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The effect of chitosan and modified atmosphere packaging on peach export and storagability

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ABSTRACT

The present study was conducted in order to lower fruit quality losses of Late Swelling peaches during shipping and storage. Fruits were harvested at the early ripe stage, dipped in two chitosan concentrations (Chi_1 and Chi_2) and packed in three types of modified atmosphere packages (MAP_1, MAP_2 and MAP_3). Fruits were held in storage room for 10 days (simulating transport or shipping conditions) or 32 day (simulating storage conditions) at 10°C ± and 85-90% RH. The treatments' effect on the fruit ability for transport and storage, as well as the fruit physicochemical characters was investigated. At 10 days, all combinations indicated general decrease in fruit electrolyte leakage and weight loss. The $Chi_1 \times MAP_2$ and $Chi_2 \times MAP_2$ treatments indicated the lowest off-flavor incidence compared to the other combinations. All treatments resulted in decreasing the percentage of unmarketable fruits after 10 days, with $Chi_1 \times MAP_2$ and $Chi_2 \times MAP_2$ indicating the lowest unmarketable value after 21 and 32 days. Fruit firmness and color change were maintained by all combinations. Most treatments resulted in high content of fruit acidity, soluble solids, chlorophyll and vitamin C, while low carotene, anthocyanin and sugars contents.

Keywords: Postharvest combined treatments, fruit quality, transport and storage- ability

1. Introduction

Next to carbohydrates, organic acids, pigments, volatiles, nutritive minerals and trace amounts of proteins and lipids, a peach fruit contains immeasurable diversity of biologically bioactive compounds such as vitamin C, carotenoids, flavonoids, and phenols that act as antioxidants, making the peach fruit very attractive to consumers for nutritive and health issues [1]. During marketing or shipping, peach fruits suffer from high susceptibility to flesh softening that make it more sensible for pathogen attack and deterioration leading to a shorter handling period and limited marketability. Therefore, postharvest practices for maintaining fruit characters of better marketing ability and extended shelf life is seriously considered. This would be accomplished by reducing quality losses due to the physiological and biochemical changes that fruits undergo after harvest. A physiological weight loss of about 20-30% is reported by [2], which is determined by both water loss, due to transpiration of the living fruit tissues, and by dry matter loss due to respiration. Also, a wide range of postharvest fruit losses are caused by several postharvest diseases. Reference [3] recorded a 15-25% decay of horticultural commodities during storage and transport caused by fungal and other microorganism's diseases.

In this respect, efforts are made to find effective and safe techniques for controlling fruit postharvest diseases and lowering quality losses. As alternative of using synthetic fungicides, postharvest treatments with a variety of organic chemical elicitors such as chitosan, salicylic acid and jasmonic acid is stated as safe applications for developing enhanced resistance to pathogen infection [4]. In the meanwhile, modified atmosphere packaging (MAP) is a preservation technique where the in-package atmosphere is modified by using polymeric films with or without perforations and the air surrounding the fruit in the package is changed to another composition (lowered level of O_2 and a heightened level of CO_2). This kind of package delays the natural deterioration of the fruit by slowing down respiration activity, ripening process and the incidence of various physiological disorders and pathogenic infestations [5]. Several studies confirmed the use of MAP conditions under cold storage for extending the fruit shelf life by limiting water loss, delaying ripening, and suppressing diseases [6]. Recently edible films have- been developed to extend the shelf life of fresh fruits such as chitosan-based edible coating that is concerned for its non-toxic, biodegradable, and biocompatible properties [7]. Chitosan can form a semi-permeable film coating that would modify the internal atmosphere and decrease transpiration losses and thus regulates the postharvest fruit quality [8] and it is also recorded to have a broad-spectrum of antimicrobial activity [9]. However, as deficiency of single chitosan coating is mostly viewed, suggestions are made to combine chitosan coating with other technique including; heat treatment, hypobaric treatment, gas fumigation, and modified atmosphere packaging [7].

