

## **EFFECTS OF EDIBLE COATING – SEMPERFRESH™, ASCORBIC ACID AND WHEY PROTEIN TREATMENT ON CERTAIN MICROBIOLOGICAL, PHYSICAL AND CHEMICAL QUALITIES OF PEELED BANANAS “MUSA SAPIENTUM”**

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### **Abstract**

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Banana is one of the most economically important fruits in the world with a huge consumption demand, as offering a valuable reserve of vitamins, potassium and energy. In this study, certain quality parameters in peeled banana was investigated by the treatment of vitamin C, Semperfresh™ (a sucrose polyester base coating), and whey protein during cold storage (277 K). According to microbiological analysis, the vitamin C application was found to be the most effective group by extending the shelf life depending on its acidic feature of bananas allowing to decrease the total mesophilic aerobic bacteria (TMAB), yeast and the mold counts with respect to control. The weight determination and the firmness results showed that the sole Semperfresh™ treatment was found to indicate a high chemical and physical quality at the end of the experiments.

*Key words:* banana, vitamin C, whey protein, Semperfresh™

### **Introduction**

Banana is the common name for herbaceous plants of the genus *Musa* and for the fruit they produce. They are native to the tropical region of Southeast Asia, and are likely to be first domesticated in Papua New Guinea. Bananas were introduced to the Americas by Portuguese sailors who brought the fruits from West Africa in the 1500s. The word banana is of West African origin, from the Wolof language, and passed into English via Spanish or Portuguese. It is one of the most economically important fruits in the world with a huge consumption demand, as since bananas are a valuable source of vitamin B<sub>6</sub>, vitamin C, and potassium. Bananas consist mainly of sugars (glucose, fructose and sucrose) and fiber, which makes them ideal for an immediate and slightly prolonged source of energy. Rather than its consumption as raw fruit, they are also processed in fried, jam and pancake style (The Australia & Pacific Science Foundation, 2002; Agroforestry net, 2010; Banana web, 2010; Economic Botany Textbook,

2009; Online Etymology Dictionary, 2005). Bananas were reported to be a well stress relieving agent and reduce the incidence of depression, stroke risk, anemia, constipation and diarrhea, heartburn, smoking ulcers, maintain healthy bones and a healthy kidney function, decrease the blood pressure and provide eyesight protection. (Banana web, 2010).

Semperfresh™ is a sucrose ester based coating, widely used in the fresh fruit and vegetable industry to reduce bruising, weight loss and preserve green color and fruit pressure in storage without delaying normal ripening processes for consumers. The Semperfresh™ was comprised of sucrose esters of fatty acids, sodium carboxymethyl cellulose and monodiglycerides of fatty acids obtained in liquid form (50/100 w/v). Semperfresh™ inhibits water loss while allowing gas exchange between the fruit and its environment. In this way, a modified atmosphere of reduced oxygen with slightly raised carbon dioxide levels is provided. This modified atmosphere effect leads to an extended green life, better color, delayed softening, fresh appearance, delayed break-

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down in storage, reduced chilling injury, reduced bruising and reduced scuffing. It also reduces weight loss and excess respiration, resulting in firmer fruits with less pitting. Semperfresh™ was reported to give sheen to cherries without leaving an oily or greasy film on fruit and widely used in the fresh pear industry to reduce bruising, weight loss and preserve green color and fruit pressure in storage without delaying normal ripening processes for consumers (Baldwin et al., 1995; Lin et al., 2003; Gonzalez-Aguilar et al., 2008; Maftoonazad et al., 2008). It was found that Semperfresh™ was effective in keeping the quality of coated bananas, together with modified atmosphere storage (Truter and Combrink, 1990).

Whey is a general term that typically denotes the translucent liquid part of milk that remains following the process (coagulation and curd removal) of cheese manufacturing. All of the constituents of whey protein provide high levels of the essential and branched chain amino acids that are important for their role in the maintenance of tissue and prevention of catabolic actions of exercise (Hoffman and Falvo, 2004; Fuente et al., 2002).

