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Development of minimally processed apple slices by hurdle technology

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Development of minimally processed apple slices by hurdle technology

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CERTIFICATE

This is to certify that the thesis entitled **'Development of minimally processed apple slices by hurdle technology'** submitted to the Faculty of the Post-Graduate School, Indian Agricultural Research Institute, New Delhi in partial fulfilment of **DOCTOR OF PHILOSOPHY** in **POST HARVEST TECHNOLOGY**, embodies the results of bonafide research work carried out by **Mr. Pushpendra Kumar**, **Roll No. 10524** under my guidance and supervision, and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help availed during the course of investigation as well as source of information have been duly acknowledged by him.

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LIST OF ABBREVIATIONS

NHB	National Horticultural Board
msl	Mean sea level
PPO	Polyphenol oxidase
POD	Peroxidase
MAP	Modified atmosphere packaging
O_2	Oxygen
CO_2	Carbon dioxide
4-HR	4-hexyl resorcinol
$CaCl_2$	Calcium chloride
mg	Milligram
CMC	Carboxymethyl cellulose
AVG	Aloe vera gel
L	Length
W	Width
Dg	Geometric mean diameter
Da	Arithmetic mean diameter
N	Newton
BI	Browning index
WI	Whiteness index
BP	Browning potential
AOAC	Association of Official Analytical Chemists
GAE	Gallic acid equivalents
ICP-MS	Inductively coupled plasma mass spectrometry
µg	Microgram
CFU	Colony forming unit
FAMEs	Fatty acid methyl esters
PLW	Physiological loss in weight
SSC	Soluble solid content

1. INTRODUCTION

Apple (*Malus domestica*) is the most popular temperate fruit in the world because of its crispy texture and sweet taste. India ranks 5^{th} in the world apple production where it is commercially grown in Himalayan regions of Jammu and Kashmir, Himachal Pradesh and Uttarakhand. In these states, the apples are grown at altitudes ranging from 1200 m to 3500 m above mean sea level (Basannagari and Kala, 2013). Its cultivation has also been extended to Arunachal Pradesh, Sikkim, Nagaland and Meghalaya in north-eastern region and nilgiri hills in Tamil Nadu. Apple production in India was estimated to be 22.65 lakh tonnes in 2016-2017 with 3.05 lakh ha of area under cultivation (NHB, 2016-17). Apples form an important part of human diet as they are a rich source of sugars, minerals, dietary fibre and functional compounds such as ascorbic acid and phenolics (Wu *et al.,* 2007). According to the National Horticulture Board, a typical apple serving weighs 100 g and contains 59 kcal with significant dietary fiber and modest vitamin C content. It is also rich in minerals like phosphorus, potassium, magnesium, calcium, sodium, iron and copper.

The consumer acceptability and quality of apple fruit is associated with their chemical composition and overall sensory appeal (Todea *et al.,* 2014). The fruit maturity and environmental factors (Markuszewski and Kopytowski, 2008), horticultural practices applied in the orchard (Amarante *et al.,* 2008) and storage conditions (Begić-Akagić *et al.,* 2011) affect the differences in the relative concentrations of individual components in different cultivars.

In apple, the taste characteristics such as flavour, bitterness and astringency are associated with the content of sugars, organic acids and phenolic compounds (Bengoechea *et al.,* 1997). The phenolic compounds are also associated with browning processes in apple with their concentration being cultivar dependent (Ayaz and Kadioglu, 1997). Fatty acids present in the fruit are useful in maintaining fruit quality and development of aroma (Fellman *et al.,* 2000). Similarly, many minerals affect the disease incidence and ripening behaviour in fruits.

There are more than 7500 known cultivars of apples, resulting in a range of desired characteristics (Elzebroek and Wind, 2008). Apart from being consumed as fresh, apple is also processed into different value added products such as apple juice, apple cider, wine, apple juice concentrate, packed natural ready-to-serve (RTS) drink and vermouth, apple purees and jams, jelly, apple butter and dried apple products (Shalini and Gupta, 2010). Of late, fresh-cut apples are one of the principal growing segments in fresh-cut food retail establishments. Minimally processed fruits and vegetables offer to consumer's convenient, highly nutritious and healthful products while maintaining the freshness of the intact products. The restaurants are the most important user of fresh-cut fruits and vegetables (Ponce *et al.*, 2008). The reason for their utilization is to condense the manpower and manage the waste (Xu *et al.*, 2003). Much research has been done to find the optimum conditions for quality maintenance of whole fruits and vegetables, but only limited information is available on fresh-cut minimally processed products. Minimal processing includes grading, washing, sorting, peeling, slicing, chopping and then packaging (Lee *et al.,* 2003; Rojas-Grau *et al.,* 2007; Martín-Belloso *et al.,* 2006). Minimally processed products are vastly nutritious and extremely delicate. For minimally processed apple slices, that are mainly used in fruit salads, the greatest hurdle to the commercial marketing is the limited shelf life which is due to the cut surface browning and excessive tissue softening as compared to the intact apples from which they are made. The consequences of browning are not only restricted to the discoloration, undesirable tastes can also be produced and loss of nutrient quality may occur (Vamos-Vigyazo, 1981). Polyphenol oxidase (PPO) has been considered one of the most damaging enzymes to quality maintenance of fresh-cut produce (Whitaker and Lee, 1995) and the control of enzymatic browning in fresh-cut products has always been considered a challenge for the food scientists (Ponting, 1960). Also, the chemical composition, physiological function and the overall quality of fresh cut apples varies depending on cultivar and fruit maturity (Lee and Smith, 1995; Soliva-Fortuny *et al.,* 2002).

Therefore, extending shelf life of minimally processed apple slices is one of the major problems in the production of fresh-cut apples. Hurdle technology is a suitable approach to develop stable minimally processed products. It is an intelligent combination of different hurdles or preservation techniques to achieve an enhanced level of product stability towards quality changes and safety (Leistner, 2000). Different preservation technologies, including cold storage, edible coatings, modified atmosphere packaging and ozonation have been used to reduce deterioration, prolong the shelf life and retain the nutritional value and freshness of fresh-cut fruits (Rocculi *et al.,* 2004). The treatment with browning inhibitors is an effective and commonly employed method for preventing the enzymatic browning in various minimally processed fruits. Browning inhibitors such as reducing agents, acidulants, complexing agents, chelating agents, enzyme inhibitors and enzyme treatments can be employed (Garcia and Barrett, 2002).

Effective preservation of minimally processed products can also be achieved using a combination of treatments such as edible coatings and modified atmospheric packaging (MAP). The edible coatings are thin layers of edible material employed to the surface of product in addition to or as a replacement for natural protective waxy coatings and to provide a barrier to oxygen, moisture and solute movement for the product. They are applied directly on the product surface by spraying, brushing and dipping to create a modified atmosphere around the product. The edible coatings have a high potential to carry active ingredients such as anti-browning agents, colorants, flavours, spices, nutrients and antimicrobial compounds that can extend product shelf life and reduce the risk of microbial growth on food surface. Edible coatings can enhance the shelf life of minimally processed products by forming a gas barrier thus resulting in a reduction in enzymatic browning and respiration (Baldwin *et al.,* 1995) and weight loss (Olivas and Barbosa-Canovas, 2005). Different types of edible coatings like whey protein, milk protein and alginate have been used to enhance the shelf life of minimally processed apple slices (Olivas and Barbosa-Canovas, 2005).