Accordingly, the present study was conducted on the late season peach cultivar; Late Swelling grown in Egypt in order to investigate the effect of postharvest combined applications of chitosan and modified atmosphere packaging on the fruit shelf life and marketability.

2. Materials and Methods

2.1. Plant materials and postharvest treatments

The present study was carried out during 2016 and 2017 postharvest seasons on Late Swelling peach (*Prunus persica* L.) cv. Fruits were harvested at the early ripe stage (during the first week of June) and immediately transported to the postharvest laboratory, washed, air dried and sorted to remove any unsuitable ones (mechanical damage, injured and discolored). Combination treatments of chitosan (Chi_1 and Chi_2) dipping and modified atmosphere packaging (MAP_1, MAP_2 and MAP_3) and the control were arranged in a randomized complete block design (RCBD) with 28 experimental units; (2 Chi × 3 MAP + control × 4 replicates). The Chi_1 was 1.5 and 3 while the Chi_2 was 3 and 6 $g.L^{-1}$ in the first and second season, respectively. The types of MAP are illustrated in Table (1). All experimental units were kept in storage room either for 10 days (simulating transport or shipping conditions) or 35 days (simulating storage conditions) at 10°C±1 and 85-90% R.H. in order to investigate the effect of the different treatments on the physicochemical characters and fruit ability for transport and storage.

2.2. Fruit physicochemical characteristics

A fruit sample of 2 kg from each replicate was taken after 10 days of storage for measuring the fruit physicochemical characteristics; Fruit firmness was measured by a pressure tester with a probe 8 mm in diameter and fruit color was recorded by Minolta, Chroma Meter CR-200 and expressed as Lightness (L*), Chroma (C) and hue angle (*h*°). the percentage of soluble solids content (SSC) was measured by hand refractometer model ATAGO, model. N-1e. Japan T. Vitamin C (VC), acidity, reducing (RS), non-reducing (NRS) and total sugars (TS) content (%) were determined [10]. Also, fruit bioactive properties were defined by determining calorimetrically fruit total chlorophyll (T Chl.), total carotenoids (T Car.), anthocyanin (Anth.) and total phenols (T Phenl.) as mg/100g and measured using a spectrophotometer (Spectronic model.20, Milton Roy Co., USA) according to [11; 12; 13;14, 2012, respectively].

2.3. Fruit transport- and storagability

Fruit transport- and storagability was expressed as the percentages of peel electrolyte leakage (EL) according to [15], off-flavor incidence recorded after 21 and 32 days, fruit weight loss and unmarketable fruits. Unmarketable fruits were estimated by sorting any defected fruit such as decayed, off-flavored, shriveled, external browning, etc. on off flavors (%) after 21 and 32 days in 2016 and 2017 seasons. In addition, storage life was estimated as number of days from zero storage time until 25-35% unmarketable fruits.

2.4. Statistical analysis

One-way analysis of variance (ANOVA) according to [16] was carried out by Statistical Analysis System (SAS). The differences among the treatments were separated and compared using the least significant differences (LSD) at 0.05 level of significance [17].

3. RESULTS

3.1. Fruit physicochemical characters

The effect of modified atmosphere packaging (MAP) combined with chitosan dipping (Chi) on the fruit physiochemical characters held for 10 days at 10oC and 85-90% R.H is presented in Tables (2, 3 and 4).

All chitosan and MAP combinations maintained peach fruits with high firmness compared to the control in both seasons. In 2016, all combinations did not significantly differ among each other and indicated higher firmness values than $Chi_2 \times MAP_1$ treatment. In 2017, all chitosan and MAP combinations resulted in higher fruit firmness than the $Chi_1 \times MAP_1$ treatment. Also, fruits treated with $Chi_1 \times MAP_2$, $Chi_1 \times MAP_3$, $Chi_2 \times MAP_2$ and $Chi_2 \times MAP_3$ did not differ among each other in their firmness and were firmer than those treated with $Chi_2 \times MAP_1$.

