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Extending shelf-life of fresh-cut apple slices by controlling browning and microbial load

Gehan Ahmed Mahmoud

Fruit Handling Research Department, Horticulture Research Institute, Agricultural Research Center, Cairo, Egypt

Abstract:. Extending shelf life and keeping quality of Anna apple slices fresh-cut by using ascorbic acid and citric acid as well as aqueous extracts of rosemary and green tea as anti-browning and antimicrobial agents were evaluated. Solutions of 2.5 and 5% were used as dipping treatments prior to storage at 4°C up to twelve days as compared to untreated control. All treatments enhanced storage ability of apple slices as compared to control. Both rosemary and green tea aqueous extracts improved overall antioxidant activities of stored apple fresh-cut by the end of the storage period. Green tea aqueous extract at 5% was the most effective treatment in terms of reducing decay and total count of microbial load as well as inhibition of both peroxidase and polyphenol oxidase enzymatic browning activities.
Keywords : Apple fresh-cut, ascorbic acid, citric acid, rosemary aqueous extract, green tea aqueous extract, browning, microbial load, antioxidants, polyphenol oxidase activity, peroxidase activity.

Introduction:

Rapid market growth for fresh cut fruits and vegetables has been observed due to the consumers increased demand for convenience, fresh-like quality and high nutritive value[1]. Apple (*Malus domestica* Borkh.) is one of the most widely consumed fruits in the world[2]. Apples are an important source of polyphenols (phenolic compounds) in the human diet and a classic example of fruit susceptibility to enzymatic browning, which is a major problem for the fruit processing industry[3].

Enzymatic browning is one of the most important reactions that occur in fruits and vegetables, usually resulting in negative effects on color, taste, flavor, and nutritional value. The reaction is a consequence of phenolic compounds' oxidation by polyphenol oxidase (PPO), which triggers the generation of dark pigments. This is particularly relevant for apples, which are rich in polyphenols and highly susceptible to enzymatic browning [3].

Mechanical damage during processing results in cellular delocalization of enzymes and their substrates, leading to biochemical deteriorations such as enzymatic browning, off-flavor, and texture breakdown[4]. Enzymatic browning is one of the most important reactions that occur in many fresh-cut fruit and vegetables. This reaction, in which phenolic compounds are oxidized, is related to PPO activity, the amount of phenolics and the presence of oxygen[5]. However antioxidants are involved in browning and maintaining the commercial value of fresh cut products [6].

Many compounds may be used to reduce polyphenol oxidase (PPO) browning in foods[7,8]. One of the most widely used compounds is ascorbic acid, because it is very effective in reducing browning, generally recognized as safe, inexpensive and consumer friendly[9,10]. Citric acid is one of the most commonly used Acidulant agents in the food and plant industry and also it is considered chelating agent as both facilitate inhibition of PPO[11].

In recent years, green tea is considered the most commonly used beverage in the market due to its nutritional values and benefits. It has polyphenolic compounds in its structure know as flavonoids. These tea flavonoids such as theaflavins, thearubigins and catechins have antioxidant properties[12].The inhibitory effect of the extracted green tea was evaluated and described as a natural inhibitor for the PPO and browning activity of the fresh cut fruits by approximately 42%[13].

Rosemary was investigated as efficient source in the reduction or inhibition of PPO and POD activity in apple as well as anti-microbial and anti-fungal activity so that the antioxidant activities of rosemary extract were reported *in vivo* and *in vitro* by [14]. It is considered alternative method for plant browning inhibition rather than using of heat and chemical reagents.

The main objective of the current study is to extend shelf life and keeping quality of fresh-cut apple slices by using ascorbic acid and citric acid as well as aqueous extracts of rosemary and green tea as anti-browning and antimicrobial agents.

Materials and Methods

This study was carried out during two successive seasons (2015 and 2016) at Horticulture Research Institute (HRI), Agricultural research Center (ARC), Egypt. Apple fruits cultivar Anna (*Malus Domestica* Borkh.), which is the main local apple genotype, were used as experimental material.

