

Original Article

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Incorporation of garlic extract as antifungal agent in psyllium based edible coating for mandarin

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Abstract

In present Research work, the methanolic extract of garlic was incorporated in locally developed Psyllium based edible coating for its application on mandarin. Different concentrations of the extract were used in the coating and quality of the fruit was monitored during storage at room temperature. The results indicated that there was least change (increase) in brix, weight loss, brix/acid ratio, pH and acidity of the fruit during storage studies. The fungal contamination was effectively controlled due to incorporation of garlic extracts at a rate of 6-8%. On the basis of these results it was concluded that the garlic extracts can be used in psyllium based edible coating and it has antifungal significant antifungal potential but at relatively higher concentrations (>6%).

History

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Edible Coating, Psyllium
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1. Introduction

Citrus fruit belonging to the family, Rutaceae, appeared on globe, 30 million years ago (Liu, Heying, & Tanumihardjo, 2012). Nippur in Mesopotamia is the origin of citrus cultivation (Anitei, 2007). The oranges having loose peel which belong to the specie Citrus reticulata Blanco are usually called mandarins (Niaz, 2004). Citrus fruit is widely cultivated all over the world with areas under cultivation and production increasing greatly from 2000 - 2010. According to FAO (2012) Brazil is the largest producer of citrus in the world, followed by China and USA, and Pakistan is at 6th position. In Pakistan, its 95% area of cultivation is in Punjab because of favorable temperature and environmental conditions with Sargodha as a leading district.

There is a huge demand from both the fresh and processed oranges by the consumer all over the world. However, it is considered as a perishable commodity and there are many hurdles in its shelf stability. The major postharvest spoilage of mandarin are due to the

incidence of pathogens *Panicillium* (green and blue mould) (Valencia-Chamorro et al., 2009, 2010). In order to enhance its shelf life a number of techniques are being used now days. The most common technologies used commercially are low temperature storage, polyethylene packaging and emulsion applications as wax coatings and chitosan (Chien & Chou, 2006; Chien, Sheu, & Lin, 2007; Contreras-Oliva, Rojas-Argudo, & Perez-Gago, 2011, 2012; Navarro-Tarazaga et al., 2007, 2008; Perez, Gago-Rojas, & Del Rio, 2002; Thakur, Kaushal, & Sharma, 2002; Valencia-Chamorro et al., 2011). Each techniques have its own disadvantages like cold storage is costly, wax (shellac and polyethylene) application may result in peel pitting, chilling injury, deformation and coating fracture (Dou, 2002; Petracek, Dou, & Pao, 1998) and are also associated with elevated levels of internal volatiles (Hagenmaier, 2002; Perez, Gago-Rojas, & Del Rio, 2003; Porat et al., 2004). Shellac require ammonia as a solvent (Navarro-Tarazaga et al., 2007; Contreras-Oliva, Rojas-Argudo, & Perez-Gago, 2011, 2012). Chitosan has non-vegetative background as it is extracted from exoskeleton of crustacean animals.

On the other hand, plant based coatings are edible, vegetative and naturally biodegradable. Psyllium seed

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and husk contain polysaccharides which can be used as a source to prepare a novel edible coating (Ahmadi et al., 2012; Banasaz et al., 2013). *Plantagoovata* is a short-stemmed annual herb that is cultivated primarily in India, having more than 200 different species worldwide (Dhar et al., 2005). Mucilage of Psyllium contains a gel forming fraction and major portion contain a highly branched arabinoxylan.

Inspired by the current trends in food industry directed towards safer and healthier foods and to produce little and environmental friendly wastage, the current research was focused to develop a novel and unique film/coating of psyllium polysaccharide extracted from its husk. The second objective was the incorporation of metanolic extracts of garlic in the film as antimicrobial agent.

2. Material and methods

The current research work was carried out in Food Microbiology Laboratory, University of Sargodha, Sargodha.