As for fruit color, the $Chi_2 \times MAP_1$, $Chi_2 \times MAP_2$ and $Chi_2 \times MAP_3$ did not significantly differ among each other and indicated higher L* than $Chi_1 \times MAP_2$ and the control in 2016, while in 2017 the value of L* was not affected by any of the treatment except that the combination $Chi_1 \times MAP_1$ revealed the lowest L* value compared to the control and all other combinations. However, a higher value of the h angle than the control was obtained by all combinations in 2017, while, in 2016 the *h*° was not affected except that Chi*1*×MAP1indicated higher *h*° than all other combinations. All chitosan and MAP combinations resulted in a significant decreased value of the chroma (C) without differing among each other.

In addition, all treatments showed a significant high acidity and fruit soluble solids content in comparison with the control, and in general the different combinations did not show differences among each other. Fruit reducing, non-reducing and total sugars content was significantly lower in treated fruits by all combinations than the control fruits in 2017 only. The different treatments generally maintained the fruit organoleptic characteristics after 10 days; most treatments resulted in high fruit chlorophyll and vitamin C, while low carotene and anthocyanin contents compared to the control. Total phenols were not affected by any of the treatments except $Chi_1 \times MAP_2$, $Chi_2 \times MAP_1$ and $Chi_2 \times MAP_2$ resulted in lower phenols content than the control in 2016.

3.2. Fruit transport- and storagability

At 10 days a general decrease in the percentage of fruit electrolyte leakage was obtained by all combinations in comparison with the control (Table 4). In addition, all treatments showed a significantly clear reduction in fruit weight loss in comparison with the control fruits which lasted for only ten days and had values of 11.9 and 11.7 % weight loss in 2016 and 2017, respectively, while values of all treatments in both seasons ranged from 0.62-2.25% without significance difference among them. Never the less, the treated fruits showed increases in the percentages in fruit weight loss with extending storage period (21 and 32 days) with the combinations; $Chi_1 \times MAP_3$ and $Chi_2 \times MAP_3$ resulting in the highest weight loss compared to all other treatments in the 2016 season (Fig.1). All chitosan and MAP combinations showed a noticeable difference among them affecting the off-flavor incidence after 21 and 32 days. The $Chi_1 \times MAP_2$ and $Chi_2 \times MAP_2$ treatments indicated the lowest off-flavor incidence compared to the other treatments, followed by $Chi_1 \times MAP_3$ and $Chi_2 \times MAP_3$, and then $Chi_1 \times MAP_1$ and $Chi_2 \times MAP_1$obtianng high off-flavor values compared to all other treatments (Fig.2). In the meantime, all treatments resulted in decreasing the percentages of unmarketable fruits after 10 days compared to the control. The $Chi_1 \times MAP_1$, Chi1×MAP_2, Chi1×MAP_3 and $Chi_2 \times MAP_2$ indicated lower unmarketable fruits than $Chi_2 \times MAP_1$ and $Chi_2 \times MAP_3$. In general, the combination of $Chi_1 \times MAP_2$ and $Chi_2 \times MAP_2$ indicated the lowest percentage of unmarketable fruits after 21 and32 days compared to the other combinations (Fig.3).