Aloe vera, generally known as a ’medicinal plant’, is known for its wide range of therapeutic properties. The most common species of aloe vera are *Aloe arborescens* and *Aloe barbadensis*. The two major liquid components of aloe vera are yellow latex (exudate) and a clear gel (mucilage) which gets from the large leaf parenchymatic cells (Ni *et al.,* 2004). The major use of aloe vera is in the cosmetic industry including treatment of wound healing, burns and scars (Serrano *et al.,* 2006). Recently the use of aloe vera as an edible coating has been reported to enhance the shelf life and delay senescence in sweet cherry and table grapes (Martinez-Romero *et al.,* 2006; Serrano *et al.,* 2006). Aloe vera based edible coatings have been shown to control moisture loss, respiration and senescence rate, softening delay and delay in browning and reduced microorganism growth in fruits such as sweet cherries, table grapes, nectarines and papaya (Ahmed *et al.,* 2009; Valverde *et al.,* 2005; Martinez-Romero *et al.,* 2006; Marpudi *et al.,* 2011). The carboxymethyl cellulose is a white to creamcolored, odorless, tasteless and free-flowing powder. Treatment with carboxymethyl cellulose can extend shelf life and maintain quality of minimally processed fruit.

Extension of storability of the minimally processed produce can be done by synergistic application of edible coating with modified atmospheres. With modified atmospheric packaging (MAP) the desired balance of oxygen and carbon dioxide is created through the control of oxygen (O_2) and carbon dioxide (CO_2) transmission of the packaging film and the respiration of the commodity. Studies have shown that the low level of oxygen (O_2) and elevated carbon dioxide (CO_2) atmospheres can slow down the enzymatic browning. Modified atmospheres should be seen as a supplement to an adequate management of temperature and humidity (Kader, 1992). To prevent anaerobic respiration, development of off flavors and odors and increasing the susceptibility to decay, it is important to choose appropriate gas composition of modified atmosphere for a particular product (Wills *et al.*, 1998). Therefore, in the present study attempts were made to obtain optimal conditions for development of minimally processed apple slices using various conditions/ treatments.

Considering the existing research gaps and practical importance of the work, a research proposal on '**Development of minimally processed apple slices by hurdle technology**' was formulated with the following objectives:

Objectives:

1. Evaluation of the nutritional composition of different apple cultivars grown at varying altitudes**.**
2. Synergistic effect of edible coatings and antibrowning agents on quality of fresh cut apple slices.
3. Extension of shelf life of minimally processed apple slices by modified atmosphere packaging.

2. REVIEW OF LITERATURE

Apple is an important temperate fruit of the world. In India, although a lot of cultivars are grown but data on their compositional characterization is sparse. Also, attempts are still underway to develop value added and stable minimally processed apple slices. These are one of the major growing segments in fresh-cut food retail establishments. The major lacuna in their marketing is the lack of shelf stability of the fresh-cut slices. Hence, the present investigation on **'Development of minimally processed apple slices by hurdle technology'** has been conducted to extend the storage life of fresh-cut apple slices by various interventions. This chapter reviews the literature available under different sub-heads.

2.1 Evaluation of Physical and Physiological Characteristics of Apple Cultivars

2.1.1 Fruit Dimensions

Chakespari *et al.* (2010) found statistically significant differences in the physical dimensions of the two Iranian apple cultivars, 'Shafi Abadi' and 'Golab Kohanz'. They attributed the differences to the individual properties of apple cultivars, cultivation and environmental conditions. The mean fruit length ranged between 51.56 to 53.09 mm whereas thickness and width were in the range of 54.86 to 59.37 and 53.03 to 57.02, respectively. The geometric, equivalent and arithmetic mean diameter of Golab Kohanz and Shafi Abadi apples were 53.11, 53.12, 53.15 mm for Golab Kohanz and 56.41, 56.42, 56.49 mm for Shafi Abadi variety, respectively.

2.1.2 Fruit firmness

Jan *et al.* (2012) harvested four apple cultivars ('Royal Gala', 'Mondial Gala', 'Golden Delicious' and 'Red Delicious') at three different maturity stages on 15 days interval as early, mid and late and recorded the fruit firmness with a penetrometer (11 mm probe) The fruit firmness varied significantly among cultivars with maximum fruit firmness (5.85 kg/cm^2) recorded for cultivar 'Red Delicious' and the minimum (5.08 kg/cm^2) for cultivar 'Royal Gala'. The fruit firmness significantly decreased from an initial of 6.62 kg/cm^2 for fresh fruits to 4.09 kg/cm^2 for fruits stored for 150 days. Harvesting stage significantly affected the firmness of apple fruit wherein the maximum fruit firmness was recorded for the early harvested fruit (5.88 kg/cm^2), followed by fruits at mid and late harvesting stage with 5.33 kg/cm^2 and 4.81 kg/cm^2, respectively.

Johnston *et al.* (2001) conducted an experiment to quantify physical change in apple texture with change in fruit temperature. The fruits of cultivars 'Granny Smith', 'Cox's Orange Pippin' and 'Royal Gala', had higher firmness values at harvest when measured at 20°C than at 0–3°C. They reported that the relationship between firmness values and fruit temperature between 0°C and 20°C were linear and positive at harvest and linear and negative for stored apples. The changes in fruit firmness with temperature were not affected by harvest maturity and orchard. Previously, Lara and Vendrell (1998) have reported maximum apple fruit firmness when stored at 1°C.

Konopacka and Plocharski (2004) carried out experiments to determine relationship between fruit firmness and texture acceptability of apples, stored under different atmospheres. The three apple cultivars ('Elstar', 'Jonagold' and 'Gloster') were stored at 3 ºC in either normal atmosphere, controlled atmosphere (3 % O_2, 5 % CO_2) and low oxygen controlled atmosphere (1.5 % O_2, 1.5 % CO_2). The firmness of the apple was significantly varied by further storage at 0 ºC and 18 ºC for different duration after storage. Fruit were then subjected to the Magness–Taylor test and sensory analysis. The effect of different storage conditions on relationship between fruit firmness and acceptability of fruit texture was observed to be dependent on apple cultivar. Higher values of firmness were observed for 'Gloster' and 'Jonagold' fruits stored under controlled atmospheric conditions.

2.1.3 Peel color

Of the seven apple cultivars studied, Drogoudi *et al.* (2008) observed a intense red peel colour of cultivars 'Starkrimson' and 'Fyriki'. Both apple cultivars were darker and more red compared to the green cultivars 'Golden Delicious', 'Granny Smith' and 'Mutsu'. The skin of bicolored cultivars 'Fuji' and 'Jonagored' was either intermediate or similar to the red or green cultivars. The flesh tissue of cultivar 'Fyriki' was the brightest and reddest. The study revealed that the more nutritious peel may be redder, darker and bluer and the more nutritious flesh have a lighter color and low level of soluble solid content. Vieira *et al.* (2009) also compared six apple cultivars grown at an altitude of 960 m. The cultivar 'Galaxy' apple showed highest value for a* and the lower values for h° and b* compare to the other cultivars, indicating the redness of the fruits compared to other cultivars.

2.1.4 Respiration rate

Fan (1999) applied ethylene action inhibitor, 1-methylcyclopropene (0.8 to 1.0 ml/L as a gas) to preclimacteric and climacteric apples (cvs. 'Delicious', 'Gala', 'Fuji', 'Jonagold' and 'Ginger Gold') for different durations (12 to 16 hr) at 20 to 24 ºC. 1-MCP substantially reduced the respiration and ethylene production. Respiration rate was about half as much in 1-methylcyclopropene treated samples (6.07 mL CO_2/kg/h) as in control samples (14.10 mL CO_2/kg/h).