Preparation of aqueous extracts

100 g. weight of each Rosemary (*Rosmarinus officinalis*) and Green tea (*Camellia sinensis*) were chopped with 200 ml distilled water (w/v) by using a domestic blender for 1 min at average speed. The mixtures were macerated during 24 h at 4ºC. After that, resulting extracts were filtered through double layered Whatman No.4 filter paper and sterilized using a 0.45 µm pore size cellulose acetate membrane filter (Cole-Parmer-47 mm). Dilutions were prepared (2.5 and 5%).

Mature apple fruits of 110 days age from full bloom were eliminated and uniform fruits were washed with water then left at room temperature to be dried. Then, the apple slices were prepared the same size with a sharp knife and transversely (2-3 mm thick). Thereafter, slices were dispensed into nine treatments as follows:

- Control
- Apple slices dipped in 2.5% ascorbic acid solution (for 2 min)
- Apple slices dipped in 5% ascorbic acid solution (for 2 min)
- Apple slices dipped in 2.5% citric acid solution (for 2 min)
- Apple slices dipped in 5% citric acid solution (for 2 min)
- Apple slices dipped in 2.5% rosemary aqueous extract (for 2 min)
- Apple slices dipped in 5% rosemary aqueous extract (for 2 min)
- Apple slices dipped in 2.5% green tea aqueous extract (for 2 min)
- Apple slices dipped in 5% green tea aqueous extract (for 2 min)

Treated slices were packed in polypropylene bags 20×20 cm^2 with 29.2 p mol/s/m^2/Pa oxygen transmission rate film and stored at 4°C and 90±5% relative humidity for 12 days. Evaluation was performed over 0, 3, 6, 9 and 12 days.

Fresh cut apple slices decay (%)

Fresh cut apple slices which were decayed by different physiological and pathological factors were periodically counted and discarded, and then percentage of slices decay was calculated in relation to the total number of slices.

Vitamin C (mg/100g. FW)

Vitamin C was determined as described by[15]. Results were expressed on mg ascorbic acid/100 g. FW.

Total flavonoids (mg/100g. FW)

Total flavonoids content was measured according to[16] and expressed as mg catechin equivalents per 100 g. fresh weight.

Total phenols (g/100g. FW)

Total phenolics were analyzed spectrophotometrically using the method described by[17]. Results were expressed as g. gallic acid /100 g. FW.

Antioxidant activities (%DPPHsc)

The antioxidant activity was evaluated by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method according to the procedure of[18]. The antioxidant activity was expressed as the percentage of decline of the absorbance, relative to the control, corresponding to the percentage of DPPH that was scavenged. The percentage of DPPH, which was scavenged (% $DPPH_{sc}$), was calculated using %$DPPH_{sc}$: %$DPPH_{sc}$ = (A_{cont}-A_{sam}) ×100/A_{cont}

Peroxidase activity (U/g.FW)

Peroxidase enzyme activity was determined according to [19]. Method and the activity was expressed as units per g fresh weight.

Polyphenol Oxidase Activity (U/g.FW)

Polyphenol oxidase (PPO) activity was determined according to [20] and the activity was expressed as units per g. fresh weight.

Microbial load determination

For enumeration of microorganisms present in each sample, 10-fold serial dilutions of each rinse water were made and 1 ml of 10^{-4} dilution was pipetted into sterile petri-dishes and molten nutrient agar (45^{o}C) was added and swirled thoroughly to allow even distribution. The colonies were counted after 24h incubation at 37^{o}C **[21]**. The average of colonies formed on duplicate plates for each treatment was recorded. The total microbial count was recorded as colony forming units per milliliter (cfu ml^{-1}).

Statistical analysis

Each experiment was repeated twice, completely randomized design was used and the represented data were averages. Results were analyzed by analysis of variance (ANOVA) and least significant difference (L.S.D. at 5% level) was also adopted according to [22].