4. Discussion

In the present study, the use of modified atmosphere packaging combined with the chitosan dipping treatments resulted in significant positive influence in enhancing the transport and storage ability of the Late Swelling peaches, as well as maintaining its physicochemical characters for a period of 10 days. For chitosan application to be more effective, it's reported to be combined with other methods such as short heating [18], short gas fumigation [19] and modified atmosphere packaging [20]. In this respect and similar to the obtained results of the present study, [21] found that the combination of chitosan and modified atmosphere packing retained membrane integrity of the litchi fruit and maintained its firmness. Also, [21] found that the combination of chitosan + MAP was effective in preventing decay, browning and retaining the pericarp color in litchi fruits compared with single MAP. They added that this combination significantly reduced polyphenol oxidase and polyphenol decarboxylase activity, retained membrane integrity, anthocyanin content and prevented the decline of pericarp color values during storage**.** Additionally, both MA packaging and chitosan coating work for maintaining the fruit moisture content which thereby would reduce weight loss due to the limitation of gas exchange. [22l.; 23]. MAP alters air composition surrounding the fruit in the package (low O_2 and high CO_2) and thus lowers respiratory activity and ethylene production, delays ripening and softening, limits weight losses, and decreases the incidence of physiological disorders and decay-causing pathogens [24]. Levels of CO_2 higher than 1% are reported to work as an antagonist of ethylene action, thus prevent its autocatalytic synthesis and consequently retard fruit ripening and deterioration [25]. Studies on peaches and nectarines showed that MAP slowed fruit tissues deterioration through decreasing respiration rate and browning development [26; 27], thereby extending storage life [28]. This is clearly shown in the result of the present study as all treatments decreased the electrolyte leakage of the Late Swelling peach tissues. Similarly, [29] packed honey peaches in different-thickness low density polyethylene bags stored at 2°C and stated that MAP treatments inhibited the climacteric peak, decreased the development of softness, and retarded the reduction of membrane integrity.

In addition, chitosan is known to slow down fruit decay by its direct toxic effect on many phytopathogens as it inhibits spore germination, germ tube elongation, and mycelia growth [30; 31]. Another way that chitosan works is the elicitation of fruits biochemical defense responses, as well as having antimicrobial properties by impeding the movement of microbial cells [32; 34]. It is also reported to decrease respiration rate [34]. In the meanwhile, chitosan reduces water loss and increases resistance to water vapor transmission because of its dense films structure that works effectively as gas barrier [35]. It is a polysaccharide with hygroscopic properties that enable the formation of a water barrier and consequently reduces external water transfer [22]. In the meantime, in the present study, physiological disorders such as off- flavor seemed to appear in all types of MAP treated fruits after 21 days of storage, limiting the storage life of the fruit according to the packaging types used. This disorder found in the MAP treatments was probably associated with the exposure of the packed fruits to high CO_2 and low O_2 levels accumulated during the longer storage period (over 3 weeks) compared to the shorter one (3 weeks). These findings go online with those of [36] working on plums. Moreover, [37] concluded that extreme reduction of O_2 concentration leads to an increase in the potential risk for the growth of pathogenic anaerobic microbes, and excessive reduction of O_2 concentration (<1%) intensifies anaerobic respiration, which leads to off-flavor production and tissue deterioration or visible tissue damage.