2.1. Chemicals and reagents

All the reagents analytical grade (Merck, BDH and Oxoid) were purchased from local market of Lahore, Pakistan and Mandarin were purchased from Hassan Citrus Orchard, Bhalwal, Sargodha (Pakistan).

2.2. Extraction and purification of *Psyllium* polysaccharide powder

Extraction and purification of *Psyllium* polysaccharide was done by following the method described by Ahmedi et al. (2012). *Psyllium* husk (50g) was added into 1L of distilled water and was gelatinized at 65°C on hot plate with magnetic stirrer.

The gel after removal of husk particles by sieving, was washed with 97% ethanol and residues were evaporated in hot air oven (Binder, Germany) followed by fine grinding into powder.

2.3. Garlic extract

The peeled garlic (200 g) and 500 ml of methanol was blended for 2 minutes and was left for 24 hours in a shaking chamber/incubator (Shin Saeng Scientific Co. Korea, Model SKIR-601 L) at 25°C at 120 rpm. Extract was concentrated in a rotary evaporator (Heidolph,

America, Model Laborota 4001 HB Digital) at 50°C for further use.

2.4. Development of coating

Psyllium based edible coating was formulated by following the method of Azarakhsh et al. (2014) with some modifications. The coating contained *Psyllium* polysaccharide powder (1.29%), Glycerol (1.16%) and calcium chloride solution (1% Ascorbic acid and 1 % Citric acid) at the rate of 2.0%. Then different concentrations of garlic extract (0%, 2%, 4%, 6% and 8%) were incorporated into the coating. The ingredients of coating were dissolved by heating at 65°C on hotplate with constant stirring.

2.5. Application of coating

The fully ripened and fresh, washed and air dried Kinnows (Mandarin) were coated by the prepared edible coating and stored at room temperature. Kinnow were then evaluated for physico-chemical and microbial quality on weekly basis during a storage period of one month.

2.6. Analysis of mandarin

The Kinnow samples from each treatment were randomly drawn after every 7 days interval during the whole period of storage to determine physico-chemical parameters and microbial quality.

2.7. Physiochemical tests

The weight loss (WL) in Kinnows was calculated according to the method of Thakur, Kaushal, & Sharma (2002) by taking average weight of 20 kinnows into account.

Total Soluble Solids, Titratable acidity and pH were determined by following the standard methods of AOAC (2000) by method no. 920.151, 942.15 and 981.12, respectively.

Sugar/Acid Ratio was calculated by taking ratio between brix and Titerateable acidity (TA) as reported by Balaswamy et al. (2011).

2.8. Microbial quality

The fungal attack on the mandarins was inspected regularly and the fungal attack on the fruit was also confirmed by taking suspension of the whole fruit in

Table 1: Mean sum of squares for physicochemical parameters of mandarin during storage

Source of variance	DF	Weight	Brix	pH	TA	Brix Ratio	Acid
Storage Period (S)	4	1202.86**	27.32**	1.63**	0.44**	240.21**	
Treatments (T)	5	53.55**	14.29**	1.19**	0.16**	24.19**	
S x T	20	90.12**	25.00**	2.32**	0.036**	84.20**	
Error	60	0.02	0.002	0.0002	0.0001	0.06	
Total	89						

** = Highly Significant $p < 0.05$

peptone water with subsequent inoculation and incubation while using potato dextrose agar by following the method of Tournas et al. (2001).

2.9. Statistical analysis

Experimental data was subjected to ANOVA analysis as recommended by Valero et al. (2013). The overall least significant differences (Fisher's LSD procedure, $p < 0.05$) were calculated using MiniTab Software Package v.16.2.1 for Windows (MiniTab, 16).

3. Results

The current research work was carried out to study the impact of garlic extract on the shelf life of the mandarin. For this purpose, edible coating from Psyllium husk was developed and various concentrations of garlic extract were incorporated into the coating. The results obtained during the entire research work were described as under.

The statistical results (ANOVA) revealed that there was a highly significant effect of storage period (weeks) and treatments on all the parameters (weight loss, brix, pH, acidity and brix acid ratio). Moreover combine effect of storage period and treatment was also found to be highly significant as shown in Table 2.