The two most popular types of protein in the supplemental form are whey and casein. Research showed that whey protein elicits a sharp, rapid increase of plasma amino acid following ingesting, while the consumption of casein induces a moderate, prolonged increase in plasma amino acids. The differences in the digestibility and absorption of these protein types indicate the ingestion of the “slow” (casein) and “fast” (whey) proteins (Campbell et al., 2007). Whey and whey derived bioactive compounds have important functional properties and enhances general health. Whey has a potent antioxidant activity, immune modulation, antimicrobial activity, improved muscle strength and body composition (Ha and Zemel, 2003).

Vitamin C (ascorbic acid) is a food additive which shows antioxidant properties. It protects the body against oxidative stress, and plays a cofactor role in several vital enzymatic reactions. Vitamin C has therefore a bactericidal effect and it was reported to be one of the major antioxidant molecules occurring naturally in foods like fruit juices and it prevents browning and improves the nutritional value, providing extraordinary health benefits to the consumer, including prevention from strokes, the common cold, oxidative damage of proteins by cigarette smoke and it acts primarily as a singlet oxygen quencher. It was also used as an external fruit preservation agent on quinces (Yurdugul, 2005).

The main aim of this research is; to extend the shelf life of peeled bananas by inexpensive techniques and to investigate changes such as microbial growth, pH, the weight loss, the total sugar content and the firmness parameters. Peeled

banana is useful in the confectionery, cake and pastry industry, as well as for the housewives in ready to eat meal preparation.

## Materials and Method

### *Fruits*

Bananas were harvested in November 2013 and November 2014 in Anamur, Turkey and were immediately transported to the laboratory prior to the experiment. The bananas were washed and their skins were peeled. The defective and bruised fruits were eliminated before the treatments. All of the experiments were repeated two times in triplicates for each.

### *Experimental methodology*

A total weight of 30 kg bananas were used in the treatment period of the experiment. The bananas were treated with eight different groups with three replicates: involving vitamin C (C); Semperfresh™(S); whey protein(W); vitamin C-Semperfresh™(C+S+W); vitamin C-whey protein (C+W); Semperfresh™-whey protein (S+W); vitamin C-Semperfresh™-whey protein (C+S+W); and the control (Co) group without any treatment. Each group was consisted up of six bananas. All bananas were kept at (277 K) in closed glass jars for the 36 days' experimental period.

### *Semperfresh™ treatment*

Semperfresh™ was obtained from ACC group (Ankara) in liquid concentrate form (50/100 w/v). As no recommended concentration of Semperfresh™ was defined for the peeled bananas by the vendor, 0.05/100, 0.1/100, 0.5/100, 1/100 and 2/100 (w/v) levels were subjected to trials with relevance to microbiological and chemical quality criteria. A taste panel, carried out prior to the experiment showed that 0.1/100 w/v can be preferred as a recommended concentration. 0.1/100 (w/v) concentration of Semperfresh™ was prepared by diluting 1 mL Semperfresh™ to 1000 mL distilled water. The solution were then mixed for 30–45 minutes with occasional stirring and then peeled bananas were treated with the solution for a period of one minute (Özden & Bayindirli, 2002). All of the samples in the related treatment groups were immersed into the water solution containing Semperfresh™.

### *Vitamin C (Ascorbic acid) treatment*

Ascorbic acid (vitamin C) was primarily used at concentration of 0.1, 0.5, 1.0, 5.0, 50 and 100 mg/L, and throughout the entire study 100 mg/L levels were applied by diluting 100 mg ascorbic acid to 1 liter distilled water (100 mg/L),

which was found to be the effective dose for retardation of the microbial growth and oxidation of bananas in a previous trial.

#### ***Whey protein***

1.0, 5.0, 50 and 100 mg whey protein, dissolved in 1000 mL distilled water, were subjected to trials with relevance to microbiological and chemical quality criteria. A 100 mg dose of whey protein was found to be sufficient and effective due to the microbiological and chemical perspective. Whey protein solution was prepared by mixing 100 mg of whey protein with distilled water by completing to 1000 mL.

#### ***Analysis of microbiological growth***

1 g of sample, obtained from a randomly selected fruit was mixed with 9 mL of sterile distilled water. After serial dilutions, the total mesophilic aerobic bacteria, mold and yeast counts were carried out on nutrient agar, potato dextrose agar and yeast agar, respectively. Each group contains three replicates. Total mesophilic aerobic bacteria plates were incubated at 310 K for 24 h, mold and yeast plates were incubated at 310 K for 24 h. The analysis was repeated in the twentieth and the thirty-sixth days.