The preservation of fruit physicochemical characteristics such as firmness, color, TSS, sugars, bioactive compounds are important to determine acceptability and marketability of the fruit. Obtained results showed positive influence of the treatments on generally maintaining the fruit physicochemical characteristics. MAP maintained fruit firmness and revealed high chlorophyll and acidity and low anthocyanin content. Similarly, [38] indicated that the MAP maintained fruit firmness of sweet cherry after harvest. The effect of MAP on fruit firmness could be attributed to the beneficial effects of atmospheres with low O_2 and/or high CO_2 content on reducing softening [39]. The softening of flesh during storage could be due to the degradation of soluble pectin by high activity of endopolygalacturonase enzyme in fruits [40]. Delaying fruit softening would be an ethylene-mediated effect as reported by [41] who referred softening reduction of plums fruit to the inhibition of ethylene production by the MAP to a greater extent than in those untreated ones. Delay in softening under MAP conditions has been observed in several climacteric fruits such as apricot, peach and nectarine [42; 43]. Also, chitosan affected positively fruit firmness over a storage period of 10 days. Several studies showed the positive influence of chitosan dipping in eliminating cell wall degradation and thus slowing down fruit softness and maintaining its postharvest firmness [44; 45]. Moreover, other investigations approved the positive influences of MAP in keeping fruit acidity from declining during storage [43; 46]. This clears the MAP effect on decreasing fruit metabolism, especially respiration rate, by reducing hydrolysis of organic acid leading to the maintenance of respiration substrates and in turn to a delay postharvest ripening process [47; 48]. Similarly, the high SSC indicated by MAP might be referred to lowered respiration rate and thus decreased sugars consumption [43; 41; 49], as well as inhibiting the climacteric peak and retarding and reducing starch conversion to sugars under cold storage conditions[29; 46]. Also, obtained results showed that chitosan kept fruit with high acidity content which means that chitosan slowed down the use of organic acids as substrates for respiration metabolism via Krebs cycle during storage [50]. Similar low acidity loss in chitosan-coated fruits during storage is stated in other studies on peach [51] and litchi [52]. Similarly, [53] revealed that the high levels of acidity in the pulp of Navel oranges coated with chitosan may be because that it works as a protective O_2 barrier or reduces oxygen supply on the fruit surface which inhibit respiration. In general, deterioration of fruits bioactive properties (Vitamin C, carotenes, anthocyanin and phenols content) was kept at minimum by the combined treatments and thus keeping the antioxidant potential of fruits 48]. Never the less, MAP generally slowed down the red color development during storage which would be attributed to the effect of the MAP conditions in slowing down fruit ripening and is evident in the high chlorophyll content and low anthocyanin and carotenoids detected in the fruits, as fruit carotenoids become apparent when chlorophyll disappears upon ripening, which is commonly accompanied by a marked biosynthesis of carotenoids [28].

In over all, it is clear that reduced O_2 level by the MAP can delay compositional changes such as fruit pigment development, softening, hardening, and development of flavor due to a decrease in the activity of oxidative enzymes such as glycolic acid oxidase, ascorbic acid oxidase, and polyphenol oxidase [5], and that modified atmosphere and chitosan dipping would enhance fruit storability by slowing down all physiological activities, especially respiration rate and the activity of fruit softening enzymes and thus delaying senescence [34; 46; 54]. Also, it should be put in consideration that, although chitosan is reviewed to induce numerous biological responses in plants and suggested to be used for sustainable crop productivity, its influence mainly depends on its chemical composition and the timing and rate of application [55].

Based on the results obtained in the present investigations, it can be concluded that among all the combinations of chitosan dipping and MAP, the Chi.1 combined with MAP especially MAP_2 was the most effective treatment in modulating biochemical changes, preserving the organoleptic quality and increasing the marketability of the Late Swelling peach fruits during cold storage, as well as postponing its storage life.

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Table 1 Types of modified atmosphere packaging

Modified atmosphere Packaging (MAP)	Thickness (mm)	slope	Area (m^2)	WVTR*	WVP** (g. mm m^{-2}. day. mmHg)	O_2 Permeability (m3m^{-1}.day.mmHg)	CO_2 Permeability (m^3m^{-1}.day.mmHg)
MAP 1 (low density polyethylene 40 micro/Nano calcium carbonate)	0.074	0.0049	0.0013	0.0003	0.0693	2.25E-05	2.53E-07
MAP 2 (low density	0.033	0.0087	0.0013	0.0002	0.0549	3.26E-06	8.48E-08

polyethylene)							
MAP 3 (low density polyethylene + liner polyethylene)	0.083	0.0017	0.0013	0.0001	0.0270	7.70E-07	1.53E-06

Table 2 Effect of chitosan (Chi) and modified atmosphere (MAP) combinations on fruit firmness, color, acidity of Swelling peach fruits after 10 days at 10˚C and 85-90 % R.H

