http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 15 (1): 10-18, 2012
ISSN 1028-8880 / DOI: 10.3923/pjbs.2012.10.18
© 2012 Asian Network for Scientific Information

Evaluation of Food Protective Property of Five Natural Products using Fresh-cut Apple Slice Model

[1,2]Faruk Lumanul Hakkim, [3]Mathiraj, [1,4,5]Musthafa Mohamed Essa, [3]Girija Arivazhagan, [1]Nejib Guizani and [2]Hyuk Song
[1]Department of Food Science and Nutrition, College of Agriculture and Marine Sciences, Sultan Qaboos University, Oman
[2]Department of Food Bioscience, Konkuk University, Chungju 380-701, Republic of Korea
[3]School of Biotechnology and Genetic Engineering, Bharathiar University, Coimbatore, Tamilnadu, India
[4]Neuropharmacology Group, Department of Pharmacology, College of Medicine, University of New South Wales, Sydney, Australia
[5]Developmental Neuroscience Laboratory, NYSIBR, 1050 Forest Hill road, Staten Island, NY, 10314, USA

Abstract: The present study evaluated the antioxidant (AA), antimicrobial and preservation effects of five plant derived natural products viz., Rosmarinic Acid (RA), p-Coumaric Acid (pCA), Trans-Cinnamic Acid (TCA), Hydroxyphenyllactic Acid (HPA) and Caffeic acid (CA) along with synthetic compounds (Ascorbic acid, gallic acid, citric acid and BHA) on fresh cut apple slices. Antimicrobial efficacy of these compounds against *Bacillus licheniformis*, *Pseudomonas vulgaris*, *Shigella boydii*, *Salmonella typhi*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Escherichia coli* was found to be concentration dependent with the maximum inhibition observed at 500 μg mL^{-1}. A considerable AA potential of these compounds was observed in *in vitro* based assay system, with RA exhibiting significantly higher effect than the other compounds at 500 μg mL^{-1}. Furthermore the compounds at 500 μg mL^{-1} significantly reduced the browning, maintained the acidic pH and restricted growth of *L. monocytogenes* even after 10 days of treatment. Ethanol accumulation in fresh cut apple slices increased significantly throughout the experimental period. Over all RA exhibited maximum effect in all the food preservation parameters studied suggesting that it has synchronized food protection effect and can be recommended as food additive.

Key words: Antioxidant, antimicrobial, rosmarinic acid, food preservation, apple slice

INTRODUCTION

Fresh fruits and vegetables are an essential part of the diet of people around the world. Nutritionists emphasize the importance of fruits and vegetables in healthy diets and researchers and governmental publicity campaigns around the world tend to recommend consumption of at least five servings of fruit and vegetables per day. Dietary important and high nutritious fruits and vegetables are may not be available in all seasons. In order to meet these demands, the food industry has focused on the development of new processing techniques for Minimally Processed (MP) fruits and vegetables products which facilitate their availability throughout the year. The quality of MP fruits is mainly dependent on the physiology of wounded tissue (Brecht, 1995). Physiological and biochemical changes in such products occur at a faster rate than in intact fruits. Mechanical injury sets off a complex series of events which result in loss of quality (Ana *et al*., 2006). Wounding stimulates respiration rate, induces ethylene synthesis, oxidation of phenols and altered enzymatic activity, leading to an accelerated loss of quality, especially colour and firmness attributes (Rolle and Chism, 1987; Kim *et al*., 1993a, b). In addition fresh-cut processing of fruit may increase microbial spoilage of fruit through transfer of peel microbial flora to fruit flesh where microorganisms can grow rapidly upon the exposure to nutrient rich juices. Control of wounding is therefore the major obstacle that must be overcome for extension of the shelf life of MP fruits.

The vast majority of fresh MP produce manufacturers use chlorine-based washing and decontamination procedures (Seymour, 1999). There is a controversy about

Corresponding Author: F. Lukmanul Hakkim, Department of Food Science and Nutrition, College of Agriculture and Marine Sciences, P.O. 34, Cams, Sultan Qaboos University, P.C. 123, Sultanate of Oman Tel: +968 2414 1231 Fax: 96824413418

the formation of carcinogenic chlorinated compounds in water (chloramines and trihalomethanes), calling into question the use of chlorine (Wei *et al*., 1995). The long-term use of commercially available synthetic antioxidants such as Butylated Hydroxy Anisole (BHA) and Butylated Hydroxyl Toluene (BHT) for the increase of shelf life of MP fruits is not possible due to its high carcinogenic effect (Grice, 1986; Wichi, 1988). It is assumed that natural compounds are 'safe' and it has been reported that the use of natural products and their derivatives have been found to be effective in improving the shelf life of MP fruits and vegetables (Ahvenainen, 1996; Gonzalez-Aguilar *et al*., 2001). To obtain a useful natural food additive, it is necessary to improve the antioxidant and antimicrobial properties and to create an odourless, tasteless and colourless product. In this study, we tested five plant based natural products viz., Rosmarinic Acid (RA), p-Coumaric Acid (pCA), Trans-Cinnamic Acid (TCA), Hydroxyphenyllactic Acid (HPA) and Caffeic Acid (CA) along with synthetic compounds (Ascorbic acid, gallic acid, citric acid and BHA) for food preservation property. Though natural products has been reported extensively as an antioxidant and antimicrobial (Albayrak *et al*., 2010) but no such data is available to support strongly their possible use in food industry as an additives. To address this issue, in the present study, we have evaluated the preservative effect of these compounds on fresh cut apple slices and their effects on reduction of browning and other quality attributes as well as its microbial decay. This study adds value on the possible use of natural products as an effective preservative.

