)	Season 2013					Season 2014				
	Weight loss %	Firmness (lb/in ²)	SSC %	Acidity %	V.C (mg/100g F.W.)	Weight loss %	Firmness (lb/in ²)	SSC %	Acidity %	V.C (mg/100g F.W.)
MA1	1.30	6.26	13.00	0.73	2.15	1.60	6.00	13.30	0.70	2.13
MA2	1.60	6.13	13.20	0.71	2.13	1.60	5.93	13.60	0.68	2.07
MA3	0.50	7.00	11.60	0.83	2.70	0.63	6.80	11.90	0.76	2.40
<i>C. saitoana</i>	1.70	6.10	13.35	0.70	2.09	1.80	6.30	13.65	0.69	2.07
<i>C. shehatae</i>	1.95	6.00	13.57	0.69	2.00	1.99	6.17	13.87	0.67	2.00
<i>C. saitoana</i> +MA1	0.70	6.80	12.00	0.80	2.40	0.90	6.60	12.30	0.78	2.30
<i>C. saitoana</i> +MA2	0.90	6.60	12.20	0.79	2.20	1.20	6.40	12.50	0.76	2.15
<i>C. saitoana</i> +MA3	0.20	7.60	11.00	0.86	3.40	0.40	7.40	11.30	0.82	2.80
<i>C. shehatae</i> +MA1	1.13	6.60	12.40	0.77	2.10	1.40	6.40	12.60	0.74	2.05
<i>C. shehatae</i> +MA2	1.46	6.40	12.60	0.74	2.05	1.76	6.20	12.80	0.72	2.04
<i>C. shehatae</i> +MA3	0.40	7.40	11.20	0.84	3.20	0.70	7.20	11.50	0.82	2.60
Control	3.50	5.20	14.00	0.65	1.60	3.60	5.00	14.50	0.60	1.40
LSD at 0.05 probability level:	0.04	0.05	0.01	0.01	0.05	0.06	0.07	0.03	0.02	0.08

MA = Modified atmosphere & MA₁ = 5 O₂ + 5 CO₂ + 90 N₂ & MA₂ = 10 O₂ + 5 CO₂ + 85 N₂ & MA₃ = 5 O₂ + 10 CO₂ + 85 N₂

Discussion

Pathological and physiological postharvest losses of peaches are very common all over the world due to fungal infection and the deterioration of quality during cold storage and marketability. Gas mixture of 10% O₂, 5% CO₂ and 85% N₂ was the most suppressive modified atmosphere on growth of *B. cinerea* and *R. stolonifer* *in vitro* comparing with naturally infected fruits (control). On the other hand, Cia *et al.* (2003) found that the atmospheres of 3% O₂ + 8, 10 or 12% CO₂ and 5% O₂ + 10 or 12% CO₂ significantly inhibited the mycelial growth of *R. stolonifer* on PDA medium, low O₂ down to 10% showed more suppression than 5%. This finding refers to importance of ratio of O₂/CO₂ reabs more than usual concept of positive increment suppression with elevating CO₂ and lowering O₂, which could be attributed to accumulation of secondary metabolites of the hyphal cells suppressing mass transfer of protoplasm into apical cells more than the anoxia effect as well as suppressing the biological function to transport electrons by the cytochromes in the mitochondria (El-Goorani and Sommer, 1981).

In vitro current study on PDA medium, *C. saitoana* and *C. shehatae* yeasts significantly inhibited the growth of *B. cinerea*, but did not affect *R. stolonifer*. El-Ghaouth *et al.* (1998) clarified how hyphae of *B. cinerea* in close proximity to the antagonistic yeast *C. saitoana* exhibited severe cytological injury, such as cell wall swelling and protoplasm degeneration.

Individual treatments of modified atmospheres or yeasts investigations revealed that modified atmosphere containing 10% O₂ + 5% CO₂ was the most suppressive treatment against gray mold and *Rhizopus* rot on peaches cold stored for 45 days during both seasons. Modified atmosphere storage showed inhibitory effect on postharvest pathogens (Spotts *et al.*, 2002). Elevated CO₂ effectively suppresses mycelia growth, spore germination, and germ tube elongation of *B. cinerea* and showed fungistatic effects against many other fungi (Wszelaki and Mitcham, 2003).