#### ***Firmness***

One fruit from each treatment group was randomly selected for the firmness test by using a fruit hardness tester (model FT011, Everwell, Japan) and results were stated in kilograms. The test was applied on three different areas from both of the two tips and the middle of the banana.

#### ***Weight retention***

Three fruits from each treatment group were weighed during storage with a digital balance (Precisa, model BJ210 C) to determine weight loss.

#### ***pH determination***

The pH values of the bananas were determined by using a portable pH meter (Isolab) at the zeroth, twentieth and thirty-sixth days. Since bananas were considered as soft, a spear type electrode was replaced the original one and the sample was pierced with the electrode to a fixed depth.

#### ***Total sugar content***

The total sugar content of the control and the other treated groups were determined by the dinitrosalicylic acid (DNS) method. 10 g of DNS was weighed and dissolved in 200 mL NaOH solution. The solution was heated up and stirred intensively. A colour stabiliser Na-K-tartrate was added by dissolve 300 g of Na-K-tartrate in 500 mL of distilled water). Both solutions were combined and stirred well and

distilled water was added to complete 1 L. 1 g of banana was put into an Erlenmeyer flask and then 10 mL of H<sub>2</sub>SO<sub>4</sub> was added. It was heated up in boiling water bath for 20 minutes and stirred periodically until hydrolysis was completed. The sample was cooled down by carefully adding 12 mL of NaOH (w = 10%). It was stirred and filtered into 100 mL volumetric flask, filled with distilled water to 100 mL. 10 mL of solution was transferred into another 100 mL volumetric flask and filled with distilled water to 100 mL. 0.5 mL from this mixture was mixed with 1.5 mL DNS reagent and 0.5 mL distilled water in duplicates. These groups were kept in water bath at 323 K in 30 minutes incubation (Nüve NB20, Turkey) and subsequently the absorbance was determined by a spectrophotometer (CADAS 50S Dr. Bruno Lange GmbH-Berlin) at a wavelength of 550 nm and the glucose concentrations in each sample was expressed as g/100 g fruit sample.

#### ***Taste panel***

20 previously trained panelists were asked to taste and then rate the bananas according to the criteria of colour, sweetness, sourness, bitterness, saltiness, odor, appearance, flavor, hardness and overall evaluation using a 10-point hedonic scale (one = dislike extremely, five = neither like nor dislike, and 10 = like extremely).