MATERIALS AND METHODS

Chemicals: The chemicals used for the study were of analytical grade and obtained from Sigma Chemical Company, USA, Himedia (Mumbai). Rosmarinic acid, caffeic acid, p-Coumaric acid, Trans cinnamic acid, Hydroxy phenyllactic acid obtained from Sigma Aldrich, USA. Compounds were dissolved in suitable solvent according to manufacturers instructions.

Microbial cultures: *Bacillus lichneformis* (MTCC No. 14580), *Pseudomonas vulgaris* (MTCC No. 13525), *Shigella boydii* (MTCC No. 29508), *Salmonella typhi* (MTCC No. 6539), *Staphylococcus aureus* (MTCC No. 9144), *Listeria monocytogenes* (MTCC No. 1143) and *Escherichia coli* (MTCC 443) cultures were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The stock cultures were maintained on Nutrient Agar medium at 37°C.

Antimicrobial activity

Agar well diffusion assay: Agar well diffusion method was used to determine the antibacterial activity of the compounds viz., RA, CA, pCA, HPA and TCA against *B. lichneformis*, *P. vulgaris*, *S. boydii*, *S. typhi*, *S. aureus*, *L. monocytogenes*, *E. coli*. Wells (5 mm in diameter) were cut from the inoculated agar medium with sterile borer. The wells were then filled with the antioxidant solutions of varying concentrations (100, 200, 300, 400 and 500 μg mL^{-1}) and incubated at 37°C for 24 h. After incubation, the plates were examined and the diameters of the clear zones were measured, including the diameter of the well, in millimeter (mm) using a ruler.

DPPH radical-scavenging system: The ability of the test solutions to scavenge DPPH radical was assessed spectrophotometrically (Gyamfi *et al*., 1999). Briefly, 50 μL of RA, CA, pCA, HPA and TCA test solutions (at 500 μg mL^{-1}) was mixed with 450 μL Tris-HCl buffer (50 mmol L^{-1}, pH 7.4) and 1.0 ml DPPH (0.1 mmol L^{-1}, in methanol), the resultant absorbance was recorded at 517 nm after 30 min incubation at 37°C. The percentage of inhibition was calculated from the following equation:

$$\% \text{ Inhibition} = [(A_0 - A_1) / A_0] \times 100$$

where, A_0 was the absorbance of the control (blank, without test solution) and A_1 was the absorbance in the presence of the test solution.

Ferric reducing antioxidant power assay (FRAP assay): The FRAP, a method for measuring total reducing power of electron donating substances, was assessed according to Benzie and Strain (1996). Briefly, 6 mL of working FRAP reagent (0.1 M acetate buffer: 0.02 M $FeCl_3$:0.01 M TPTZ =10:1:1) prepared freshly was mixed with 20 μL of RA, CA, pCA, HPA and TCA (500 μg mL^{-1}). The absorbance at 593 nm was recorded after 30 min incubation at 37°C. Gallic acid, Ascorbic acid, BHA and Citric acid were used as the standard at 0.2 mg mL^{-1}.

β-carotene-linoleic acid bleaching: The ability of the extracts to prevent the bleaching of β-carotene was assessed (Koleva *et al*., 2002). Briefly, 0.2 mg β-carotene in 1 mL chloroform, 20 mg of linoleic acid and 200 mg of Tween 20 were transferred into a round-bottom flask. After removal of chloroform, 50 mL distilled H_2O was added and the resulting mixture was stirred vigorously. Six mililiter aliquots of the emulsion were transferred to tubes containing either 50 μL (500 μg mL^{-1}) compounds or 50 μL (0.2 mg mL^{-1}) BHA or 50 μL (0.2 mg mL^{-1}) ascorbic acid or 50 μL (0.2 mg mL^{-1}) citric acid. After mixing, an aliquot was transferred into a cuvette and the absorbance (Abs^0) at 470 nm was recorded. The remaining

samples were placed in a water bath at 50°C for a period of 2 h. Thereafter, the absorbance of each sample was measured at 470 nm (Abs^{120}). The data (n = 3) are presented as antioxidant activity % (AA%) values, calculated using the following equation:

$$AA\% = [1 - (Ab^0_{sample} - Abs^{120}_{sample})/(Ab^0_{control} - Abs^{120}_{control})] \times 100$$

Anti-browning assessment

Apples: Red delicious apples were chosen for this study for its susceptibility to browning and were purchased from a local wholesale product distributor. Fruits were stored briefly at 4°C until processing.

Apple slice preparation: Apples were selected for good colour, size and free from any spoiled part by microorganism or injury by transporting or storage. After rinsing with water, fruits were sliced using slicer. At start of an experiments, a transverse cut was made, at least 1 cm from the skin end (to exclude the effects of bruising), exposing fresh surface. Apple slices 0.5-1 cm were dipped for 5 min in test solutions (RA, CA, pCA, HPA and TCA at 500 μg mL^{-1}), drained and placed in glass petri dishes. Control samples were dipped in distilled water. The dishes were sealed to retard photooxidation and stored for 8 h, 24 h and 10 days at 5°C for shelf life study.