2.2 Evaluation of the Nutritional Composition of Different Apple Cultivars

2.2.1 Total soluble solids, titratable acidity, pH and ascorbic acid

Campeanu *et al.* (2009) reported total acidity ranged between 0.12 % and 0.34 % in the ten apple cultivars studied, the highest being in 'Jonathan' apple cultivar. They also reported ascorbic acid in the range of 25.75 mg/100g to 77 mg/100g with cultivars 'Mutzu' and 'Jonathan' showing the highest values. Hussain *et al.* (2014) reported a TSS range of 9.5 to 11.4°B with pH between 3.44 to 3.89 and acidity in the range of 0.15 to 0.28 %. Jan *et al.* (2012) studied quality attributes of four apple cultivars (Royal Gala, Mondial Gala, Golden Delicious and Red Delicious) at three different maturity stages at 15 days interval as early, mid and late. The cultivar 'Red Delicious' recorded the highest vitamin C (12.49 mg/100g) whereas cultivar Mondial Gala showed the highest titratable acidity (0.56 %). They reported an increase in total soluble solids with a corresponding decline in the titratable acidity and ascorbic acid during 150 day storage of the apple cultivars at 5±1°C.

Nour *et al.* (2010) evaluated 15 apple cultivars for their quality parameters. They reported soluble solids content in the range of 10.8 % ('Prima') to 16.5 % ('Red Boskoop'). The average content of titratable acidity in the apple cultivars was 0.26 %. In general the ascorbic acid content in all tested apple cultivars was low (average 6.18 mg/100g) with the exception 'Red Boskoop' (18.7 mg/100g) and 'Idared' (11.4 mg/100g) in which the vitamin C content exceeded 10 mg/100 g.

Wu *et al.* (2007) analyzed eight apple cultivars ('Delicious', 'Golden Delicious', 'Ralls', 'Fuji', 'QinGuan', 'Jonagold', 'Granny Smith' and 'Orin') for the sugars, phenolic compounds, organic acids, amino acids and fatty acids by high-performance liquid chromatography and gas chromatography. The cultivar 'Delicious' showed a low level of soluble solids compared to the others. The maximum total soluble solid was observed in cultivar 'Ralls' (14.68 %) followed by Fuji (13.84 %). The pH for the studied cultivars averaged to 3.87 with maximum in cultivar 'Qin Guan' (4.16) and minimum in green cultivar 'Granny Smith' (3.40).

2.2.2 Antioxidant activity and total phenolic content

Drogoudi *et al.* (2008) measured total antioxidant activity and total phenols in pulp and peel tissues of seven apple cultivars. Pulp of cultivar 'Fyriki' showed 82 % and 67 % higher antioxidant activity and total phenolic content, respectively than the other cultivars studied. In fruit peel tissue, the highest antioxidant activity and total phenolic content (up to 64 % more) were found in cultivar 'Starkrimson'. Overall, it was observed that fruit peel showed 1.5 to 9.2 times higher total antioxidant activity and 1.2 to 3.3 times higher total phenolic content as compared to the pulp fraction. Later, Vieira *et al.* (2009) also reported a huge variation in total phenolic content among the six apple cultivars (105.4–269.7 mg GAE 100/g FM) they studied.

Manzoor *et al.* (2012) appraised 5 apple cultivars namely, 'Red Delicious', 'Golden Delicious', 'Kashmiri Amri', 'Kala Kulu' and 'Sky Spur' for antioxidant activity and total phenolics in the peel and pulp fractions. The total phenolic contents ranged from 1,907.5–2,587.9 mg GAE/100 g in peel and 1,185.2–1,475.5 mg GAE/100 g in pulp. The peel of cultivars 'Red Delicious' and 'Kala Kulu' showed significantly higher contents of total phenolics (2,587.9 and 2,274.8 mg/100 g, respectively), followed by 'Golden Delicious' (2,102.4 mg/100 g) and 'Kashmiri Amri' (2,097.1 mg/100 g). In case of pulp, the highest amount of total phenolics (1,475.5 mg/100 g) was observed in cultivar 'Red Delicious' and the lowest in 'Kashmiri Amri' (1,185.2 mg/100 g). The average antioxidant content in peel and pulp fractions was found to be 22.1 g/100 g and 14.2 g/100 g, respectively. Kołodziejczyk *et al.* (2010) reported the total polyphenol content range of 161.9 to 882.4 mg/kg FW with hydroxycinnamic acid as the major phenolic compounds in the 22 apple cultivars studied.

Todea *et al.* (2014) investigated the polyphenols in different apple cultivars ('Golden Delicious', 'Auriu de Cluj', 'Prima', 'Florina', 'Generos', 'Starkrimson' and

'Productiv de Cluj') by the high performance liquid chromatography. Variation in concentration of major polyphenols (quercetin, epicathechin and catechin) was observed in different cultivars studied with maximum content of polyphenols in 'Idared' and 'Jonathan' cultivars. Quercetin was found to be the principal polyphenol, in all investigated apple cultivars, followed by epicathechin and catechin. In contrast, Wu *et al.* (2007) reported the predominance of chlorogenic acid and protocatechuic acid in the eight apple cultivars studied.

2.2.3 Sugars, organic acids and fatty acids

In the ten apple cultivars studied by Campeanu *et al.* (2009), sugars ranged between 9.53 and 12.34 %. Similarly, Hussain *et al.* (2014) also reported sugars to be in the range of 10.70 to 15.13 % in the three cultivars they studied whereas, Nour *et al.* (2010), reported a range of 9.5 % to 15.03 %. Jan *et al.* (2012) found that the total sugars increased with increase in storage duration of apples. The initial value of 9.31 % total sugars recorded in early mature fruit, increased to 12.98 % in late maturing fruits.

Begić-Akagić *et al.* (2011) analyzed ten traditional and two international commercial apple varieties using HPLC, for individual sugars and organic acids. They quantified fructose to be the most abundant sugar in apple fruit, followed by glucose, sucrose and sorbitol. Malic acid and citric acid were predominant among the individual organic acids in both traditional and international apple varieties, followed by fumaric and shikimic acid. In general, it was observed that the traditional apple cultivars had higher sugar content and low level of organic acids in comparison to international ones with traditional cultivars being sweeter and good for direct consumption.

Nour *et al.* (2010) evaluated 15 apple cultivars for organic acids by a reversed-phase HPLC method. The average value of malic acid for the fifteen apple cultivars was 919 mg/100 g. Citric acid was present in lesser quantities with the average content of 21.47 mg/100 g. Higher values of citric acid were reported in cultivar 'Starkrimson' (49.1 mg/100 g) and 'Early Red' (47.8 mg/100 g) while cultivars 'Patul' (7.7 mg/100 g) and 'Aura' (3.5 mg/100 g) cultivars showed lower values.

Wu *et al.* (2007) identified fructose and glucose as the major monosaccharides in the eight apple cultivars analyzed. Malic, quinic and citric were the predominant organic acids detected. Cultivars 'Granny Smith' and 'Ralls' showed maximum organic acid content amongst all the cultivars. They have also reported palmitic acid and linoleic acid to be the two most dominant fatty acids in apples that account for about 70–80 % of the total fatty acids. They found cultivar ‘Qin Guan’ to be relatively low in α-linolenic acid, whereas cultivar ‘Orin’ was found to be a good source.

2.2.4 Mineral content

Mineral elements were reported to vary within the apple varieties (Campeanu *et al.,* 2009). Nitrogen content varied between 0.67 % and 0.11 %, phosphorous between 0.15 % and 0.23 % and potassium between 0.40 % and 0.75 %. The values for calcium varied between 2.5 mg/100g and 7.8 mg/100g while iron ranged between 0.2 mg/100g and 0.28 mg/100g in the ten cultivars studied. Hussain *et al.* (2014) observed sodium 0.8 mg/100g in cultivar ‘Golden Delicious’, 0.4 mg/100g in ‘Nazak Badan’ and 0.6 mg/100g in cultivar ‘Five Star’. The potassium content were recorded as, 160 mg/100g in ‘Golden Delicious’, 155 mg/100g in ‘Nazak Badan’ and 178 mg/100g in ‘Five Star’.