Result and Discussion:

Decay (%)

Results revealed that the decay percentages were gradually increased significantly by increasing the storage period at room temperature (Table 1). The lowest decay percentage was recorded by the treatment with

green tea aqueous extracts at 5 % (4.53%). On the other hand, the highest decay percentage was recorded by the control (31.47%). The overall observation showed that green tea was the best aqueous extracts treatment for keeping fresh cut apple slices from decay.

Table (1): Decay percentages of apple fresh cut slices as affected by natural browning inhibitors.

Period / Treatments	Zero time	3 days	6 days	9 days	12 days	Mean
Control	0.00	11.00	30.33	53.33	62.67	31.47
Ascorbic acid (2.5%)	0.00	3.67	12.33	26.00	30.00	14.47
Ascorbic acid (5%)	0.00	3.33	10.67	22.33	26.00	12.47
Citric acid (2.5%)	0.00	2.33	8.67	20.33	23.33	10.93
Citric acid (5%)	0.00	1.33	8.33	14.67	19.33	8.73
Rosemary aqueous extract (2.5%)	0.00	1.67	9.33	13.67	16.33	8.2
Rosemary aqueous extract (5%)	0.00	1.67	8.00	10.67	15.33	7.13
Green tea aqueous extract (2.5%)	0.00	0.00	6.67	9.67	13.00	5.86
Green tea aqueous extract (5%)	0.00	0.00	5.00	7.00	10.67	4.53
Mean	0.00	2.77	11.03	19.74	24.07	
L.S.D. 5%	Period =0.490		Treatment=0.658		Interaction=1.471	

Vitamin C (mg/100g. FW)

Data in Table (2) showed that, vitamin C content decreased gradually during the period of storage up to 12 days, however control achieved the lowest ratios of vitamin C content through storage of apple fresh cuts. By the end of the storage period (12 days), ascorbic acid at concentration of 5% gave the best result of vitamin C content (8.159 mg/100g. FW) followed by green tea aqueous extract at 5% (6.707 mg/100g. FW), whereas untreated samples recorded the lowest value (3.289 mg/100g. FW).

Table (2): Vitamin C content (mg/100g. FW) of apple fresh cut slices as affected by natural browning inhibitors.

Period / Treatments	Zero time	3 days	6 days	9 days	12 days	Mean
Control	13.267	10.618	8.764	6.460	3.289	8.479
Ascorbic acid (2.5%)	19.098	15.902	12.728	9.591	4.631	12.390
Ascorbic acid (5%)	23.121	17.517	13.939	10.548	8.159	14.657
Citric acid (2.5%)	15.690	13.616	11.013	8.237	4.664	10.644
Citric acid (5%)	17.110	14.420	11.616	8.713	5.272	11.426
Rosemary aqueous extract (2.5%)	18.879	13.416	10.863	8.118	5.200	11.294
Rosemary aqueous extract (5%)	19.080	15.080	12.111	9.104	5.412	12.158
Green tea aqueous extract (2.5%)	19.156	14.927	11.996	9.013	5.610	12.141
Green tea aqueous extract (5%)	19.739	15.631	12.525	9.431	6.707	12.806
Mean	18.349	14.569	11.729	8.802	5.438	
L.S.D. 5%	Period=0.473		Treatment=0.634		Interaction=1.418	

[8]Reported that, once the ascorbic acid has been oxidized to dehydroascorbic acid, quinones can again accumulate and undergo browning, therefore, these treatments may reduce fresh cut browning by maintaining higher vitamin C. Previous reports showed that the individual effect of ascorbic acid is temporary, other alternatives should be searched to control browning[6].

Total phenols (g./100g. FW)

It can be observed from the results in Table (3) that, total phenols content of apple fresh cut slices revealed high level in average by the starting of the experiment (zero time). Phenols content increased gradually during the period of storage starting of 3 days up to 12 days. However, after 12 days of storage at 4°C control

fresh cut apple slices gave the highest value of phenols content, while all dipping treatments reduced it by different ratios. Rosemary aqueous extract at concentration of 5% showed the best result in the total phenols content of fresh cut apple slices (0.124 g./100g. FW) followed by 5% green tea aqueous extract (0.135g./100g. FW) by the end of the storage period.