Absorbance measurement of browning: Apple slices treated with inhibitory agents were crushed using mechanical grinder and centrifuged at 12000 rpm for 10 min and supernatant was collected. Absorbance of supernatant were recorded at 420 nm using UV/visible spectrophotometer (Ozoglu and Bayindirli, 2002).

Microbiological analysis: Ten grams of fresh apple slices were treated with compounds (RA, CA, pCA, HPA, TCA at 500 μg mL^{-1}) along with the synthetic antioxidants (Butylated hydroxy anisole, Ascorbic acid, Gallic acid and Citric acid at 500 μg mL^{-1}). Then *L. monocytogenes* (565 CFU mL^{-1}) were inoculated in the compound treated apple slices, packed and stored at 5°C. Apple slices were then removed aseptically from each package after 8, 24 h and 10 days of experimental period and transferred into a sterile plastic tube to which 15 mL of 0.1 g/100 mL sterile peptone water was added. The sample and peptone solution were blended for 5 min in a homogenizer. Serial dilutions were made using sterile peptone water and then pour onto plate count agar (PCA) for plating. Colonies were then counted and recorded as colony forming units per g sample (CFU/g). Samples were plated in duplicate.

pH Measurement: Apple slices were treated with compounds as described for browning measurement. The pH values of the samples were recorded using a glass electrode pH- meter before and after inoculation and during the time course. Five gram of sample were blended with 10 mL of de-ionized water. Measurements were done by inserting the probe into the solution.

Ethanol analysis: Apple slices were treated with RA, CA, pCA, HPA and TCA at 500 μg mL^{-1}. Five gram of treated samples were blended with 10 mL of de-ionized water and centrifuged at 12000 rpm for 10 min. The ethanol content of collected supernatant was recorded with UV/visible spectrophotometer at A_{340} (Davis and Chace, 1969).

Statistical analysis: All data were expressed as Mean±SD. The statistical significance was evaluated by student's t-test. The level of significance was considered at $p<0.001$ and $p<0.05$.

RESULTS AND DISCUSSION

Antimicrobial activity: Table 1 shows the antibacterial activity of five natural products (RA, CA, pCA, HPA and TCA) along with four synthetic antioxidants (BHA, AA, citric acid and GA) at different concentrations (100-500 μg mL^{-1}) on selected pathogens (viz., *E. coli*, *S. boydii*, *S. aureus*, *P. vulgaris*, *B. licheniformis* and *S. typhi*). All tested compounds exhibited growth inhibition and the effect was found to be concentration dependent (data not shown). All compounds showed maximum inhibition zones (from 7-24 mm) at a concentration of 500 μg mL^{-1} (Table 1). This concentration was therefore used to test antioxidant potential, anti browning, pH and ethanol measurements. The possible mechanism by which the compounds exhibit the antibacterial activity could be attributed to the breakdown of cell wall lipo polysaccharides (Russel, 1991). The high antimicrobial activity of the phenolic components may be further explained in terms of the alkyl substitution into the phenol nucleus which is known to enhance the antimicrobial activity of phenols (Pelczar *et al*., 1988). The results obtained suggest that the compounds tested in this study may have potential use in food conservation as main or as adjuvant antimicrobial compounds in order to assure the production of microbiologically stable foods.

Antioxidant potential

DPPH radical scavenging activity: The antioxidant activity of natural antioxidants has been shown to be involved in the termination of free radical reactions and

Table 1: Antibacterial effect of natural products and synthetic antioxidants

Compounds (500 μg mL^{-1})	*S. boydii*	*S. aureus*	*P. vulgaris*	*B. lichneformis*	*S. typhi*	*L. monocytogenes*	*E. coli*
Rosmarininc acid	13±2.1	11±2.3	12±1.6	13±1.8	12±2.3	24±1.3	14±2.4
Caffeic acid	11±2.4	8±1.9	10±2.2	11±1.5	9±2.5	13±1.4	13±1.7
p-Coumaric acid	10±1.5	12±1.4	13±1.5	15±1.8	12±22.5	11±1.4	11±1.8
Hydroxy phenyllactic acid	11±2.4	8±2.3	10±2.1	9±2.1	11±2.5	13±1.6	8±1.3
Trans cinnamic acid	11±1.4	9±1.1	10±1.4	13±1.7	12±1.4	15±2.1	14±2.3
Ascorbic acid	9±2.1	9±1.4	-	10±1.5	9±1.4	10±1.6	7±1.3
BHA	12±1.4	8±1.5	12±2.4	11±2.8	11±2.1	20±2.4	13±2.5
Citric acid	10±2.2	9±1.5	10±2.5	12±2.7	10±1.7	15±2.3	14±2.1
Gallic acid	8±1.1	7±1.5	10±1.7	10±1.8	11±1.3	13±1.2	9±1.3

Values are presented as Mean±SD (n = 2)