3.1. Weight loss

The effect of treatments and storage period (Figure 1) on weight loss of the mandarins revealed that in all the

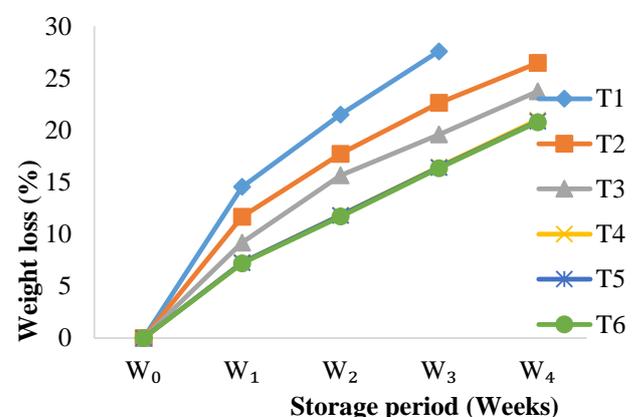
Table 2: Fungal contamination on mandarin during storage

Treatments	W ₀	W ₁	W ₂	W ₃	W ₄
T ₁	Nil	Nil	+	+	+
T ₂	Nil	Nil	Nil	+	+
T ₃	Nil	Nil	Nil	+	+
T ₄	Nil	Nil	Nil	Nil	+
T ₅	Nil	Nil	Nil	Nil	Nil
T ₆	Nil	Nil	Nil	Nil	Nil

treatments there was a gradual decline in weight with storage period of 4 weeks. Moreover, the non-coated mandarins (T₁) suffered severe weight loss (27.58%) and were discarded due to deterioration after 3 weeks of storage followed by T₂ (coated without garlic extract) with a mean weight loss of 26.46% at 4th week of storage.

3.2. Brix

The results pertaining to the effect of treatments and storage period on brix of the Mandarins showed that uncoated Mandarins (T₁) demonstrated highest increase in brix at the end of 3rd week with a value of 16.07 °Brix. The results further revealed that the brix was gradually increased with the passage of storage time in all the treatments. The increment in brix after 4 week of storage period in various treatments was from 12.13 to 15.78 (coated but no garlic extract), 12.06 to 15.54 (coated with incorporation of 2% garlic extract), 12.09 to 14.45 (coated with incorporation of 4% garlic extract), 12.04 to 14.37 (coated with incorporation of 6% garlic extract) and 12.07 to 14.35 (coated with incorporation of 8% garlic extract) as shown in Figure 2. Its clear from the results that there was also a positive impact of incorporation of garlic extracts into coating on change in brix of the fruit.

**Figure 1: Effect of storage and treatments on weight loss during storage of mandarin**

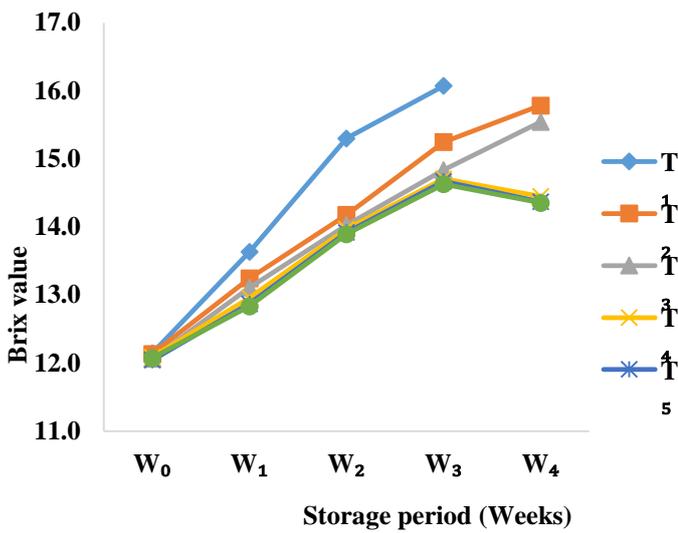


Figure 2: Effect of treatment and storage period on brix of mandarin during storage