Table (3): Total phenols content (g./100g. FW) of apple fresh cut slices as affected by natural browning inhibitors.

Period / Treatments	Zero time	3 days	6 days	9 days	12 days	Mean
Control	0.211	0.175	0.185	0.191	0.247	0.202
Ascorbic acid (2.5%)	0.205	0.123	0.121	0.169	0.209	0.165
Ascorbic acid (5%)	0.217	0.141	0.168	0.203	0.198	0.186
Citric acid (2.5%)	0.218	0.143	0.185	0.176	0.189	0.182
Citric acid (5%)	0.177	0.135	0.151	0.167	0.171	0.160
Rosemary aqueous extract (2.5%)	0.163	0.112	0.175	0.204	0.212	0.173
Rosemary aqueous extract (5%)	0.174	0.097	0.099	0.084	0.124	0.116
Green tea aqueous extract (2.5%)	0.178	0.096	0.098	0.115	0.176	0.133
Green tea aqueous extract (5%)	0.199	0.084	0.128	0.129	0.135	0.135
Mean	0.194	0.123	0.146	0.159	0.185	
L.S.D. 5%	Period =0.0148		Treatment=0.0198		Interaction=0.044	

Total flavonoids (mg/100g. FW)

Results in Table (4) showed that, total flavonoids decreased gradually during the period of storage up to 12 days. However, control fresh cut apple slices gave the lowest mean of flavonoids content (12.394 mg/100g.), while all dipping treatments increased it. Green tea aqueous extract at concentration of 5% showed the highest mean of flavonoids content (34.573 mg/100g.).

Table (4): Total flavonoids content (mg/100g. FW) of apple fresh cut slices as affected by natural browning inhibitors.

Period / Treatments	Zero time	3 days	6 days	9 days	12 days	Mean
Control	17.344	8.9827	9.8050	13.759	12.078	12.394
Ascorbic acid (2.5%)	34.353	21.854	19.442	17.449	15.542	21.728
Ascorbic acid (5%)	40.153	34.283	40.105	17.708	14.017	29.253
Citric acid (2.5%)	34.353	53.735	33.917	19.738	15.977	31.544
Citric acid (5%)	41.128	38.283	20.379	10.104	11.418	24.263
Rosemary aqueous extract (2.5%)	41.153	26.503	21.854	15.649	14.924	23.796
Rosemary aqueous extract (5%)	54.053	40.761	33.509	8.6161	16.179	30.624
Green tea aqueous extract (2.5%)	45.489	39.382	22.801	26.629	16.208	30.102
Green tea aqueous extract (5%)	49.843	44.220	34.286	30.558	13.958	34.573
Mean	39.763	34.223	26.111	17.801	14.478	
L.S.D. 5%	Period =0.7035		Treatment=0.9438		Interaction=2.110	

Antioxidant activity (%)

Data in Table (5) shows the effect of antioxidant inhibitors treatments on fresh cut slices of apple that may affect the browning of apples. This data showed a significant decrease in the antioxidant activity during period of storage for control and all treatments with different levels. It is obvious that, untreated samples gives the lowest mean of antioxidant activity (56.56%) while the other all treatments improved its activity. Natural antioxidant inhibitors positively affected the antioxidant activity of stored apple fresh cut slices by the end of the storage period which recorded 78.68, 77.08, 73.36 and 67.80% using aqueous extracts of rosemary (2.5%), rosemary (5%), green tea (5%) and green tea (2.5%), respectively.

Table (5): Antioxidant activity (%) of apple fresh cut slices as affected by natural browning inhibitors.