The contents of potassium and calcium in apple peel and pulp as observed by Manzoor *et al.* (2012) ranged from 695.3–980.9 to 443.6–790.1 mg/100 g and 35.6–72.1 to 19.8–48.9 mg/100 g, respectively. The highest content of potassium was exhibited in peel and pulp of ‘Golden Delicious’ cultivar (980.9 and 790.1 mg/100 g, respectively), whereas, least (695.3 and 443.6 mg/100 g, respectively) in ‘Sky Spur’ cultivar. The concentration of calcium was highest in peel (72.1 mg/100 g) and pulp (48.9 mg/100 g) of cultivar ‘Red Delicious’ while least in peel (35.6 mg/100 g) and pulp (19.8 mg/100 g) of cultivar ‘Sky Spur’. Magnesium and sodium ranged from 35.7–65.9 to 2.9–7.3 mg/100 g for peel while values of 15.6–34.8 to 5.3–10.8 mg/100 g were recorded for pulp of different apple cultivars, respectively. In case of trace elements, the content of iron ranged from 1.2–2.4 mg/100 g in peel and in pulp 0.8–2.1 mg/100 g whereas zinc ranged from 0.4–1.2 mg/100 g in peel and 0.2–0.9 mg/100 g in pulp of different apple cultivars. It was reported that the cultivar ‘Golden Delicious’ had the highest content of both of these trace elements and the lowest was reported in ‘Sky Spur’ cultivar. Nour *et al.* (2010) evaluated 15 apple cultivars for Na, Ca, Mg, P, Fe, Mn, Cu, Al, Cr, Zn and Sr contents by ICP-MS following a microwave digestion. They reported that the Na content varied between 82.25 mg/100 g (‘Mutzu’) and 160.85 mg/100 g (‘Florina’). Calcium varied between 1.70 mg/100 g

('Starkrimson') and 8.74 mg/100g ('Prima') while Fe content ranged between 0.19 mg/100 g ('Ionagold') and 0.40 mg/100 g ('Cadel' and 'Early Red').

Horsley *et al.* (2014) determined elements of three different apples (green, yellow-red and wild) by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). The potassium contents of apples varied from 8510 mg/kg (green apple) to 11942 mg/kg (yellow-red apple). While magnesium contents of the three apples ranged between 505 mg/kg (wild apple) and 734 mg/kg (wild apple), the phosphorus contents were between 683 mg/kg (green apple) to 1145 mg/kg (yellow-red apple).

Studies on inductively coupled plasma optical emission spectrometry (ICP-OES) for determining mineral composition of five Romanian cultivars of apple by Oana *et al.* (2014) revealed that the concentrations of the elements found in whole fruit, flesh and peel was found to be comparable for most of the elements of interest on a dry weight basis. For the whole fruit, flesh and peel fractions, they obtained values (in mg/kg) of 8290, 28434 and 30350 K; 8.14, 7.66 and 8.31 Na; 241, 231 and 280 Ca; 244, 226 and 347 Mg; 3.18, 1.65 and 3.08 Cu; 2.82, 2.50 and 2.94 Fe; 1.30, 0.35 and 2.29 Mn and 1.57, 1.71 and 2.51 Zn, respectively.

Todea *et al.* (2014) quantified various minerals by ICP-MS and ICP-OES in different apple cultivars ('Golden Delicious', 'Auriu de Cluj', 'Prima', 'Florina', 'Generos', 'Starkrimson' 'Productiv de Cluj'). Potassium (31976 mg/kg) was found to be the predominant among the macro elements, while iron (3.68 mg/kg) and copper (3.57 mg/kg) were the major microelements.

2.2.5 Polyphenol oxidase (PPO) activity

Kołodziejczyk *et al.* (2010) analyzed twenty two apple cultivars for their polyphenol oxidase (PPO) activity. The level of polyphenol oxidase (PPO) activity varied between 5 and 240 U/g FW. About half of the twenty two cultivars showed polyphenol oxidase activity below 50 U/g FW. The apple cultivars 'Selena' 'Angold' and 'Gold Milenium' showed the maximum polyphenol oxidase (PPO) activity (between 125-133 U/g FW) while 'Topaz', 'Rebella', 'Enterprise' 'Šampion', 'Rewena' and 'Gerlinde' showed the lowest.

2.3 Effect of Altitude on the Nutritional Composition of Different Fruits

Correia *et al.* (2016) harvested four blueberry cultivars ('Goldtraube', 'Duke', 'Ozarkblue' and 'Bluecrop') at two different altitudes: up to 636 meters (high altitude) and up to 217 meters (low altitude) for three consecutive years from the middle of May to the end of July. Free sugars, protein, fat, dry mass, organic acids, ascorbic acid and energy contents were analysed using HPLC-UV-DAD and spectrophotometric methods. Differences in the concentrations of organic acids, free sugars and ascorbic acid were found among the harvesting years while the altitude had no influence on the composition of fruits. Fructose was the most abundant sugar and citric acid the main organic acid in the blueberry fruits. The cultivar 'Ozarkblue' had the highest volume and mass while highest fat and crude protein was in 'Bluecrop' cultivar. The sucrose and organic acids was highest in 'Goldtraube' and fructose content in 'Duke' cultivar.

Solarte *et al.* (2014) sampled three white-fleshed genotypes ('Guavatá Victoria', 'Regional Blanca' and 'Ráquira Blanca') and one pink-fleshed genotype of guava fruit ('Regional Roja') from three altitudes (1570, 1720 and 1890 msl) and studied the effect of altitudes on fruit quality. At lower altitudes where the high radiation and warmer temperature are predominant, the fruit weight was higher for 'Regional Roja' and 'Guavatá Victoria' cultivars, accompanied by rapid colour change from green to yellow. In general, the organic acid content increased with altitude except in case of 'Regional Roja' genotype.

Chand (1962) reported that the total soluble solids content varies with altitude in different apple cultivars. At 1500-2000 msl , total soluble solid content varied in cultivars as 'Red Delicious' (13.28 %), 'Royal Delicious' (15.01 %), 'Richared' (14.03 %) and 'Golden Delicious' (12.10 %), whereas it was 14.10, 14.35, 15.00 and 11.50 %, respectively at 2250-2750 msl. They also observed that the acid content varied between 0.20 and 0.41 %. Rana (1978) reported total soluble solid content in 'Red Delicious', 'Royal Delicious', 'Richared' and 'Golden Delicious' cultivars to be between 10.50 and 12.50 % in higher hill zones of Himachal Pradesh. Some scientists reported that the apples grown in higher elevations (i.e. Kashmir valley and Kinnaur districts of Himachal Pradesh) have higher vitamin C (Dang *et al.,* 1974; Sharma, 1996) than those grown in high and mid hills of Himachal Pradesh (Bhardwaj and Kaushal, 1990; Sharma, 1996). There is good colour development in apples at higher elevations associated with more availability of sunlight (Sharma, 1996). In 1987, Kanwar observed that the apples grown at lower elevations (mid hills) take less time for optimum maturity in comparison to fruits grown at higher elevations and in dry temperate zone because of higher temperature in mid hills.