Treatments		Firmness (N)		Color						Acidity (%)	
				L*		*h*˚		C			
Chi	MAP	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
0	0	28.4	26.2	63.9	61.8	110.7	60.8	18.5	13.9	0.44	0.31
1	1	52.1	31.0	64.2	57.4	118.6	72.0	14.7	11.5	0.54	0.36
1	2	50.5	41.4	63.6	59.0	112.2	80.5	14.8	11.0	0.55	0.39
1	3	53.8	40.7	64.3	60.9	112.7	77.7	14.4	10.8	0.55	0.34
2	1	46.5	34.5	65.5	60.6	111.3	73.5	14.7	11.1	0.54	0.37
2	2	52.4	40.2	65.4	61.1	112.7	81.21	14.5	11.2	0.49	0.36
2	3	54.0	39.8	65.5	62.9	111.5	78.4	14.8	10.8	0.54	0.38
LS D 0.05		4.7	2.9	1.4	3.5	3.6	5.4	1.3	0.92	0.03	0.04

Table 3 Effect of chitosan (Chi) and modified atmosphere (MAP) combinations on fruit SSC, reducing sugars (RS), non-reducing sugars (NRS), total sugars (TS), total chlorophyll (T Chl.) of Swelling peach fruits after 10 days at 10˚C and 85-90 % R.H.

Treatments		SSC (%)		RS (%)		NRS (%)		TS (%)		T Chl. (mg.100g^{-1})	
Chi	MAP	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
0	0	13.4	12.2	2.18	2.30	7.43	7.60	9.61	9.90	107	67
1	1	14.7	12.8	1.90	1.92	6.62	6.45	8.52	8.37	177	101
1	2	15.2	12.2	1.92	1.96	7.34	6.74	9.25	8.71	128	122
1	3	14.8	12.4	1.92	2.04	7.30	6.47	9.22	8.50	106	108
2	1	14.7	12.8	1.93	1.97	7.45	6.43	9.38	8.40	147	93
2	2	14.5	12.7	1.89	1.77	7.22	6.95	9.11	8.72	170	114
2	3	14.5	12.6	1.92	2.15	6.91	6.13	9.02	8.29	162	124
LS D 0.05		0.51	0.65	0.7	0.4	0.92	0.76	0.98	0.67	38	27

Table 4 Effect of chitosan (Chi) and modified atmosphere (MAP) combinations on fruit vitamin C (V.C), anthocyanin (Anth.), total carotenoids (T Car.), total phenols (T Phenl.), electrolyte leakage (EL) of Swelling peach fruits after 10 days at 10˚C and 85-90 % R.H.

Treatments		V.C (%)		Anth. ($mg.100g^{-1}$)		TCar. ($mg.100g^{-1}$)		T Phenl. ($mg.100g^{-1}$)		EL (%)	
Chi	MAP	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
0	0	13.9	13.0	3.1	4.3	0.22	0.35	0.44	0.57	78.9	89.3
1	1	14.4	16.5	1.3	2.9	0.16	0.26	0.31	0.44	61.4	75.8
1	2	16.1	15.0	1.2	2.9	0.15	0.25	0.20	0.43	57.9	67.3
1	3	14.9	16.8	1.2	2.8	0.16	0.26	0.63	0.48	58.0	70.4
2	1	16.5	15.6	1.2	2.5	0.17	0.26	0.20	0.49	71.8	74.7
2	2	17.6	14.9	1.3	2.7	0.15	0.27	0.20	0.38	50.7	60.1
2	3	14.9	13.8	1.2	2.5	0.16	0.25	0.42	0.43	56.7	65.8
LS D 0.05		1.3	0.86	0.32	0.76	0.04	0.06	0.24	0.21	7.4	3.8