Period / Treatments	Zero time	3 days	6 days	9 days	12 days	Mean
Control	69.44	59.80	61.52	53.72	38.32	56.56
Ascorbic acid (2.5%)	87.88	71.60	76.60	84.08	38.20	71.67
Ascorbic acid (5%)	88.84	64.04	85.72	89.76	53.68	76.40
Citric acid (2.5%)	77.36	65.92	86.28	76.68	38.56	68.96
Citric acid (5%)	87.32	77.84	94.08	54.44	50.08	72.75
Rosemary aqueous extract (2.5%)	82.76	64.32	86.52	87.12	78.68	79.88
Rosemary aqueous extract (5%)	93.08	71.92	83.00	78.56	77.08	80.73
Green tea aqueous extract (2.5%)	87.04	80.88	84.92	76.48	67.80	79.43
Green tea aqueous extract (5%)	91.20	81.68	74.60	72.88	73.36	78.75
Mean	85.00	70.88	81.48	74.88	57.32	
L.S.D. 5%	Period =2.28		Treatment=3.08		Interaction=6.88	

[4]Showed that, ascorbic acid increased antioxidant activity (%$DPPH_{sce}$) of apple fresh-cuts during storage. It has been suggested that, ascorbic acid exerts protection by: (1) acting as an oxygen scavenger, removing molecular oxygen and avoiding polyphenol oxidase-catalyzed reactions and (2) self-oxidation to avoid oxidation of phenol compounds or reduction of enzymatically formed oquinones to their precursor diphenols. Therefore, it was possible the ascorbic acid treatment reduced cut surface browning in apple slices by increasing antioxidant capacity.

Peroxidase activity (units per g. fresh weight)

The changes in POD activity in fresh-cut apple slices were clearly distinguished by the different treatments (Table 6). Data reveled that, POD activity recorded the highest mean (0.235 U/g.FW) at zero time then dropped dramatically by the beginning of storage. During storage, POD activity increased gradually since it reached the highest mean (0.214 U/g.FW) at the end of the storage period. During storage, treatments reduced POD activity, there was significant difference between treated and untreated apple slices till the end of storage. Control samples gave the highest activity (0.284 U/g.FW) after 12 days of storage followed by rosemary treated slices at 2.5% (0.255 U/g.FW), while green tea (5%) seems to be the most effective treatment regarding inhibition of POD activity (0.162 U/g FW) followed by green tea at 2.5% (0.173 U/g.FW) and then 5% rosemary aqueous extract (0.192 U/g.FW).

Table (6): Peroxidase activity (units per g. fresh weight) of apple fresh cut slices as affected by natural browning inhibitors.

Period / Treatments	Zero time	3 days	6 days	9 days	12 days	Mean
Control	0.317	0.223	0.245	0.235	0.284	0.261
Ascorbic acid (2.5%)	0.317	0.205	0.203	0.182	0.241	0.229
Ascorbic acid (5%)	0.288	0.184	0.146	0.194	0.216	0.206
Citric acid (2.5%)	0.205	0.275	0.223	0.212	0.206	0.224
Citric acid (5%)	0.210	0.213	0.166	0.139	0.196	0.185
Rosemary aqueous extract (2.5%)	0.201	0.163	0.237	0.167	0.255	0.205
Rosemary aqueous extract (5%)	0.178	0.152	0.169	0.151	0.192	0.168
Green tea aqueous extract (2.5%)	0.198	0.158	0.203	0.211	0.173	0.189
Green tea aqueous extract (5%)	0.203	0.141	0.169	0.117	0.162	0.158
Mean	0.235	0.190	0.196	0.179	0.214	
L.S.D. 5%	Period =0.0096		Treatment=0.01297		Interaction=0.029	

This result is consistent with those of [23] who reported that, the ascorbic acid-treated fresh-cut apple sample showed high activity immediately after processing, but the activity decreased until eighth day of storage. With the exception of the high POD activity on treatment day, these results are consistent with those of [24] who reported that, the presence of ascorbic acid effectively reduced the POD activity in the fresh-cut cantaloupe

melon. The researchers also indicated that the reduced POD activity in the fruit treated with ascorbic acid could be the result of a lower oxidative stress on the fruit surface due to the antioxidant ability of ascorbic acid.