2.4 Effect of Browning Inhibitors on Quality of Minimally Processed Apple Slices

Arias *et al.* (2007) investigated the mechanism of action of 4-hexylresorcinol and ascorbic acid on polyphenol oxidase which catalyzed the oxidation of phenolic substrate. It was found that incubation of polyphenol oxidase (PPO) with 4-HR diminishes strongly polyphenol oxidase activity due to the high affinity of 4-HR for polyphenol oxidase, as irreversible inactivation of polyphenol oxidase. The authors suggested two mechanisms by which ascorbic acid can prevent enzymatic browning: first in the absence of polyphenol oxidase substrates it inactivates polyphenol oxidase irreversibly, probably through the binding to its active site, particularly in its *oxy* form. Second, in the presence of polyphenol oxidase substrates, ascorbic acid reduces polyphenol oxidase oxidized reaction substrates which results in a lag phase when measuring polyphenol oxidase activity as observed by the dark product formation. Thus, they suggested that concurrent use of both 4-hexylresorcinol and ascorbic acid on polyphenol oxidase, results in additive prevention of enzymatic browning.

Luo *et al.* (1995) reported a positive effect of ascorbic acid and 4-hexylresorcinol for inhibiting the enzymatic browning of vacuum packaged ‘Red Delicious’ apple slices at 0.5 ºC for a period of 8 weeks. The browning inhibition by combination of ascorbic acid and 4-hexylresorcinol was comparable to the application of sulphite. In addition, the synergistic effect of ascorbic acid and 4-hexylresorcinol did not bleach the fruit tissue resulting in fresh-like apperance of the cut apple slices. The treatment also reduced the total microbial counts by more than ten fold as compared to the control apple slices. Moreover, the apple slices treated with combination of ascorbic acid and 4-hexylresorcinol had a slightly firmer texture compared to the control and no adverse effects on the total soluble solids and titratable acidity of the apple slices was observed. Overall the authors suggested that the combination of ascorbic acid and 4-hexylresorcinol is a promising anti-browning treatment for minimally processed apples.

Buta *et al.* (1999) tested the combinations of enzymatic inhibitors (4-hexylresorcinol, from 0.0005 to 0.005 M), reducing agents (ascorbic acid or isoascorbic acid, from 0.1 to 0.5 M), sulfhydryl compounds (*N*-acetylcysteine,

cysteine hydrochloride, homocysteine and *S*-carbamylcysteine from 0.01 to 0.1 M), calcium salts (lactate, chloride, glucoheptanoate, tartrate and propionate from 0.025 to 0.1 M) and antimicrobial compound (benzoate, from 0.005 to 0.05 M), containing calcium to extend storage life, retard the enzymatic browning of slices of ‘Red Delicious’ apple stored at 5°C and 10°C. The apple slices treated with the combinations of anti-browning compounds maintained higher level of sugars and organic acids at 5 ºC and 10 ºC.

Kaur and Kapoor (2000) tested the different browning inhibitors like 4-hexylresorcinol, ascorbic acid and banana leaf extract either alone or in different combinations on apples (cv. ‘Royal Delicious’), potatoes and mushrooms. The obtained results were compared with sulphite treated samples, which are banned due to their harmful effects. It was reported that a mixture of 4-hexylresorcinol, ascorbic acid and banana leaf extract significantly inhibited the enzymatic browning during storage at 0 ºC for four weeks and the activity of polyphenol oxidase was retarded. The firmness and colour of treated samples were comparable to the fresh samples. The total viable microbial, mould and yeast were reduced. Overall, the results confirmed that the effectiveness of these tested formulation was found to be better than sulphites treatment.

Tortoe *et al.* (2007) studied the efficacy of ascorbic acid, citric acid, calcium chloride, sodium ascorbate, sodium chloride and cysteine, alone or in combinations for prevention of browning and decay on fresh-cut ‘Golden Delicious’ apple, at 4 ºC and 10 ºC stored for a period of 14 days. The apple slices treated with ascorbic acid and citric acid alone showed traces of browning at 4 ºC after 7 days of storage. Only ascorbic acid and ascorbic acid + sodium chloride samples had moderate browning while all other samples got heavily discoloured. It was observed that application of ascorbic acid alone was effective in minimizing browning at 10 ºC, although it was minimal after fourteen days of storage. Enzymatic browning was less severe at 4 ºC than 10 ºC in all treatments. Microbial decay was observed only in samples treated with 0.025 M cysteine, 0.25 M citric acid and the combination of 0.025 M cysteine and 0.25 M citric acid at 10 ºC after 14 days of storage in addition to the control samples which showed decay at both temperatures (4 and 10 ºC).

Bieganska-Marecik and Czapski (2007) treated the minimally processed apple slices of ‘Jonagold’ cultivar with solutions containing browning inhibitors (citric acid, ascorbic acid, sodium chloride, calcium chloride, 4-hexylresorcinol, sucrose, sodium lactate and calcium lactate). Apple slices treated with 0.005 % 4-hexylresorcinol, 1 % ascorbic acid, 0.5 % calcium chloride, 20 % sucrose effectively retarded the browning and tissue softening of apple slices during storage.

Zuo *et al.* (2008) treated apple cultivar ‘Fuzi’ with ascorbic acid (0.1 and 0.5 %), citric acid (0.1 and 0.5 %) and their combinations (0.1 % ascorbic acid + 0.5 % citric acid, 0.5 % ascorbic acid+0.1 % citric acid and 0.5 % ascorbic acid + 0.5 % citric acid). Reduction in lightness was observed in all treated samples stored at 4 ºC and 90 % RH for 9 days. In contrast, a*, b* and chroma values increased during the storage. The authors reported 0.5 % ascorbic acid, 0.5 % ascorbic acid + 0.5 % citric acid and 0.5 % ascorbic acid + 0.1 % citric acid to be most effective treatment for control of browning.

Aguayo *et al.* (2010) dipped fresh-cut ‘Braeburn’ apple slices in calcium ascorbate solution (0, 2, 6, 12 and 20 %, w/w) for 2 min and stored in air for 28 days at 4 ºC. The changes in antioxidant were measured using ferric reducing ability of plasma (FRAP), free radical scavenging activity (by DPPH), polyphenolic content and ascorbic acid content. Changes in sensory quality, browning and microbial counts were analyzed to indicate eating quality. The ascorbic acid content decreased by more than 50 % in calcium ascorbate treated slices (6, 12 and 20 %), Similar patterns were observed for FRAP and DPPH values. Control and 2 % calcium ascorbate treated slices stored in air showed poor sensory quality, high incidence of browning and microbial deterioration thus resulting in a short shelf life (<7 days) of the cut slices. However, apples slices dipped in 6, 12 and 20 % calcium ascorbate, packaged in air had a shelf life of 21–28 days.

Chiabrando and Giacalone (2011) investigated the effect of 1-MCP (1 µL/L), ascorbic acid (1 %, w/v) + calcium chloride (1 %, w/v), citric acid (1 %, w/v) + calcium chloride (1 %, w/v) towards inhibition of browning in fresh-cut apples of cultivars ‘Golden Delicious’, ‘Scarlet Spur’ and ‘Granny Smith’. The results demonstrated that 1 % citric acid + calcium chloride and 1 % ascorbic acid + calcium chloride treatments were effective inhibitors of polyphenol oxidase activity for all cultivars.

Keenan *et al.* (2012) assessed different apple cultivars, namely, 'Gala', 'Granny Smith', 'Alwa', 'Gloster', 'Topaz', 'Shampion', 'Shampion MCP', 'Ariwa', 'Jonica', 'Idared', 'Cortland', 'Braeburn', 'Bramley' and 'Rajka' for suitability to prepare ready-to-eat products. They selected the cultivars on the basis of physico-chemical and sensory attributes in response to processing. Apple wedges were dipped in a browning inhibitor (Natureseal, 6 % w/w for 2 min), vacuum packaged and stored at 4 ºC. Among all the cultivars studied by them, 'Gala' and 'Elstar' scored highest for sensory acceptability.