Yeasts *C. saitoana* and *C. shehatae* were the most suppressive individual treatments against peaches decay during prolonged cold storage period for 45 days. Several studies indicated different biocontrol mechanisms, such as production of toxins, which act on the cell walls of phytopathogens (Coelho *et al.*, 2003), production of enzymes that degrade the pathogen cell walls (Chanchaichaovivat *et al.*, 2008).

The combination between modified atmosphere and *C. saitoana* did not show increase in suppressive effect against *B. cinerea* or *R. stolonifer* than each individual treatment when treated peaches were cold stored for 45 days. However, Tian *et al.* (2004) showed that the combination between modified atmosphere and yeasts varied effectiveness against postharvest pathogens, where combination of controlled atmospheres containing 3% O₂ + 3% or 8% CO₂ with yeast *Trichosporon* sp. provided beneficial effects against gray and blue mold diseases of apple and pears.

In vivo, the physicochemical properties of peaches treated with yeasts and kept under modified atmosphere conditions were estimated for the naturally infected peaches as well as in the artificially inoculated ones to clarify the effect of these fungi on fruit quality and how these treatment affecting directly or indirectly maintained fruit quality.

Results showed that MA (5% O₂ + 10% CO₂ + 85% N₂) combined biological control of two yeasts antagonist *Candida saitoana* or *Candida shehatae* were able to high protect peach fruits against both *B. cinerea* (gray mold) and *Rhizopus stolonifer* (rhizopus mold) incidence and protected fruit skin from decay therefore stopped up of damaged fruits and reduced loss of weight, firmness, prevented changes in titratable acidity (TA), Soluble Solids Content (SSC) and preserved vitamin C and thus delaying ripen process and maintain quality peach fruits.

The percentage of weight loss was found to be very low at the end of the storage period in fruits treated with MA (5% O₂ + 10% CO₂ + 85% N₂) combined *Candida saitoana* or *Candida shehatae* yeast. Weight loss in MA was very low mainly due to high relative humidity around the fruits inside the sealed polyethylene bags, which prevent water loss due to transpiration (Serrano *et al.*, 2005) and when combine MA with yeasts which decreases the decay and so, decrease weight loss. So, MA enhanced the effect of yeast to maintain fruit quality. Inversely in artificially infected fruit with *B. cinerea* (gray mold) and *R. stolonifer* (rhizopus rot) as decay increase respiration rate and decrease weight loss. The same results reported by (Valero and Serrano, 2010) of sweet cherry and (Díaz-Mula *et al.*, 2011) of plum fruits as modified atmosphere reduced weight loss.

The highest firmness value was obtained by MA (5% O₂ + 10% CO₂ + 85% N₂) combined with the two yeasts *Candida saitoana* or *Candida shehatae* treatments. MA delayed the decline of firmness due to a direct effect of high CO₂ and low O₂ on inhibiting of the enzymes responsible for the decomposition of the walls cells, since delay in softening under MA conditions (Akbulak and Eris, 2004). Furthermore *Candida saitoana* or *Candida shehatae* yeasts work as a protective layer of growth mycelium and therefore stopped up of damaged fruits and improves quality, but firmness in infected fruits with *B. cinerea* and *R. stolonifer* probably decreased by fungal infection as cell wall break down by progress of time. Present findings were consistent with that of MAP maintained fruit flesh firmness of peach (Pablo and Trujillo, 1997), peach and nectarine (Zoffoli *et al.*, 1998) and plum (Guan and Dou, 2010; Díaz-Mula *et al.*, 2011).

In the present investigation, it was observed that the fruit treated with MA (5% O₂ + 10% CO₂ + 85% N₂) combined with two yeasts *Candida saitoana* or *Candida shehatae* have minimum increase in soluble solids content (SSC) at 45 days of storage. Probably MA could be related to reduce respiration, delay in metabolic activity this delayed ripening and yeasts as biological control protector of the surface of fruits by suppressing directly or indirectly fungus growth, which when combined with MA control fungal infection and reduce decay development so, increases the protection of the fruits of pollution patients and thus maintains the quality of the pass-through. Meanwhile, in infected fruits with *B. cinerea* and *R. stolonifer* as the increase of microbial spoilage, degradation of fruits and over ripen and senescence led to an increase SSC probably it could be related to increases in metabolic activity. These data confirm previous reports about MA storage slowed down compositional changes associated with ripening as delayed the increase in TSS of peach and nectarine (Zoffoli *et al.*, 1998; Akbulak and Eris, 2004), peach (Arbol *et al.* 2010) and (Díaz-Mula *et al.*, 2011) of plum fruits. Moreover, studies on MAP slowed down the respiration rate of peach (Pablo and Trujillo,

1997), cherry (Tian *et al.*, 2004), peach and nectarine (Zoffoli *et al.*, 1998; Akbudak and Eris, 2004) and (Valero and Serrano, 2010; Díaz-Mula *et al.*, 2011) of plum fruits.