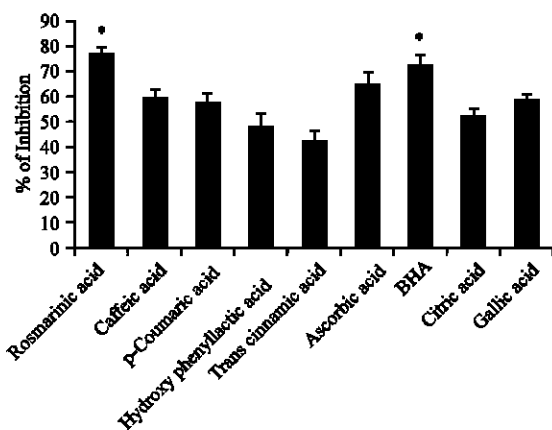

Fig. 1: DPPH radical scavenging activity of five natural products and synthetic antioxidants. Data are presented as Mean±SD of each of three replicates (n = 3). *indicates the significant at p<0.001

reducing power (Shimada *et al*., 1992; Tanaka *et al*., 1988). Free radicals involved in the process of lipid peroxidation, play a cardinal role in numerous chronic diseases and are implicated in premature ageing. The DPPH molecule contains stable free radical which has been widely used to evaluate the radical scavenging ability of antioxidants. It is possible to determine the antiradical potential of antioxidants by measurement of the decrease in the absorbance of DPPH• at 517 nm. Figure 1 shows the capacities of five natural products (RA, CA, TCA, HPA and pCA at 500 μg mL^{-1}) with synthetic antioxidants (BHA, AA, Gallic acid and Citric acid at 500 μg mL^{-1}) to scavenge the stable DPPH free radical (by donation of a hydrogen atom). At a dose of 500 μg mL^{-1}, all the compounds were capable of scavenging DPPH• free radicals. From the percentage scavenging values, it can be seen that the RA (76.2%) was the most potent scavenger followed by CA (59.1%) > pCA (57.1%) > HPA (48.2%) > TCA (42.1%). Among the five compounds tested RA showed maximum scavenging activity than the positive controls. The results of the DPPH• free radical scavenging assay suggest that all the tested components are capable of scavenging free radicals via. electron or hydrogen-donating mechanisms and thus should be potent enough to prevent the initiation of deleterious free radical mediated chain reactions in susceptible matrices e.g., biological membranes. Compared to synthetic antioxidants the natural products have the advantage of being very safe and will not exhibit any harmful effects.

Ferric reducing ability: Antioxidant activity has been reported to be concomitant with reducing power. Determination of ferric/antioxidant power is a simple direct test for measuring of antioxidant capacity and the reaction is reproducible and linearly related to the concentration of the antioxidant presence. This method was initially developed to assay plasma antioxidant capacity but can be used for natural compounds too. The total antioxidant potential of the sample was determined using a FRAP assay (Benzie and Strain, 1996) as a measure of "antioxidant power. This assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe^{2+}-tri-pyridyltriazine complex from colorless oxidized Fe^{3+} form by the action of electron donating antioxidants. As can be seen in Fig. 2, the tested compounds were capable of reducing the Fe^{3+} ions into Fe^{2+}. The order of efficacy was RA > CA > pCA > HPA > TCA. Among the compounds RA showed high reducing power than positive controls. In most of the case herbal extracts/natural compounds, their antioxidant activity has been attributed to their ability to break the free radical chain by donating a hydrogen atom (Albayrak *et al*., 2010). The reducing properties are generally associated with the presence of reductones, which also react with certain precursors of peroxide, thus, preventing peroxide formation.

β-Carotene-linoleic acid bleaching: Iron (III) reduction and synthetic free radical scavenging models are valuable tools to indicate the potential antioxidant activity of plant extracts/compounds, however, these systems do not use a food or biologically relevant oxidizable substrate so, no direct information on an extract's/compounds protective

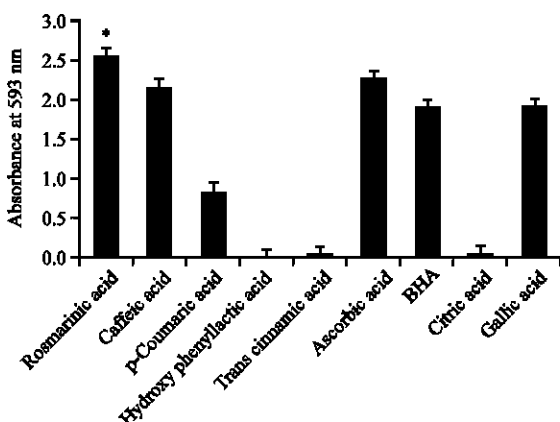

Fig. 2: Reducing power ability of five natural products and synthetic antioxidants. Data are presented as Mean±SD of each of three replicates (n = 3). *indicates the significant at $p<0.001$