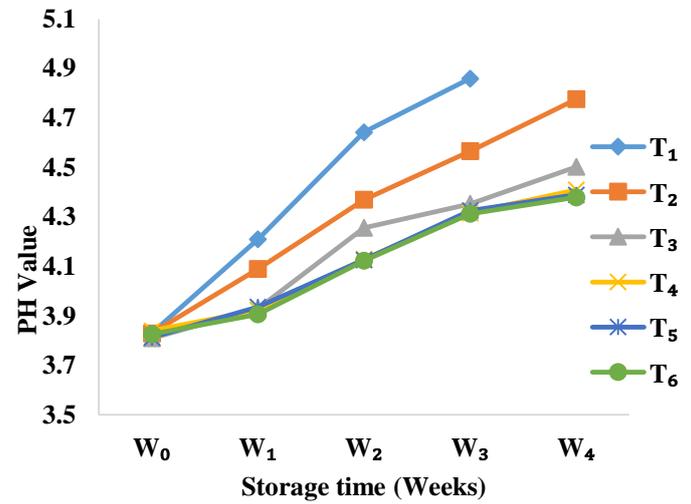


Figure 3: Effect of treatment and storage period on pH of mandarin during storage

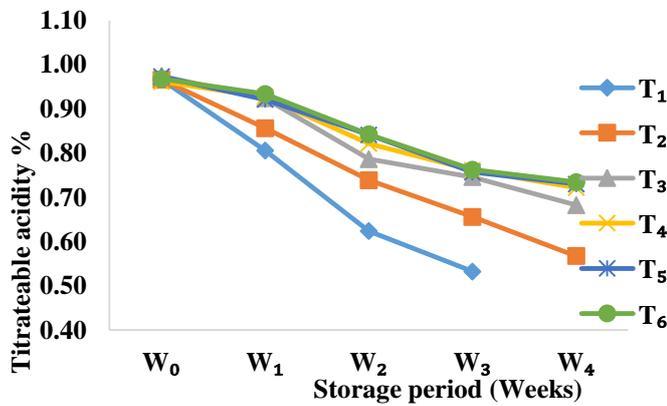


Figure 4: Effect of treatment and storage period on acidity of mandarin during storage

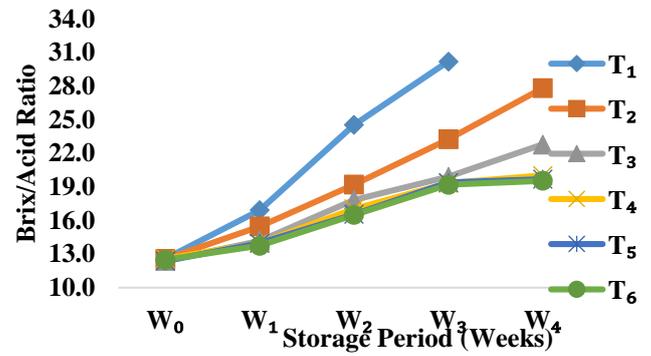


Fig 5: Effect of treatment and storage period on brix acid ratio of mandarin during storage

3.3. pH and titratable acidity

There was an increasing trend for pH and ultimately decreasing trend for acidity in all the treatments with the passage of storage period. However, the significantly higher increment in pH (from 3.83 to 4.86) and decline in acidity (0.97-0.53% citric acid) was observed in the non-coated mandarin. Moreover, the results for change in the mean pH value and acidity of the mandarin coated with 4%, 6% and 8% garlic extract were at par. Results for pH and acidity are shown in Figure 3 and Figure 4, respectively.

3.4. Brix/acid ratio

The combine effect of treatments and storage period as demonstrated in Figure 5 revealed that the brix to acid ratio was gradually increased with the passage of

storage period in all the treatments. The highest increment in the ratio was observed in T₁ (non coated) followed by T₂ (coated with incorporation of 2% garlic extract) whereas the lowest change was observed in T₆ (coated with incorporation of 8% garlic extract) during storage period of 4 weeks.