Polyphenol oxidase activity (units per g. fresh weight)

The changes in PPO activity in fresh-cut apple slices were clearly distinguished by the different treatments (Table 7). During storage, treatments reduced PPO activity, there was significant difference between treated and untreated apple slices till the end of storage. Control samples gave the highest activity (0.649 U/g.FW) after 12 days of storage, while slices treated with both concentrations of green tea aqueous extract were the lowest (0.254 at 5% and 0.278 U/g.FW at 2.5%).

Table (7): Polyphenol oxidase activity (units per g. fresh weight) of apple fresh cut slices as affected by natural browning inhibitors.

Period / Treatments	Zero time	3 days	6 days	9 days	12 days	Mean
Control	0.650	0.575	0.621	0.549	0.649	0.609
Ascorbic acid (2.5%)	0.634	0.307	0.371	0.365	0.529	0.441
Ascorbic acid (5%)	0.564	0.206	0.346	0.348	0.496	0.392
Citric acid (2.5%)	0.326	0.367	0.170	0.237	0.405	0.301
Citric acid (5%)	0.312	0.195	0.230	0.413	0.567	0.343
Rosemary aqueous extract (2.5%)	0.300	0.155	0.314	0.409	0.482	0.332
Rosemary aqueous extract (5%)	0.228	0.157	0.324	0.442	0.466	0.323
Green tea aqueous extract (2.5%)	0.289	0.350	0.330	0.442	0.278	0.338
Green tea aqueous extract (5%)	0.319	0.121	0.167	0.205	0.254	0.213
Mean	0.402	0.270	0.319	0.379	0.459	
L.S.D. 5%	Period =0.010		Treatment=0.014		Interaction=0.031	

Although it has been recognized that PPO is the main enzyme related to enzymatic browning on fresh-cut apples, it is also necessary to study the changes in POD enzymes as they can also contribute to the discolouration in fresh-cut products[23]. In addition, POD is involved in the last step in the polymerization of cinnamyl alcohols to form lignin and directly involved in the induction of defense mechanisms [25].

Polyphenol oxidase (PPO) and peroxidase (POD) are the enzymes involved in the browning process. Browning occurs almost instantly when the cell structure is destroyed, and the enzyme and substrate are mixed. PPO catalyses the hydroxylation of monophenols (monophenolase) and oxidation of o-diphenols to o-quinones (diphenolase), which subsequently polymerise to yield undesirable brown pigments in the presence of oxygen[26]. POD, an indicator of quality deterioration such as flavour loss and various biodegradation reactions, is also relevant to enzymatic browning since diphenols may function as reducing substrate in the enzyme reaction and could promote darkening in fruit and vegetable products during processing and preservation. Although POD is limited by the availability of electron acceptor compounds like hydrogen peroxide, its involvement in browning of various fruits and vegetables has been reported[27,28,29,30].

Several studies have focused on the inhibition of enzymatic browning by ascorbic acid. Ascorbic acid can reduce o-quinones, produced by PPO-catalysed oxidation of polyphenols, back to dihydroxy polyphenols and has been widely used as an antibrowning agent for processing of fruits and vegetables. However, the effect of ascorbic acid is temporary since once it is added, it is completely oxidised and o-quinones could accumulate, leading to browning pigment formation[6,10]. Therefore, ascorbic acid is insufficient in controlling browning and maintaining the commercial value of fresh-cut products.

There were many experiments *in vitro* that proved that phenolic compounds were usually major contributors of antioxidant capacities of plants. Rosmarinic acid, ferulic, caffeic, chlorogenic, vanillic, p-hydroxybenzoic acid, p-coumaric acid, protocatechuic acid, and so on were identified to contribute to the antioxidant potential of *Lycopus lucidus* and tea by using DPPH and NO scavenging assays[31,32].