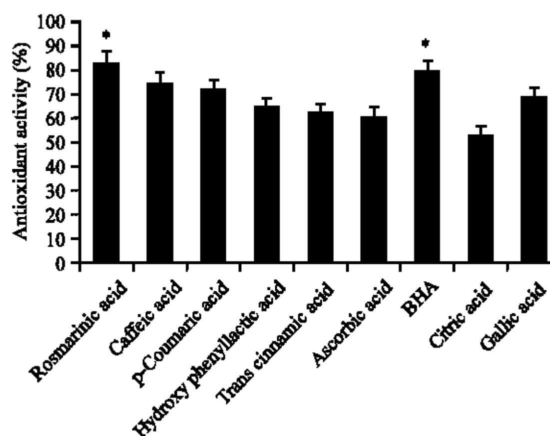

Fig. 3: Antioxidant ability of five natural products and synthetic antioxidants. Data are presented as Mean±SD of each of three replicates (n = 3). *indicates the significant at $p<0.001$

action can be determined (Dorman *et al*., 2003). Therefore, it was considered important to assess the inhibitory effect of the five natural products along with the positive controls on the oxidation of β-carotene. In this assay, oxidation of linoleic acid produces hydroperoxide-derived free radicals which attack the chromophore of β-carotene, resulting in a bleaching of the reaction emulsion. Extract/compounds capable of retarding/inhibiting the oxidation of β-carotene may be described as a free radical scavenger and primary antioxidant (Liyana-Pathirana and Shahidi, 2006). Figure 3 shows that all the compounds were capable of inhibiting the bleaching of β-carotene by scavenging linoleate-derived free radicals. The order of efficacy was RA (82.2%) followed by CA (74%) > pCA (71%) > HPA (64%) > TCA (62%). Among the five compounds tested RA showed maximum scavenging activity than the positive controls. This suggests that the compounds may have potential use as antioxidative preservatives in emulsion-type systems.

Effect of compounds on browning of apple slice: Browning is the main physiological disorder that impairs sensory properties and discourages consumer to purchase fresh-cut apples. Enzymatic browning reactions in fruits are primarily catalyzed by polyphenol oxidase in the presence of oxygen (Martinez and Whitaker, 1995). In this study, quantification of browning in apple slices treated with five natural products (RA, CA, pCA, HPA, TCA at 500 μg mL^{-1}) along with the synthetic antioxidants (BHA, AA, GA and Citric acid at 500 μg mL^{-1}) stored for 8, 24 h and 10 days at 5°C were done. The dose concentration 500 μg mL^{-1} was chosen based on the data obtained from antibacterial activity experiments. The inhibitory effect of various anti-browning agents based on absorbance measurement is shown in Fig. 4. All the compounds inhibited the browning of apple slices at each time intervals at 5°C in the following order of RA > CA > pCA > HPA > TCA. Among the five natural products tested RA exhibited a significant inhibition ($p<0.001$) than other compounds and positive controls. The positive controls citric acid and AA have been reported extensively for their anti-browning activity in minimally processed fruits and vegetables (Son *et al*., 2001). Citric acid is a chelating agent and acidulant, reducing pH and chelating copper in the active site of polyphenol oxidase and, therefore, inactivating the enzyme polyphenol oxidase (Gurbuz and Lee, 1997). AA is believed to be a free radical scavenger and to prevent browning owing to its reducing power (Lee, 1999) and it prevents enzymatic browning by reducing the quinone products to their original polyphenol compounds (Son *et al*., 2001). Similarly the observed anti-browning effect of the compounds could be due to reducing capability, radical scavenging properties and its chelating effect on active site of polyphenol oxidase.

Survival of *Listeria monocytogenes* inoculated in apple slices: Food materials are prone to both microbial and oxidative spoilage and therefore it is desirable to use a preservative with both antioxidant and antimicrobial properties (Lin *et al*., 2005). In this study, the survival of *L. monocytogenes* inoculated into natural products (RA, CA, pCA, HPA, TCA at 500 μg mL^{-1}) along with the synthetic antioxidants (Butylated hydroxy anisole, Ascorbic acid, Gallic acid and Citric acid at 500 μg mL^{-1}) treated apples slices were

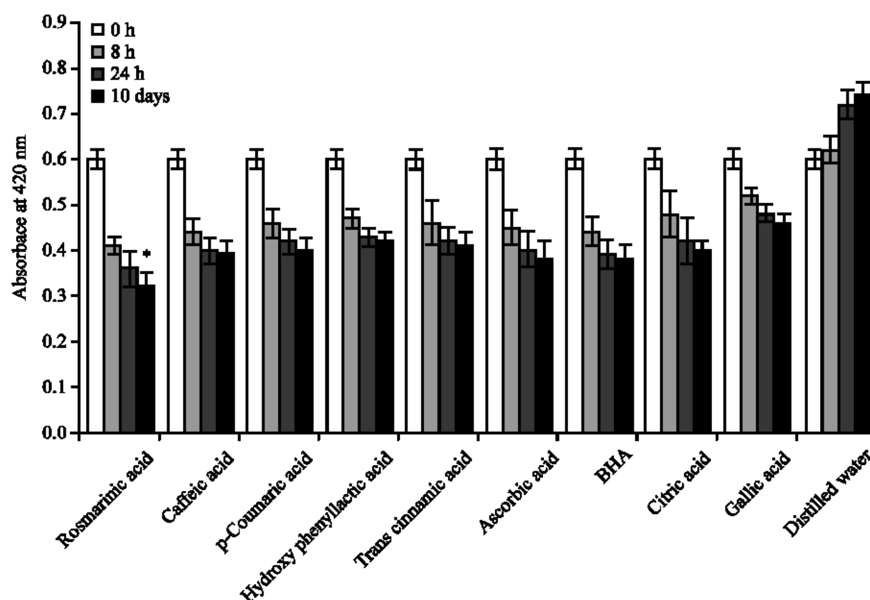

Fig. 4: Anti browning effect of five natural products and synthetic antioxidants on fresh cut apple slices. Data are presented as Mean±SD of each of three replicates (n = 3). *indicates the significant at p<0.001