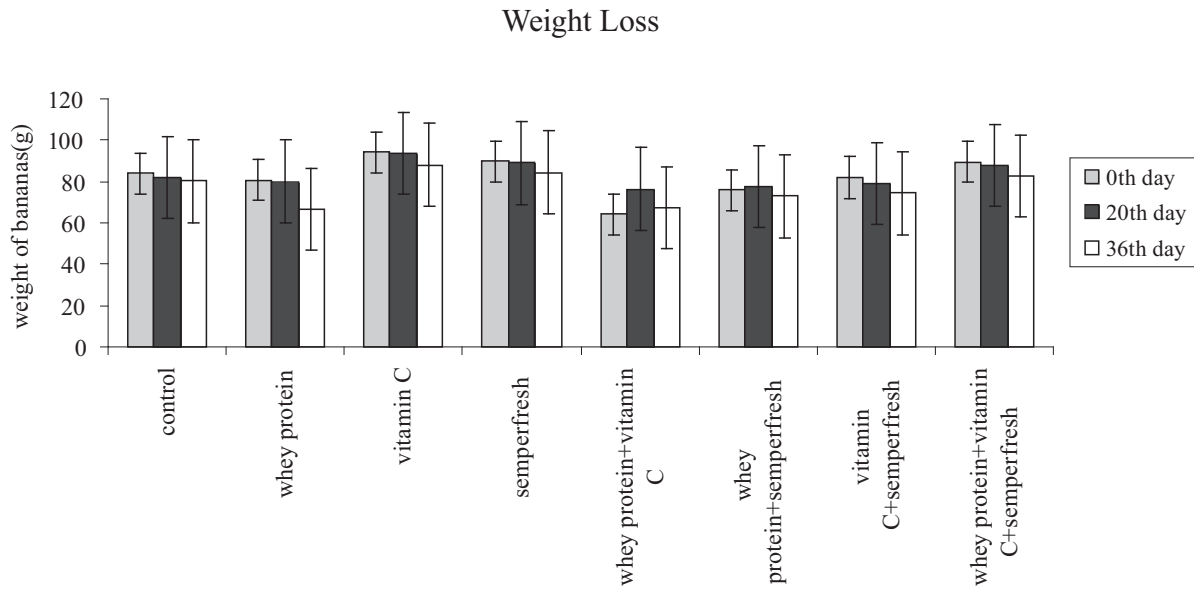
#### ***Statistical analysis***

The method of analysis of variance (SPSS) was applied to the data, obtained from each treatment to detect the significance of differences at 5% level of significance ( $p < 0.05$ ).

## **Results and Discussion**

#### ***The microbial assay***

According to the total mesophilic aerobic bacteria counts, with respect to the control; an approximate reduction of two-log cycle was observed in the sole treatment of S. In addition, the vitamin C application has also reflected an important antimicrobial activity on fruits, as the comparison of 0<sup>th</sup> day and 36<sup>th</sup> day results indicated a significant difference within the same group, with lowering the microbial counts ( $p < 0.05$ ) (Figure 1) together with the combined treatment of S+W and a sharp decline in the combination of C+W and in the sole C treatments was observed (Figure 1). The enumeration of the total mesophilic aerobic bacteria revealed that the triple treatment S+C+W has no significant reduction on the total mesophilic aerobic bacteria counts. In contrast, the combined applications of C+W and S+W and C alone were found to be effective on reducing total mesophilic aerobic bacteria in the thirty-six days' period. In a previous study carried out on quince fruits by Yurdugul(2005) a reduction



**Fig. 1. The effect of sole and combined treatment of Semperfresh™, vitamin C and whey protein on the total mesophilic aerobic bacteria (TMAB) levels of bananas, with respect to control**

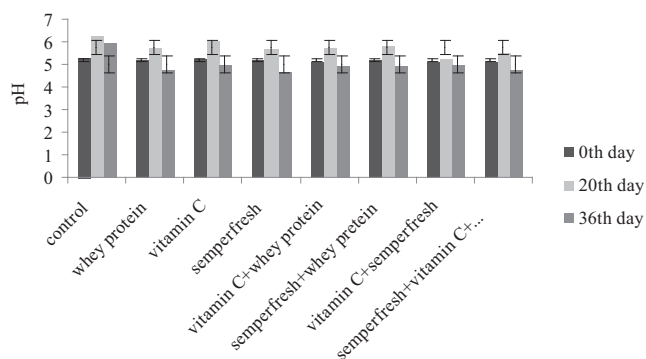
**Table 1**

**The statistical evaluation of the treatment groups, obtained by one-way ANOVA test**

Application Groups	Type	Sum of Squares	dF	Mean Square	F	Sig.
Whey protein, Vitamin C and Semperfresh	Between Groups	7 789	6	1 298	.779	.602
	Within groups	20 000	12	1 667		
	Total	27 789	18			
Whey protein	Between Groups	17 855	6	2 976	1 043	.445
	Within groups	34 250	12	2 854		
	Total	52 105	18			
Vitamin C	Between Groups	30 289	6	5 048	3 462	.032
	Within groups	17 500	12	1 458		
	Total	47 789	18			
Semperfresh	Between Groups	17 526	6	2 921	1 402	.291
	Within groups	25 000	12	2 083		
	Total	42 526	18			
Whey protein and Vitamin C	Between Groups	18 697	6	3 116	1 324	.319
	Within groups	28 250	12	2 354		
	Total	46 947	18			
Whey protein and Semperfresh	Between Groups	17 105	6	2 851	.977	.481
	Within groups	35 000	12	2 917		
	Total	52 105	18			
Vitamin C and Semperfresh	Between Groups	9 447	6	1 575	.687	.664
	Within groups	27 500	12	2 292		
	Total	36 947	18			

of one-one and a half log cycle was obtained by means of the triple treatment of S+C and cold storage, replaced by whey protein up to a period of 35 days, which is in line with these findings. However, in another study performed by Moreira et al (2009) the application of casein and CMC coatings did not cause significant reductions in the counts of mesophilic aerobic microorganisms with reference to control samples. All these findings were in line with the statistical analysis ( $p < 0.05$ ) (Table 1).