Total count of microbial load

Results in Figure (1) showed that, total count of microbial load increased by increasing storage period. Control gave the maximum total count (115.000 x 10^4 cfu ml^{-1}) by the end of storage period, while green tea aqueous extracts (5 and 2.5%) gave the best results (22.667 and 34.667 x 10^4 cfu ml^{-1}, respectively) followed by rosemary aqueous extracts (44.000 at 5% and 54.000 x 10^4 cfu ml^{-1}at 2.5%) and then citric acid solutions (74.667 and 89.333 x 10^4 cfu ml^{-1} using 5 and 2.5%, respectively). On the other hand, ascorbic acid solutions gave the lowest effect recording highest total count of microbial load (99.000 and 105.667 x 10^4 cfu ml^{-1} at 5 and 2.5%, respectively).

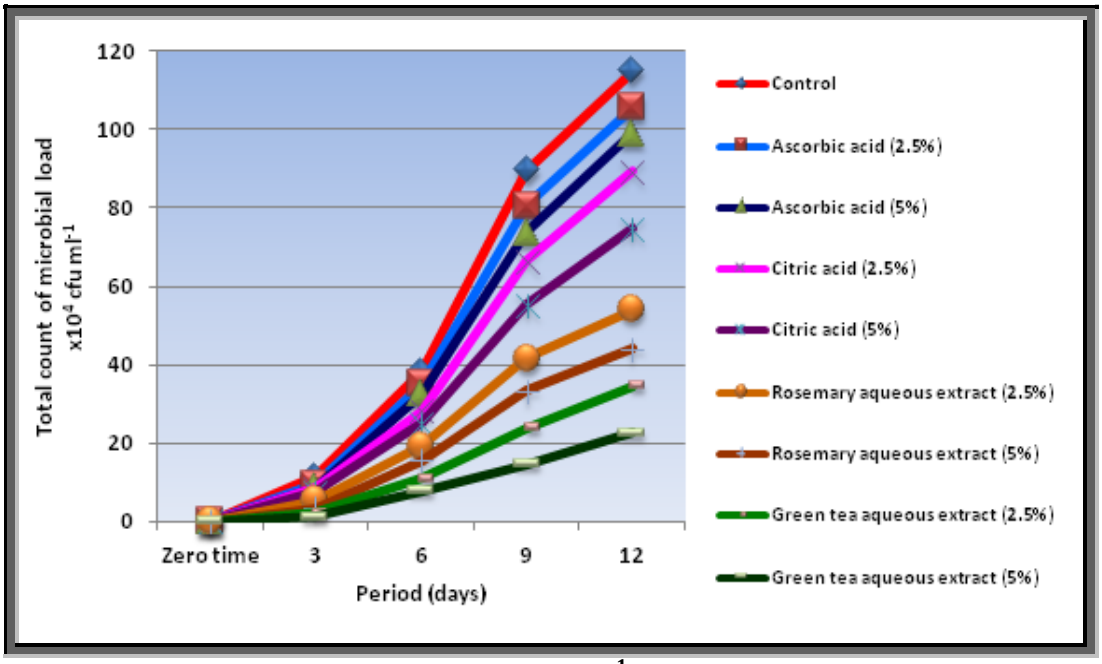

Figure (1): Total count of microbial load (cfu ml^{-1}) on apple fresh cut slices as affected by natural browning inhibitors.
L.S.D. at 5%; Period=0.808, Treatment=1.084, Interaction= 2.424

[33]Mentioned that, relatively new aspect further to be considered in the overall quality and safety measurement of a food sample is to assess the microbial survival potential in selected foods.

It is observed that, total count of bacteria in apple sample that treated with water extract of rosemary is very low and little counts of bacteria exist throughout 0 time to 12 days, so rosemary water extract has the ability to reduce the count of the bacteria. This result is approved and supported by [34] who mentioned that, rosemary extract proved to be efficient in reducing both the enzymatic browning (PPO) and microbial counts during the preservation of apple juice by refrigeration at 4°C, indicating the potential applicability of such essential oil as a food preservative.

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