Li *et al.* (2015) tested sodium chloride (6 g/L) and potassium ascorbate, sodium ascorbate, calcium ascorbate and ascorbic acid as anti-browning agent (ascorbate concentration ranges from 1.2 to 40 g/L to 0.2 mol/l) to inhibit the development of enzymatic browning on the surface of minimally processed apple slices (cv. Granny Smith). The calcium, sodium and potassium ascorbate were equally effective in inhibiting enzymatic browning. The solution of 6 g/L sodium chloride requires only half the concentration of ascorbate to maintain the same postharvest life of minimally processed apple slices and the sodium chloride had a very low cost that means only half of the cost of ascorbate.

2.5 Effect of Edible Coatings on Quality Changes of Minimally Processed Apple Slices

Olivas *et al.* (2007) investigate the efficacy of alginate edible coatings, namely, alginate, alginate-butter-linoleic acid and alginate acetylated monoglyceride-linoleic acid, to preserve the quality of fresh cut apple slices of 'Gala' cultivar stored at 5 ºC and 85 % relative humidity. Overall, the results confirmed that alginate edible coatings extended the shelf-life of minimally processed apples without causing anaerobic respiration. All coatings were effective in reducing the weight loss during storage. The texture of the apple slice was not influenced by coatings, while untreated apple slices showed a drastic dceline in texture during storage. The coatings also reduced the enzymatic browning of 'Gala' slices during storage.

Ergun and Satici (2012) investigated the effects of aloe vera coating (0, 1, 5 and 10 % w/v) on red-coloured 'Red Chief' and green-coloured 'Granny Smith' apples stored for six months at 2°C. Aloe vera coating substantially retarded the increase in weight loss for 'Granny Smith' cultivar but did not affect weight loss for cultivar 'Red Chief'. The coating also retarded the green colour loss for cultivar 'Granny Smith' but was not effective for cultivar 'Red Chief'. The total soluble solid and titratable acidity was higher for 'Granny Smith' treated with aloe vera (5 and 10 %).

2.6 Synergistic Effect of Edible Coatings and Anti-Browning Agents on Quality of Fresh-cut Apple Slices

Lee *et al.* (2003) investigated the effect of edible coatings in combination with anti-browning agents (0.5 g/100 mL carrageenan + 1 g/100mL ascorbic acid + 0.02 g/ 100mL oxalic acid, 0.5 g/100mL carrageenan + 1 g/100mL ascorbic acid + 0.5g/ 100mL citric acid, 5 g/100mL WPC + 1g/100mL ascorbic acid + 0.02 g/100mL oxalic acid and 5 g/100mL WPC + 1g/100mL ascorbic acid + 1g/100mL calcium chloride) on apple slices (cv. 'Fuji') during storage at 3 ºC for two week. The results confirmed that the edible coatings with the anti-browning agents effectively prolonged the shelf life of minimally processed apple slices by two week when packed in trays and stored at 3 ºC. The edible coatings in combination with different anti-browning agents effectively maintained the colour of slices during storage and the addition of calcium chloride significantly retarded the loss of firmness during storage. These treatments also showed positive sensory analysis results and a reduction of microbial count. Whey protein concentrate (5 g/100 mL) containing ascorbic acid (1 g/ 100 mL) + calcium chloride as anti-browning agents (1 g/100 mL), was the most effective preservation treatment in terms of sensory quality after two week.

Chauhan *et al.* (2011) soaked apple slices in a solution containing citric acid (200 ppm), ascorbic acid (200 ppm) and sodium benzoate (200 ppm) for 10 min followed by coating with edible coatings made up of shellac and aloe gel, separately and/or in combination. They reported significant reduction in respiration rate, ethylene synthesis and electrolyte leakage rate in coated samples. Polyphenol oxidase (96.1 U/g/min) and peroxidise activity (211.2 U/g/min) were minimum in aloe gel treated samples followed by the slices coated with combination of shellac + aloe gel (98.1 and 221.5 U/g/min) and shellac alone (105.7 and 230.7 U/g/min). The control samples showed maximum values for polyphenol oxidase (122.8 U/g/min) and peroxidise activity (288.5 U/g/min). The coated samples also showed lesser changes in values for firmness, L*, a* and b* and microbiological during storage at 6 ± 1 ºC for 30 days.

Qi *et al.* (2011) studied the effect of edible coatings in combination with the anti-browning compounds (1 % chitosan, 2 % ascorbic acid + 0.5 % $CaCl_2$ and 2 % ascorbic acid + 0.5 % $CaCl_2$ + 1 % chitosan) on minimally processed apple slices for 8 days at 5 ºC. The enzymatic browning and tissue softening were effectively controlled by chitosan (1 %) + ascorbic acid (2 %) + 0.5 % calcium chloride.

Pilon *et al.* (2013) studied the effect an anti-browning solution and with a chitosan edible coating on the quality of fresh-cut apple (cv. 'Gala'). The fruits treated with 1 % ascorbic acid + 2 % citric acid + chitosan maintained a better colour upto six days of storage, similar to those treated with 1 % ascorbic acid + 2 % citric acid without chitosan. The rate of CO_2 production was slightly reduced during storage in all samples. The researchers reported that application of edible coating was most effective in inhibiting the growth of moulds and yeasts (1.7 CFU/g). The coliforms and *Salmonella* were not detected throughout the study.

Song *et al.* (2013) applied the aloe vera edible coating in conjunction with an anti-browning agent to preserve the quality of minimally processed apple slices during storage for 16 days at 4 ºC. The minimally processed apple slices coated with the aloe vera showed delayed enzymatic browning and decreased weight loss and softening compared to the untreated samples. The aloe vera edible coating containing 0.5 % cysteine was most effective in delaying enzymatic browning and the reduction of microbial load.

Altisent *et al.* (2014) studied the effect of browning inhibitors on fresh-cut slices of six apple cultivars ('Golden Smoothee', 'Modì', 'Ariane', 'Granny Smith', 'Fuji Kiku 8'and 'Pink Lady'). The apple slices were treated with ascorbic acid solution (1 and 2 %), ascorbic acid (1 %) + citric acid solution (1 %), NatureSeal solution (4 %), PomFresh (2 and 6 %) solution and water as a control. The treated apple slices were packed in polypropylene trays and stored for 7 days at 4 ºC. As whole apples, it was found that the improved cultivars (Modi, Fuji Kiku 8, Ariane and Pink Lady) were notable for their sensory characteristics. Cultivars 'Modì' and 'Ariane' presented the highest content of total phenols and ascorbic acid. Cultivars 'Modì' and 'Fuji Kiku 8' showed high suitability for minimal processing with good sensory quality even after 7 days of storage. NatureSeal antioxidant treatments demonstrated the best results in terms of visual assessment, sensory quality and physicochemical parameters.

2.7 Effect of Combined Methods on Quality of Fresh-cut Apple Slices

Jang and Moon (2011) investigated the effects of ascorbic acid and ultrasound on changes of peroxidase and polyphenol oxidase activity of fresh-cut apple (cv. 'Fuji') during storage for 12 days at 10 ºC. Combined treatment of ascorbic acid and ultrasound was found most effective for inactivating peroxidase, monophenolase and diphenolase while the individual treatment of ascorbic acid or ultrasound had limited inhibitory effect on the enzymes.

Huque *at al.* (2013) demonstrated that 'Granny Smith' apple slices treated with nitric oxide gas (10 µL/L) had retarded enzymatic browning during storage at 5 ºC. The samples showed inhibition of polyphenol oxidase activity, lower level of total phenols, reduced rate of respiration and reduced ion leakage but no significant effect on the ethylene production and lipid peroxide level and hydrogen peroxide levels was observed as a result of the treatment.