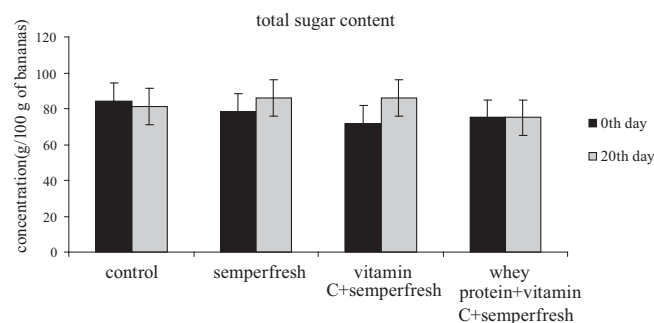
The total mesophilic aerobic bacteria and mold counts revealed that the triple treatment (S+C+W) has shown no significant reduction. The mold counts indicated that S+W and C+W treatment groups showed the largest rise in microbial activity when compared to yeast and total mesophilic aerobic bacteria counts (Figure 2). In another study, S was found to be effective at the range between 0.1/100–1/100 concentrations during inhibition of the mycelial growth of the mold *Colletotrichum musae* (Al Zaemey et al., 1993), but found not to be effective on the development of storage rots caused by *Alternaria* and *Fusarium* spp. of melons in another study conducted by Edwards and Blennerhassett (1990). All these findings were in line with the statistical analysis ( $p < 0.05$ ) (Table 1).



**Fig. 2. The effect of sole and combined treatment of Semperfresh™, vitamin C and whey protein on the mold counts levels of bananas, with respect to control**

The total mesophilic aerobic bacteria and mold counts revealed that the triple treatment (S+C+W) has no significant reduction in yeast growth at the end of the thirty-six days' period, as opposite to the results of Yurdugul (2005) on quinces. A comparable result obtained by yeast counts indicated the same situation and it was observed that the S+W application reduced the microbial growth effectively with respect to the control (Figure 3). On the other hand, C, S and C+W treatment groups showed reduced microbial activity with respect to the other treatment groups involving

control at the end of the thirty-sixth days. However, when the S+W application was considered, a sharp decrease in the eighteenth day counts of mold and yeast was observed. This might be due to the cooperative activity of S+W, as the anaerobic nature of Semperfresh™ provided the lactose to be converted into the lactic acid by certain species of the yeast present on the fruits. All these findings were in line with the statistical analysis ( $p < 0.05$ ) (Table 1).



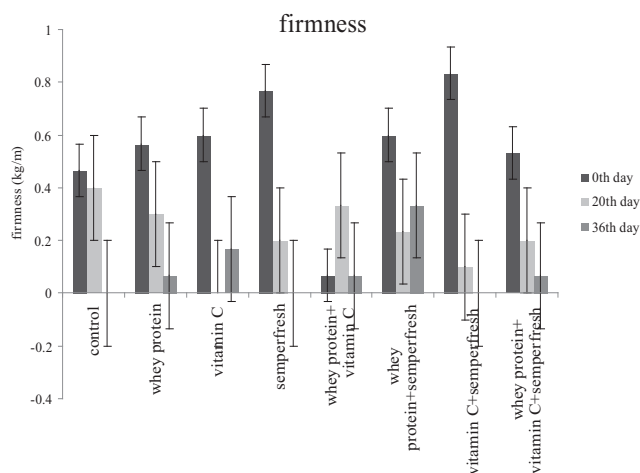
**Fig. 3. The effect of sole and combined treatment of Semperfresh™, vitamin C and whey protein on the yeast counts levels of bananas, with respect to control**

Vitamin C application alone was found to be the most effective treatment when considering all the microbial analysis; including the total mesophilic aerobic bacteria, yeast and mold counts. This result was almost in line with the findings of Yurdugul (2005) on quinces. All these findings were in line with the statistical analysis ( $p < 0.05$ ) (Table 1).