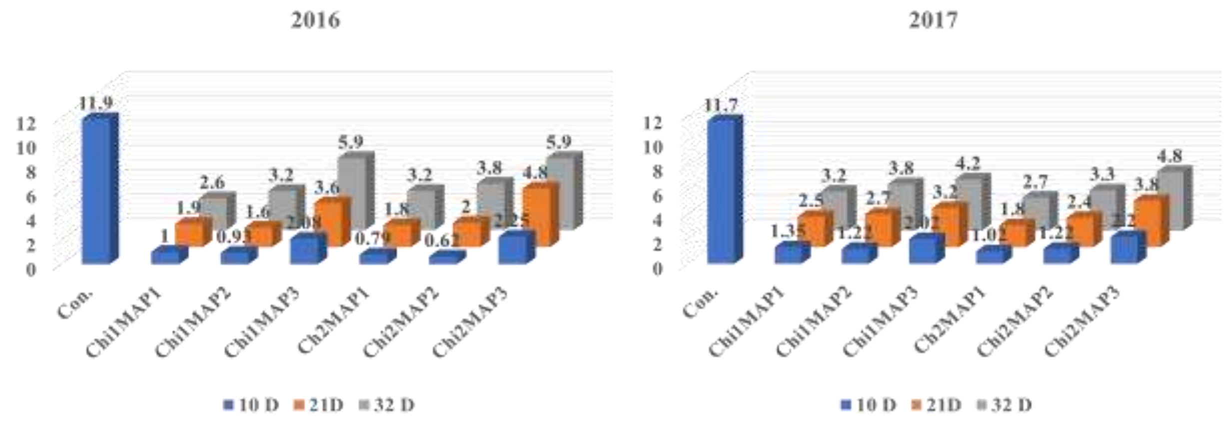

Fig. 1 Effect of combinations of chitosan (Chi) and modified atmosphere (MAP) on fruit weight loss (%) of Swelling peach fruits after 10, 21 and 32 days at 10˚C and 85-90 % R.H.

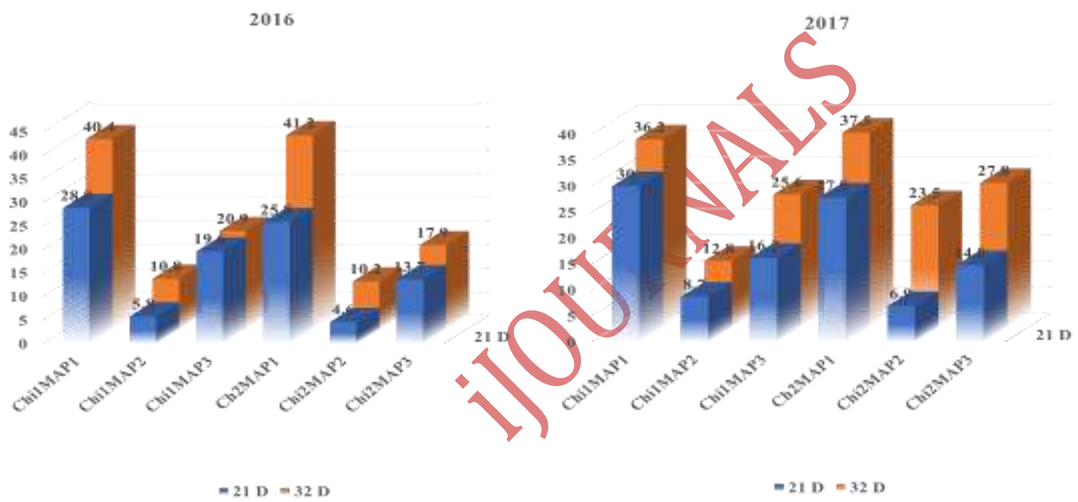

Fig. 2 Effect of MAP and chitosan treatments on off flavors (%) after 21 and 32 days.