2.8 Extension of Shelf Life of Minimally Processed Apple Slices by Modified Atmosphere Packaging

Gunes and Hotchkiss (2002) evaluated the effects of reduced oxygen and elevated carbon dioxide and abusive temperatures on the survival and growth of *E. coli* O157:H7, molds and yeast and the visual changes in quality of minimally processed apples (cv. 'Delicious'). High carbon dioxide (>15 %) and low oxygen (<1 %) inhibited the growth of microorganisms on apple slices at 15 ºC and 20 ºC. However, pathogen population increased by 1 log cycle after two weeks of storage at normal atmospheres. The modified atmosphere also resulted in reduced browning and better appearance of the apple slices in comparison to the samples stored in air and ambient carbon dioxide atmospheres. Thus, the authors concluded that *E. coli* O157:H7 may grow on minimally processed apple slices in air but are inhibited in modified atmospheres with high concentration of carbon dioxide and abusive temperature.

Cocci *et al.* (2006) investigated the effect of anti-browning solution (1 % ascorbic acid and 1 % citric acid) and modified atmosphere packaging (5 % O_2, 5 % CO_2 and 90 % N_2O) on functional properties of fresh cut apples (cv. 'Golden Delicious') during eight days of storage at 4 ± 1 ºC. Treated samples retained twenty fold higher ascorbic acid content in comparison to the control at the time of initial storage that remained highest until 6th day of storage. Moreover, the anti-browning treatment resulted in an increase in the antioxidant activity of apple slices, while modified atmosphere had a negative effect on the antioxidant activity levels. Positive correlation was observed between ascorbic acid and total phenol, the total phenol being higher in treated samples than control during the storage due to the reducing action of ascorbic acid that prevented total phenol degradation. Synergistic effect of modified atmosphere and anti-browning agents on the physical and chemical attributes (colour and texture) of the apple slices was thus evident by the study.

3. MATERIALS AND METHODS

The present investigation entitled **'Development of minimally processed apple slices by hurdle technology'** was undertaken in the Division of Food Science and Postharvest Technology at ICAR-Indian Agricultural Research Institute, New Delhi during 2014-19. Analytical facilities were availed from the Divisions of Food Science and Postharvest Technology, Soil Science and Agricultural Chemistry, Agricultural Chemicals and Microbiology, IARI, New Delhi. The treatment details and methodology for execution of these studies have been presented in this chapter.

3.1 Experimental Materials and Chemicals

The apple cultivars were harvested at commercial maturity in the month of August-September, from Regional Research Station, Dr. YSPUH&F, Seobagh, Kullu (Himachal Pradesh) (Plate 1) and Chatani, Kullu. The aloe vera plant was procured from a nursery near IARI campus and used for aloe vera gel coating preparation. Chemicals used for the study were procured from Sigma, Merck and SRL Technologies, India.

To fulfill the objectives, four experiments were conducted. The details of the experiments are discussed below.

3.2 Evaluation of Nutritional Composition of Different Apple Cultivars Grown under Indian Conditions

For conducting this experiment, twenty two apple cultivars i.e. five non-red viz., 'Golden Delicious', 'Granny Smith', 'Winter Banana', 'Goldspur' and 'Stark Spur Golden' and seventeen red viz., 'Royal Delicious', 'Top Red', 'Oregon Spur-II', 'Starkrimson', 'Well Spur', 'Red Chief', 'Super Chief', 'Red Gold', 'Royal Gala', 'Scarlet Spur-II', 'Scarlet Gala', 'Early Red-I', 'Gale Gala', 'Spartan', 'Vance Delicious', 'Silver Spur' and 'Red Delicious' were procured from Regional Research Station, Dr. YSPUH&F, Seobagh, Kullu, Himachal Pradesh (Plate 4 to 9). The orchard is located at a height of 1000-1400 msl (31°58'56" longitude and 77°7'48" latitude). The apples were harvested at commercial maturity. After harvesting and sorting, the fruits were packed in cartons, transported to New Delhi within 24 h and stored at 2°C and 80 - 90 % relative humidity (Plate 2 and 3) at the Division of Food Science and Postharvest Technology, ICAR-Indian Agricultural Research Institute, for further observations. The fruits were evaluated for physical, physiological and nutritional attributes.

3.3 Evaluation of Nutritional Composition of Apple Cultivars Grown at Different Altitudes

For conducting this experiment, three cultivars, namely, 'Golden Delicious', 'Royal Delicious', 'Red Gold', were harvested at commercial maturity from orchards located at two different altitudes (1000-1400 msl and 1500-1800 msl) from Seobagh (1000-1400 msl) and Chatani (1500-1800 msl), Kullu, Himachal Pradesh. After harvesting and sorting, the fruits were packed in cartons, transported to New Delhi within 24 h, and stored at 2°C and 80 - 90 % relative humidity at the Division of Food Science and Postharvest Technology, ICAR-Indian Agricultural Research Institute, New Delhi for further observations.

3.4 Synergistic Effect of Edible Coatings and Anti-Browning Agents on Quality of Minimally Processed Apple Slices

For execution of this experiment, freshly harvested apples of the commercial cultivar 'Royal Delicious' were procured from Seobagh, Kullu, Himachal Pradesh (Plate 10). After washing thoroughly with water, the apples were cored and sliced using a hand operated apple slicer (Plate 11) followed by treatment with anti-browning agents and edible coatings alone as well as in combination. The anti-browning agents used were:

1. 4-hexylresorcinol (0.01 %) + Ascorbic acid (0.5 %) + $CaCl_2$ (0.2 %)
2. Ascorbic acid (1 %) + $CaCl_2$ (1 %)
3. Cysteine (0.1 %)

The edible coatings applied on the apple slices were:

1. Carboxymethyl cellulose (CMC)
2. Aloe vera gel (AVG)

Preparation of aloe vera edible coating: Aloe vera leaves were procured from a nursery near IARI campus. After washing with tap water, the leaf peel was removed. The inner transparent tissues were blended and then filtered (Fig. 1. The pure aloe vera homogenates were diluted with distilled water to get the desired concentrations of 5 %, 10 %, 15 %, 20 %, 25 %, 50 %, 75 % and 100 % (w/v). Based on preliminary

Plate 1. View of the apple orchard at Seobagh, Kullu, Himachal Pradesh

Plate 2. Storage of harvested apples in cold room

Plate 3. Harvested apple fruits

Plate 4. Variation in apple cultivars studied

‘Scarlet Spur Red Delicious’

‘Red Chief’

‘Well Spur’

‘Stark Spur Golden’

Plate 5 Harvested fruits of different apple cultivars

'Super Chief'

'Vance Delicious'

'Starkrimson'

'Gale Gala'

'Gold Spur'

'Scarlet Gala

Plate 6 Harvested fruits of diferent apple cultivars

,


'Golden Delicious' **'Red Gold'**

'Silver Spur' **'Sparton' cultivars**

'Early Red One' **'Royal Gala'**

Plate 7 Harvested fruits of different apple cultivars

'Oregon Spur II' **'Winter Banana'**

'Top Red' **'Royal Delicious'**

'Granny Smith' **'Golden Delicious'**

Plate 8 Harvested fruits of different apple cultivars

‘Scarlet Spur Red Delicious’

‘Royal Delicious’

‘Golden Delicious’

‘Granny Smith’

‘Red Gold’

Plate 9. Harvested fruits of different apple cultivars

Plate 10. Harvested fruits of apple cultivar 'Royal Delicious'