### Weight retention

Compared with the control samples, the vitamin C, the Semperfresh™ and the triple combination of whey protein-vitamin C-Semperfresh™ (W+C+S) showed a low amount of weight loss during storage respectively (Figure 4). Weight loss is caused by respiratory weight loss and evaporation of water from the fruit (Amarante et al 2001). The main mechanism contributing to weight loss is the evaporation of water activated by a gradient of water vapor pressure at different locations in fruit, observed in cherries (Yaman & Bayındırlı 2001). Water diffuses preferentially through a liquid aqueous phase in the cuticle, where water conductance is considerably higher, rather than through pores (Ben-Yehoshua et al 1985; Amarante et al 2001). Generally, coatings which exhibit good water vapor barrier properties are highly hydrophobic in nature (Park et al 2004). A dip solution, composed of 1/100 (w/v) calcium chloride, 0.75/100 (w/v) ascorbic acid and 0.75/100 (w/v) cysteine and carrageenan application together with an edible coating was found to prevent weight

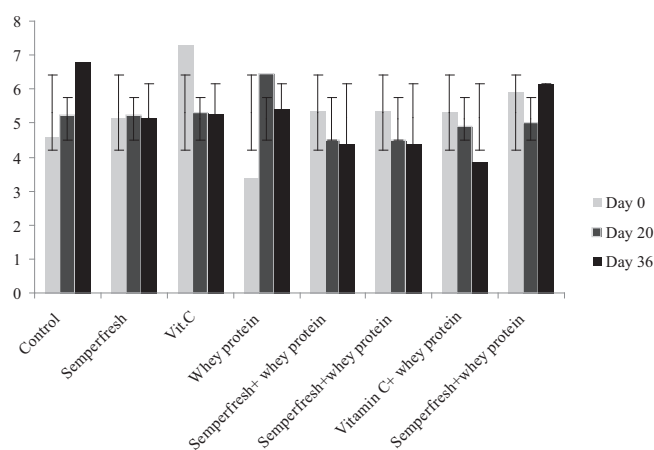
loss in bananas with respect to control (Bico et al 2009). In another study, conducted by Baez-Sañudo et al. (2009) the weight losses of the bananas, treated with one-methylcyclopropene and a chitosan based coating, accounted for an average of 6.5/100 of the initial weight, which were almost in line with our study. Clearly, relatively low weight loss in Semperfresh™ -coated bananas contributed to maintain the better quality of fruit during cold storage. All these findings were in line with the statistical analysis ( $p < 0.05$ ) (Table 1).



**Fig. 4. The effect of sole and combined treatment of Semperfresh™, vitamin C and whey protein on weight retention of bananas, with respect to control**

#### **Firmness determination**

According to the firmness test; the whey protein-Semperfresh™ combination, the vitamin C and the triple treatment; preserved the texture of the bananas by inhibiting water loss with respect to uncoated application groups at the end of 36<sup>th</sup> days (Figure 5). Hardness during ripening in climacteric fruit, such as banana, is generally attributed to degradation of the cell wall and loss of turgor pressure in the cells reduced by water loss (Lin et al., 2003; Lohani et al., 2004; Khin et al., 2007). Immediately softening occurs in five days, during the preservation of the breadfruit, in a research conducted by Worrell et al. (2002), after coating application. In addition, a fruit coating, mango carnauba was reported to retain fruit firmness, which is in line with these findings (Dang 2008) and with Semperfresh™ in Ankara pears (Sumnu and Bayindirli, 1994), on the other hand, no significant difference was observed in a study carried out in asparagus (Fuchs et al., 2008). All the findings were in line with the statistical analysis ( $p < 0.05$ ) (Table 1).