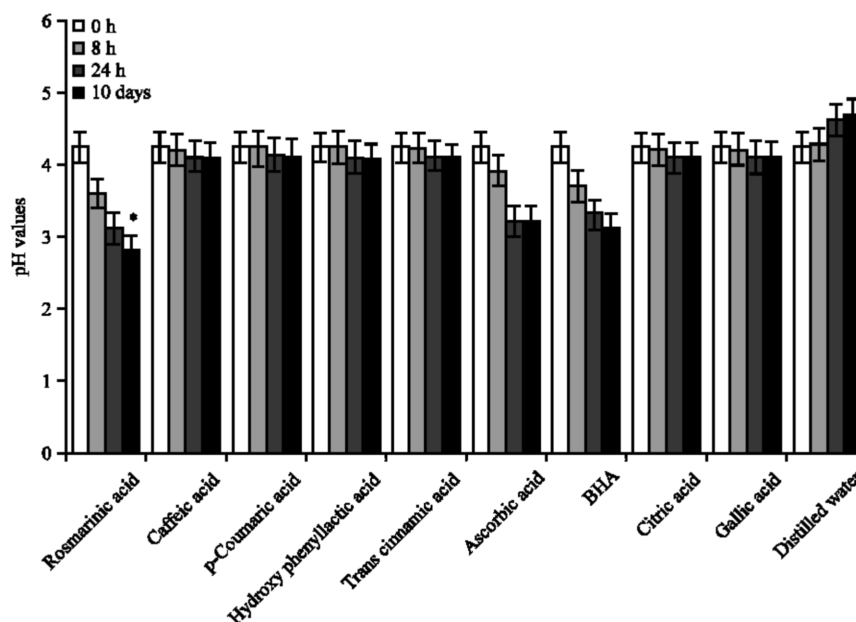

Fig. 5: Edible coating effect of five natural products and synthetic antioxidants on pH of fresh cut apple slices. Data are presented as Mean±SD of each of three replicates (n = 3). *indicates the significant at p<0.001

evaluated. Total viable count (CFU mL^{-1}) at different time intervals namely 8, 24 h and 10 days were measured. After 8 h treatment there was drastic change in microbial growth in all treated apple slices when compared to control (Table 2). Subsequent incubation with the treatment of natural products enabled the growth of microbes on the surface of apple slices; the growth was lower than that of control and apple slices treated with synthetic antioxidants. Overall the RA showed a lower number (28 CFU mL^{-1}) of *L. monocytogens* even after 10 days of incubation at 5°C than the other treated compounds. It infers that among the natural products and synthetic antioxidants, RA was found to be effective microbial growth inhibitor and it maintains the quality of apple slices.

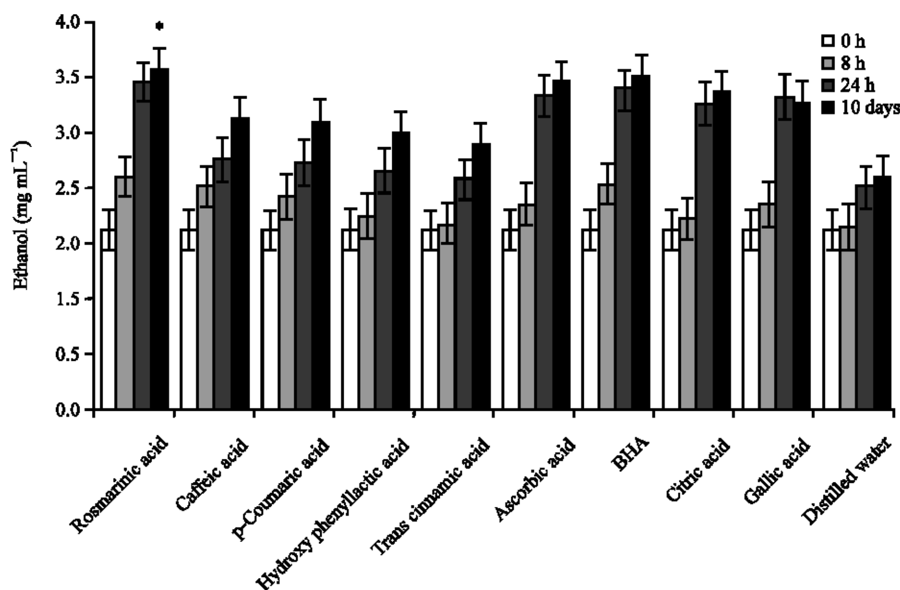

Fig. 6: Edible coating effect of five natural products and synthetic antioxidants on ethanol accumulation in fresh cut apple slices. Data are presented as Mean±SD of each of three replicates (n = 3). *indicates the significant at $p<0.001$

Table 2: Coating effect of five natural products and synthetic antioxidants on growth of *Listeria monocytogens* inoculated in apple slices