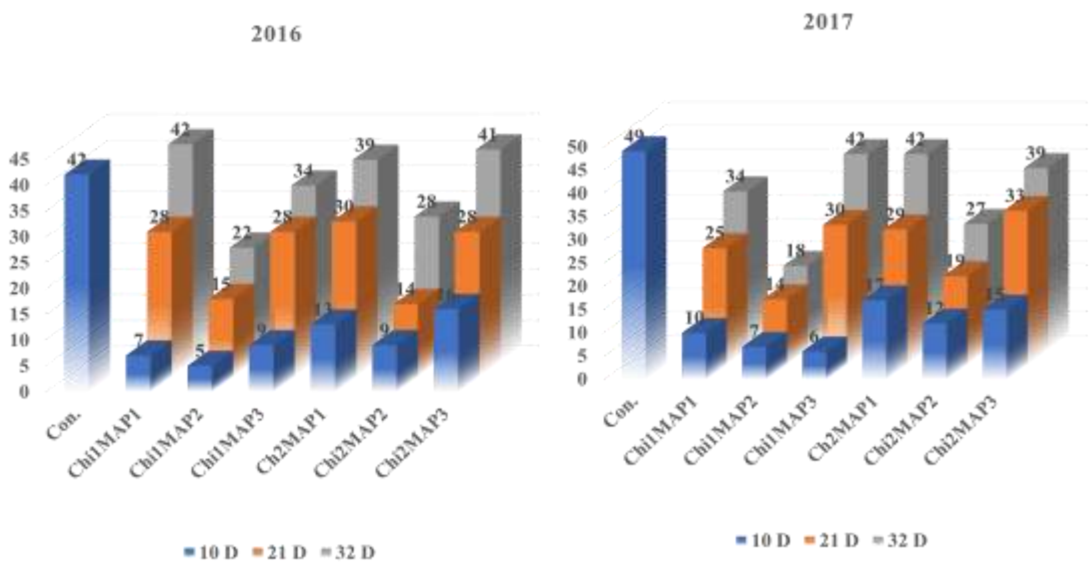

Fig. 3 Effect of MAP and chitosan treatments on Unmarketable fruits % after 10, 21 and 32 days.

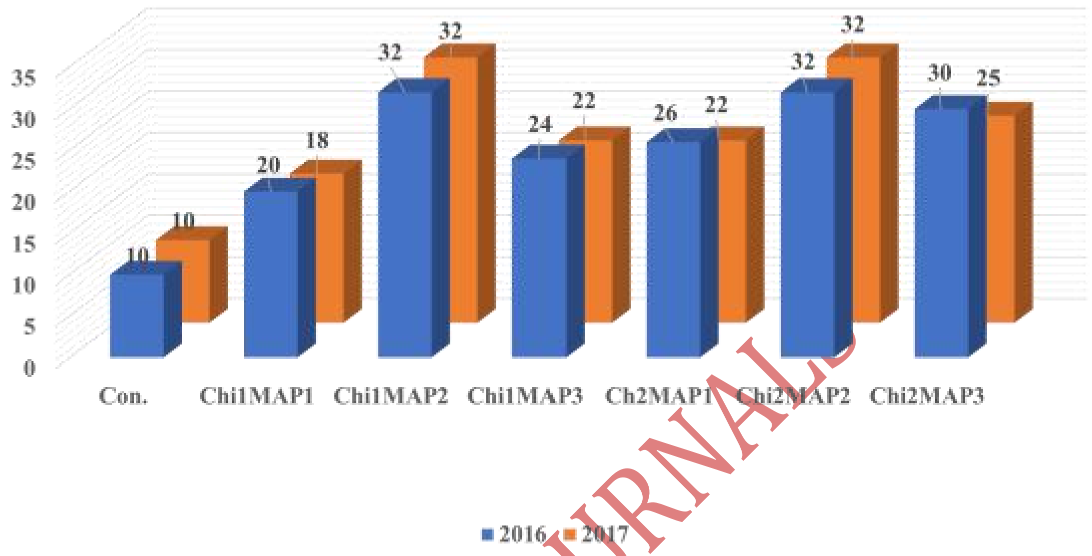

Fig. 4 Effect of MAP and chitosan treatments on storage life (days).