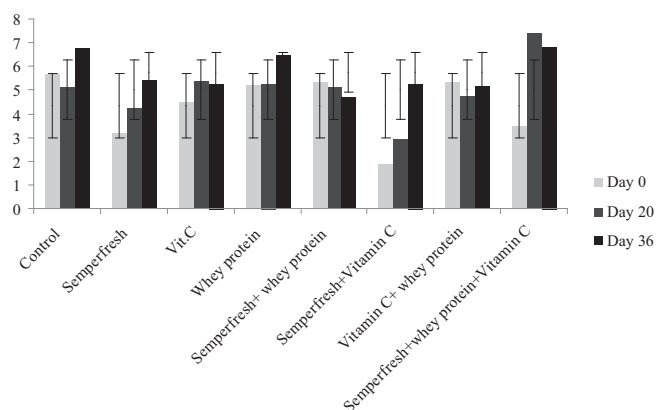
**Fig. 5. The firmness values of the application groups**

#### **pH**

According to the pH values determined in all treatment groups, a slight increase was observed between the range of the initial to 20<sup>th</sup> day measurements (Figure 6). The 36<sup>th</sup> day measurements revealed a slight decline in all application groups as obtained in the other studies such as breadfruit coating (Worrell et al., 2002) and Semperfresh™ was found to be more effective in delaying changes in pH, in the study of Tasdelen and Bayindirli (1998) on the quality of tomatoes, similar with the findings presented in this study, with respect to the control. However, an increase in pH was observed in a study conducted by Sumnu and Bayindirli (1995) on apricots, after 10 days' period. In another study, conducted on Ankara pears, coated with different concentrations of Semperfresh™ or with Johnfresh™ edible coatings after storage for five months at zero degrees centigrade, to determine the effects of coatings on poststorage quality; the coating levels were found to be effective for extending the poststorage life and delaying ripening. Both and Johnfresh™ treated pears had better titratable acidity than the control pears (Sumnu and Bayindirli, 1994). Low pH was obtained at the treatments of vitamin C, Semperfresh™ and the triple treatment. All the findings were in line with the statistical analysis ( $p < 0.05$ ) (Table 1).

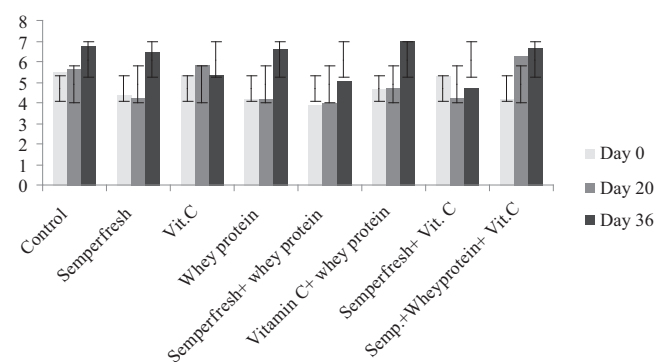
#### **The sugar content**

According to the total sugar content graph indicates that a slight decrease is observed in control group both on the 20<sup>th</sup> and the 36<sup>th</sup> day results (Figure 7). The triple application revealed that the total sugar content was constant up to 36<sup>th</sup> day whereas a little rise is exactly observed on 36<sup>th</sup> day. This increase is mainly due to the anaerobic barrier created in the fruit by the effect of Semperfresh™, which do not al-



**Fig. 6. Changes in pH values in all treatment groups**

low the gas exchange between the fruit and the external environment. A similar result was obtained in the study of Worrell (2002) on breadfruit, in which a relevant increase was observed in sole Semperfresh™ and sole StaFresh™ in the total sugar content; as well as in Yurdugul (2002) with sole Semperfresh™ on quinces. All the findings were in line with the statistical analysis (Table 1) ( $p < 0.05$ ).



**Fig. 7. The total sugar content levels of sole semperfresh; vitamin C+Semperfresh™ ; whey protein+Semperfresh™ +vitamin C compared with control**

## Conclusion

With respect to the control group, the microbiological analysis indicated that in average, vitamin C application was found to be the most effective treatment, whereas in combined treatments (S+C ; C+W ; S+W) vitamin C showed a co-working activity, together with the whey protein and sucrose esters from Semperfresh™ resulting in higher micro-

bial counts than those of single and triple treatments. The vitamin C was found to be effective in weight determination, total sugar content, pH, firmness and taste panel results, on the contrary, the Semperfresh™ alone coating treatment of bananas was also deserved to be considered as an effective method depending on its low amount of water loss from the fruits.

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