	Total viable cont (CFU mL^{-1})			
Compounds treatment (500 µg mL^{-1})	0 h	8 h	24 h	10 days
Control	565	UC	UC	UC
Rosmarinic acid	565	-	12±1.1**	28±3.2**
Caffeic acid	565	-	23±1.5**	41±3.5**
p-Coumaric acid	565	-	42±2.6**	63±3.6**
Hydroxy phenyllactic acid	565	-	61±2.8**	78±4.1**
Trans cinnamic acid	565	3±0.3	264±10.3*	385±6.7*
Ascorbic acid	565	8±0.6	UC	UC
BHA	565	-	112±8.1	146±4.2
Citric acid	565	153±3.4	UC	UC
Gallic acid	565	7±1.1	UC	UC
Distilled water	565	118±4.2	UC	UC

Values are presented as Mean±SD (n = 2). * indicates the level of significance at $p<0.001$, ** indicates the level of significance at $p<0.05$. UC: Uncountable

Effect of compounds on pH of apple slices: The quality of apple slices treated with five different natural products (RA, CA, pCA, HPA, TCA at 500 µg mL^{-1}) along with the synthetic antioxidants (BHA, AA, GA and Citric acid at 500 µg mL^{-1}) was evaluated. For the Food industry it is mandatory to maintain the acidic pH of processed foods which eventually inhibit the growth of food spoilage pathogens and extend the shelf life. Figure 5 shows the pH of apple slices at different time intervals namely 8, 24 h and 10 days at 5°C after treatment with compounds. Results show that all the samples were maintained at acidic condition for the whole period of study. RA treatment was more efficient in maintaining more acidic environment (pH 2.8) even after 10 days than the other natural and synthetic compounds. Similarly anthocyanin (polyphenol) has been reported for providing acidity in food preservation and it can able to maintain the pH 3 (Giusti and wrolstad, 2003). It may be due to presence of carboxyl group in the tested compounds and it facilitates to manifest acidic nature.

Effect of compounds on ethanol production in apple slices: Ethanol production in the minimally processed fruits and vegetables enables them to withstand for long time due to its antibacterial effect. In some cases, the addition of anti-browning agents to minimally processed fruits may lead to reduction in ethylene accumulation which ultimately causes deterioration of fruits and vegetables (Dao and Dantigny, 2011). In the food industry the ethanol emitters are commonly used to extend the shelf-life of packaged apple turnovers (Smith *et al*., 1987), pita bread (Black *et al*., 1993), packed sliced rye bread

(Salminen *et al*., 1996), English-style crumpets (Daifas *et al*., 2003) and pre-baked buns (Franke *et al*., 2002). Therefore, in this study it was considered imperative to test the effect of natural products (RA, CA, pCA, HPA and TCA at 500 μg mL^{-1}) along with the synthetic antioxidants (Butylated hydroxy anisole, Ascorbic acid, Gallic acid and Citric acid at 500 μg mL^{-1}) on ethanol accumulation in apple slices. Figure 6 shows the ethanol content at different time intervals namely 8, 24 h and 10 days. Considerable accumulation of ethanol was observed in apple slices treated with all the compounds. However, the RA treatment showed higher ethanol accumulation than the other treatments. RA treated apples was able to accumulate 3.52 mg mL^{-1} of ethanol. It has been reported that ethanol dips and vapours were used to control postharvest diseases and for long term storage of apples (Spadaro *et al*., 2004).

In conclusion, RA exhibited maximum effect in all the food preservation parameters viz. antimicrobial, AA potential and extending shelf life of fresh cut apple slices as compared to other compounds and positive controls. The outcome of this study is promising as it showed that RA has synchronized food protection effect and it can be recommended as food additive.

REFERENCES

Ahvenainen, R., 1996. New approaches in improving the shelf life of minimally processed fruit and vegetables. Trends Food Sci. Technol., 7: 179-187.

Albayrak, S., A. Aksoy, O. Sagdic and E. Hamzaoglu, 2010. Compositions, antioxidant and antimicrobial activities of *Helichrysum* (Asteraceae) species collected from Turkey. Food Chem., 119: 114-122.

Ana, A., F.A. Tomas-Barberan and I.G. Maria, 2006. Minimal processing for healthy traditional foods. Trends Food Sci. Technol., 17: 513-519.

Benzie, I.F.F. and J.J. Strain, 1996. The Ferric Reducing Ability of Plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Anal. Biochem., 239: 70-76.

Black, R.G., K.J. Quail, V. Reyes, M. Kuzyk and L. Ruddick, 1993. Shelf-life extension of pita bread by modified atmosphere packaging. Food Aust., 45: 387-391.

Brecht, J.K., 1995. Physiology of lightly processed fruits and vegetables. Hort. Sci., 30: 18-22.

Daifas, D.P., J.P. Smith, B. Blanchfield, B. Cadieux, G. Sanders and J.W. Austin, 2003. Effect of ethanol on the growth of *Clostridium botulinum*. J. Food Prot., 66: 610-617.

Dao, T. and P. Dantigny, 2011. Control of food spoilage fungi by ethanol. Food Control, 22: 360-368.

Davis, P.L. and W.G. Chace Jr., 1969. Determination of alcohol in citrus juice by gas chromatographic analysis of headspace. Hortic. Sci., 4: 117-119.

Dorman, H.J.D., A. Peltoketo, R. Hiltunen and M.J. Tikkanen, 2003. Characterization of the antioxidant property of de-odourzede extract from selected Laminaceae herbs. Food Chem., 83: 255-262.