Fruits treated with MA (5% O₂ + 10%CO₂ + 85%N₂) combined with two yeasts *Candida saitoana* or *Candida shehatae* showed higher retention of Titratable acidity (TA) during storage. This could be due to the delaying in physiological ageing and alteration in metabolism, which resulted in higher retention of acidity. Meanwhile, fruits infected with *B. cinerea* and *R. stolonifer* had high changes of acidity probably due to high respiratory rate and therefore organic acids consumption quickly and related to increases in metabolic activity. The findings are in agreement with studies on MAP retarded the decrease in titratable acidity of several fruits such as peach and nectarine (Akbudak and Eris, 2004), loquat (Amorós *et al.*, 2008), plum (Díaz-Mula *et al.*, 2011) and (Khorshidi *et al.*, 2011 and Serradilla *et al.*, 2013) of Cherry.

The maximum retention of ascorbic acid (vitamin C) was observed with MA (5% O₂ + 10%CO₂ + 85%N₂) combined with biological control with yeasts *Candida saitoana* or *Candida shehatae* treatments because these treatments reduced the oxidation in the peach fruits, but ascorbic acid in *B. cinerea* and *R. stolonifer* infected fruits were decreased probably by fungal infection. These results are similar to that reported by Lin *et al.* (2008) who found that the decrease in vitamin C level was associated with reduced capability of preventing oxidative damage and with the incidence of physiological disorders during storage. Similar finding have reported that jujube fruit treated with yeast, *R. paludigenum* raised ascorbic acid in 21 days storage at 25° C (Wang *et al.*, 2009). Also, MAP maintained vitamin C of peach (Pablo and Trujillo, 1997), peaches and nectarines (Zoffoli *et al.*, 1998) and (Amorós *et al.*, 2008) of loquat fruits.

Market-life is most influenced by contamination with microorganisms. Fruits treated by MA (5% O₂ + 10%CO₂ + 85%N₂) with either *C. saitoana* or *C. shehatae* yeasts had the best physicochemical properties indicating high marketability than other treatments. This finding could be contributed to the effect of biological control agents inhibit exists spores on surface of fruits and reduce in physiological disorders as inhibit the infection of peach fruits with gray and rhizopus molds. Also, MA may be play a vital role to control compositional changes and decrease fruit metabolism activity, including respiration rate, leading to maintenance of respiration substrates and in turn to a delay of the postharvest ripening process and aging. These processes result in delaying the ripening process with no change in quality characters of the peach fruits or minimum quality loss expressed by minimum physiological loss in weight, retard softening and help in maintain changes in acidity, soluble solids content, preserves vitamin C by delaying the ripening process and with a minimum quality loss and so longer storage and market life. Previous finding by (Pablo and Trujillo, 1997) on peach, (Zoffoli *et al.*, 1998) on peach and nectarine, (Serrano *et al.*, 2005) on cherry and (Valero and Serrano, 2010) on plum supported our result that MAP used for extending postharvest life of fruits.

In conclusion, the combinations of active MA (5% O₂ + 10%CO₂ + 85%N₂) and biological control as antagonistic yeasts, *Candida saitoana* or *Candida shehatae* were useful to inhibit growth of gray mold (*Botrytis cinerea*) and Rhizopus rot (*Rhizopus stolonifer*) and slow down compositional changes associated with ripening through a delay in the losses of weight and the decrease in acidity, maintain fruit flesh firmness, soluble solids content and vitamin C and slowed deterioration through decreasing fruit decay and in turn, maintain postharvest quality during cold storage, long distance shipping for export and thus extend of market-life of peach fruits. So, a combination of MA and antagonistic yeasts could be adopted and regarded safe and could be used instead of chemical fungicides and tested on semi, then commercial scale.

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