3.5. Microbial analysis

Microbial analysis showed that non-coated mandarins (T₁) were spoiled very rapidly and were discarded in 3rd week as compared to coated mandarin. In case of coated mandarins, the fungal attack was observed in case of T₂ (coated without incorporation of garlic extract), T₃ (coated with incorporation of 2% garlic extract) and T₄ (coated with incorporation of 4% garlic extract) after 3 to 4 week of storage. However, there was no fungal attack in case of T₅ (coated with incorporation

of 6% garlic extract) and T₆ (coated with incorporation of 8% garlic extract) after 4 weeks of storage at room temperature as shown in Table 3. It is obvious from the results that garlic extract at a rate of 6-8% showed effective antifungal activity.

4. Discussion

The changes in physio-chemical parameters of fruits during storage are due a number of factors and the parameters like pH, acidity, weight loss, brix and brix to acid ratio are interlinked with each other. After harvesting of the fruits, the process of ripening continues which results in water loss due to transpiration and ultimately there is loss in weight of the fruit during post harvest handling. In current study there was also a loss in weight during storage of mandarins which might be due to the same factor (due to loss in water by transpiration from the rind epidermal cells which caused reduction in fruit weight). However, the reduced weight loss in case of coated mandarins might be due to the reason that the film served as barrier for evaporation of the water. These results are in line with the findings of a number of scientists (Alam & Paul, 2001; Contreras-Oliva, Rojas-Argudo, & Perez-Gago, 2012; Dhall, 2013; Thakur, Kaushal, & Sharma, 2002; Mishra et al., 2010; Shamloo et al., 2015; Valencia-Chamorro et al., 2010, 2011) who also reported loss in water after post harvest handling while working on storage stability of various fruits and observed less water loss due to coating.

Similarly, change in brix (total soluble solids) might be due to decline in water contents and conversion of polysaccharides into simple sugars. The results are justified by the findings of Medeiros et al. (2012) who also reported increment in brix of citrus fruits during post harvest handling and suggested that this might be due to conversion of polysaccharides into simple sugars and loss in water during ripening. There is an inverse relationship between pH and acidity. Similarly, the brix to acid ratio is affected by change in sugar contents or due to change in acid contents (pH, acidity). The change in these parameters during this research work might be due to change in sugar contents (as brix was increased and acidity was decreased during study).

The results of the present research work are hence justified by the findings of a number of researchers who

have highlighted various findings and reasons in this regard. They had reported decline in water contents (Contreras-Oliva, Rojas-Argudo, & Perez-Gago, 2012; Dhall, 2013; Shamloo et al., 2015) and increment in sugar (TSS) contents (Lee and Kader, 2000; Manzano & Diaz, 2001; Shamloo et al., 2015; Thakur, Kaushal, & Sharma, 2002) during storage of fruits.

The results of current research work clearly illustrated that there was no fungal attack in case of mandarins which were coated by incorporation of garlic extracts. These results revealed that the garlic extracts possessed antifungal potential which might be due to the presence of volatile compounds in garlic extract such as allicin, diallyl sulfide, diallyl disulfide, and low amounts of nonvolatile, water-soluble sulfur compounds. These results are also justified by the findings of Lanzotti (2006) and Ponce et al. (2008) who also reported antimicrobial potential of garlic extracts due to the presence of volatile as well as non-volatile compounds. Gayani, Hyun-Jin, & Myong (2009) and Seydim & Sarikus (2006) also reported reduced microbial growth with incorporation of garlic oil in the coating developed for application on fruits. From the results of current study, it was concluded that the garlic extracts possessed antifungal potential and these can effectively be used in edible coatings as also suggested by Pranoto, Salokhe, & Rakshit (2005a) and Pranoto, Rakshit, & Salokhe (2005b) who also observed the suitability of incorporation of garlic oil (extracts) in different coating materials.

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