Franke, I., E. Wijma and K. Bouma, 2002. Shelf-life extension of pre-baked buns by an active packaging ethanol emitter. Food Addit. Contam., 19: 314-322.

Giusti, M.M. and R.E. Wrolstad, 2003. Acylated anthocyanins from edible sources and their applications in food systems: A review. Biochem. Eng. J., 14: 217-225.

Gonzalez-Aguilar, G.A., C.Y. Wang and J.G. Buta, 2001. Inhibition of browning and decay of fresh-cut radishes by natural compounds and their derivatives. Lebensm. Wiss. Technol., 34: 324-328.

Grice, H.C., 1986. Safety evaluation of butylated hydroxytoluene (BHT) in liver, lung and gastrointestinal tract. Food Chem. Toxicol., 24: 1127-1130.

Gurbuz, G. and C.Y. Lee, 1997. Color of minimally processed potatoes as affected by modified atmosphere packaging and anti browning agents. J. Food Sci., 62: 572-576.

Gyamfi, M.A., M. Yonamine and Y. Aniya, 1999. Free radical scavenging action of medicinal herbs from Ghana: *Thomingia sangulnea* on experimentally induced liver injuries. Gen. Pharmacol., 32: 661-667.

Kim, D.M., N.L. Smith and C.Y. Lee, 1993a. Apple cultivar variations in response to heat treatment and minimal processing. J. Food Sci., 58: 1111-1114.

Kim, D.M., N.L. Smith and C.Y. Lee, 1993b. Quality of minimally processed apple slices from selected cultivars. J. Food Sci., 58: 1115-1117.

Koleva, I.I., T.A. van Beek, J.P.H. Linssen, A. de Groot and L.N. Evstatieva, 2002. Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. Phytochem. Anal., 13: 8-17.

Lee, C.Y., 1999. Enzymatic Browning Reaction. In: Encyclopedia of Food Science and Technology, Francis, F.J. (Ed.). Wiley, New York, USA., pp: 494-515.

Lin, Y.T., R.G. Labbe and K. Shetty, 2005. Inhibition of *Vibrio parahaemolyticus* in seafood systems using oregano and cranberry phytochemical synergies and lactic acid. Innov. Food Sci. Emerg., 6: 453-458.

Liyana-Pathirana, C.M. and F. Shahidi, 2006. Antioxidant properties of commercial soft and hard winter wheats (*Triticum aestivum* L.) and their milling fractions. J. Sci. Food Agric., 86: 477-485.

Martinez, M.V. and J.R. Whitaker, 1995. The biochemistry and control of enzymatic browning. Trends Food Sci. Technol., 6: 195-200.

Ozoglu, H. and A. Bayindirli, 2002. Inhibition of enzymatic browning in cloudy apple juice with selected anti browning agents. Food Control, 13: 213-221.

Pelczar, M.J., Chan and E.C.S. Krieg, 1988. Control Microorgarisms, the Control of Microorganisms by Physical Agents. McGraw-Hill International, New York, pp: 469-509.

Rolle, R.S. and I.G.W. Chism, 1987. Physiological consequences of minimally processed fruits and vegetables. J. Food Qual., 10: 157-177.

Russel, A.D., 1991. Mechanisms of bacterial resistance of nonantibiotics: Food additives and food pharmaceutical preservatives. J. Applied Bacteriol., 71: 191-201.

Salminen, A., K. Latva-Kala, K. Rendell, E. Hurme, P. Linkot and R. Ahvenainen, 1996. The effect of ethanol and oxygen absorption on the shelf-life of packed sliced rye bread. Packag. Technol. Sci., 9: 29-42.

Seymour, I.J., 1999. Review of Current Industry Practice on Fruit and Vegetable Decontamination. Campden and Chorleywood Food Research Association, UK., Pages: 38.

Shimada, K., K. Fujikawa, K. Yahara and T. Nakamura, 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem., 40: 945-948.

Smith, J.P., B. Ooraikul, W.J. Koersen, F.R. Van De Voort, E.D. Jackson and R.A. Lawrence, 1987. Shelf-life extension of a bakery product using ethanol vapor. Food Microbiol., 4: 329-337.

Son, S.M., K.D. Moon and C.Y. Lee, 2001. Inhibitory effects of various anti browning agents on apple slices. Food Chem., 73: 23-30.

Spadaro, D., A. Garibaldi and M.L. Gullino, 2004. Control of *Penicillium expansum* and *Botrytis cinerea* on apple combining a bio control agent with hot water dipping and acibenzolar-S-methyl, baking soda, or ethanol application. Postharvest Biol. Technol., 33: 141-151.

Tanaka, M., C.W. Kuie, Y. Nagashima and T. Taguchi, 1988. Application of antioxidative Maillard reaction products from histidine and glucose to sardine products. Bull. Japanese Soc. Sci. Fisher., 54: 1409-1414.

Wei, C.I., T.S. Huang, J.M. Kim, W.F. Lin, M.L. Tamplin and J.A. Bartz, 1995. Growth and survival of *Salmonella montevideo* on tomatoes and disinfection with chlorinated water. J. Food Prot., 58: 829-836.

Wichi, H.P., 1988. Enchanced tumpr development by Butylated Hydroxyanisole (BHA) from the perspective of effect on fore stomach and oesophageal squamous epithelium. Food Chem. Toxicol., 26: 717-723.