Plate 11. Slicing of apple using apple slicer

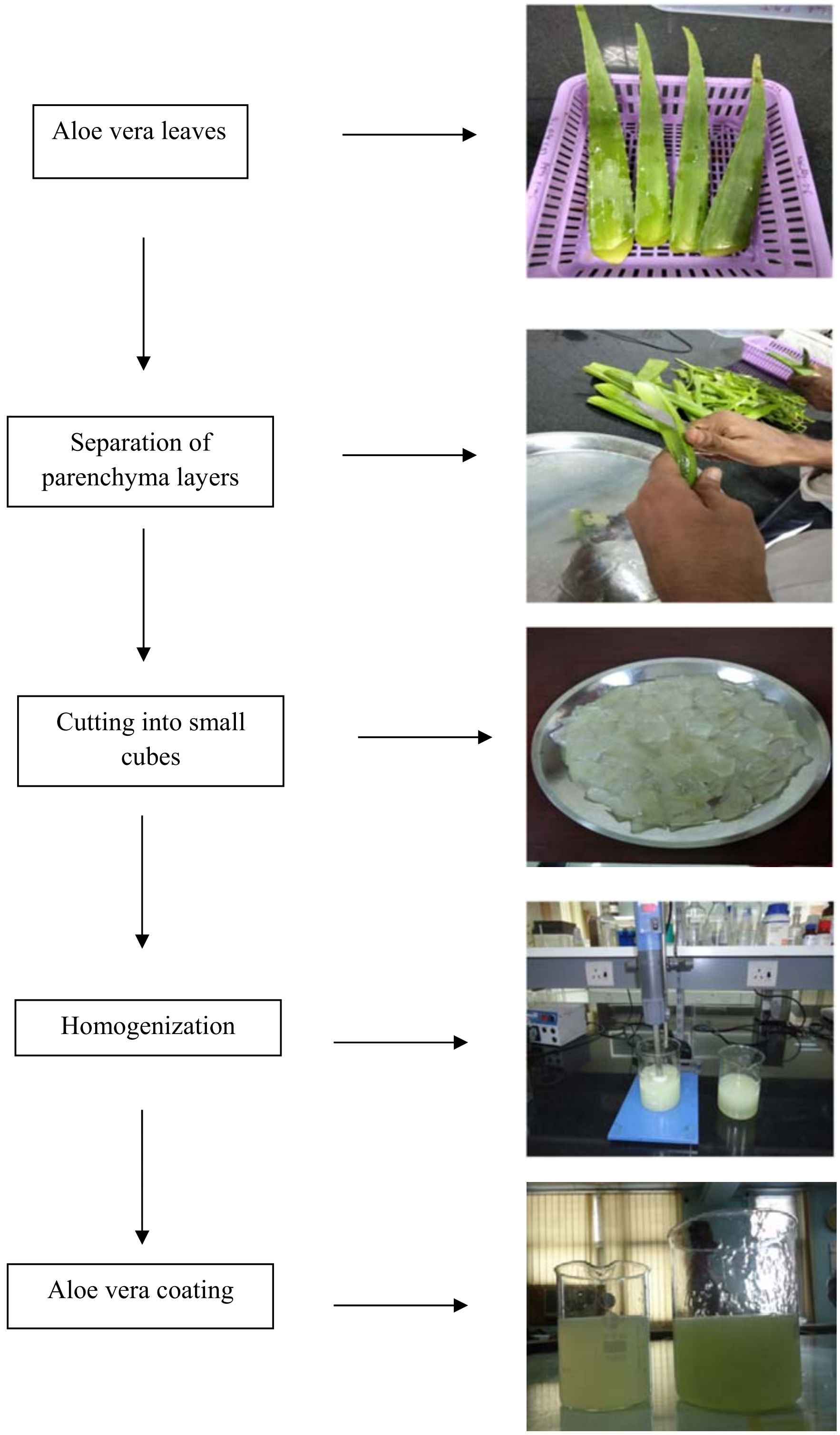

Fig. 4.1 Flow sheet of aloe vera coating preparation

sensory acceptability studies, the threshold for sensory acceptance was established at 15 % due to a bitter taste that was perceived at higher concentrations.

Preparation of CMC coating: Carboxymethyl cellulose coating was prepared by solubilizing carboxymethyl cellulose powder (1 g per 100 ml) in distilled water at 75 °C under magnetic stirring for 30 min.

The treatment combinations of browning inhibitors and edible coatings for apple slices were as follows:

Treatment	Components
T_0	Distilled water (control)
T_1	1 % ascorbic acid + 1 % $CaCl_2$
T_2	0.01 % 4-hexylresorcinol + 0.5 % ascorbic acid + 0.2 % $CaCl_2$
T_3	0.1 % cysteine
T_4	15 % AVG
T_5	15 % AVG + 0.01 % 4-hexylresorcinol + 0.5 % ascorbic acid + 0.2 % $CaCl_2$
T_6	15 % AVG + 1 % ascorbic acid + 1 % $CaCl_2$
T_7	15 % AVG + 0.1 % cysteine
T_8	1 % CMC
T_9	1 % CMC + 0.01 % 4-hexylresorcinol + 0.5 % ascorbic acid + 0.2 % $CaCl_2$
T_{10}	1 % CMC + 1 % ascorbic acid + 1 % $CaCl_2$
T_{11}	1 % CMC + 0.1 % cysteine

The treated as well as control samples were immersed for 5 min. in cold solution of the anti-browning agent/ edible coating/ water (Plate 12) (Fig. 2). The excess of solution was drained off followed by air-drying of the slices (Plate 13). Slices were packaged in polypropylene containers, shrink wrapped (Plate 14) and stored under 5 ± 2°C. The apple slices were analyzed every day for a period of seven days for different quality attributes as detailed in section 3.6.

3.5 Effect of Modified Atmospheres on Quality of Minimally Processed Apple Slices

For conducting modified atmosphere packaging (MAP) studies of apple slices, the fruits of cultivar 'Royal Delicious' were washed, cored and sliced using a hand-operated apple slicer. The minimally processed apple slices were immediately immersed into a cold solution of 4-hexylresorcinol (0.01 %) + ascorbic acid (0.5 %) + $CaCl_2$ (0.2 %) for 5 min. The excess solution was drained and the apple slices were air-dried. All the treated slices were packed in polypropylene trays (150 x 116 x 37 mm), flushed with the set modified gas compositions and sealed with a polyamide film (Plate 15) using a modified atmosphere packaging machine (Dansensor, Germany). The modified atmosphere conditions were: air (T_0), O_2: 1.5 %, CO_2: 2.5 % (T_1), O_2: 1.5 %, CO_2: 5 % (T_2), O_2: 1.5 %, CO_2: 7.5 % (T_3), O_2: 3 %, CO_2: 2.5 % (T_4), O_2: 3 %, CO_2: 5 % (T_5), O_2: 3 %, CO_2: 7.5 % (T_6), O_2: 5 %, CO_2: 2.5 % (T_7), O_2: 5 %, CO_2: 5 % (T_8), O_2: 5 %, CO_2: 7.5 % (T_9). The sealed trays were stored at 5 ± 2 °C up to 42 days and samples were analyzed for various quality parameters. Three replicate packets were used for each treatment per storage interval. The sealed trays were stored at 5 ± 2 °C up to 42 days and samples were analyzed for various quality parameters as detailed in section 3.6.

3.6 Observations Recorded and Methodology

3.6.1 Fruit weight and dimensions

Fruit weight was recorded with the help of an electronic balance. Measurement of the fruit dimensions of different cultivars of apple was done using verniercalliper (Mityoto, Japan). To determine the average size of the fruits, three linear dimensions, namely length (L) - equivalent distance of the stem to the calyx, width (W) - the longest dimension perpendicular to L, and thickness (T) - the longest dimension perpendicular to L and W, were measured. The geometric mean diameter (D_g) and arithmetic mean diameter (D_a) were calculated using the following equations (Mohsenin, 1986):

$$D_g = \sqrt[3]{LWT}$$

$$Da = (L + W + T)/3$$

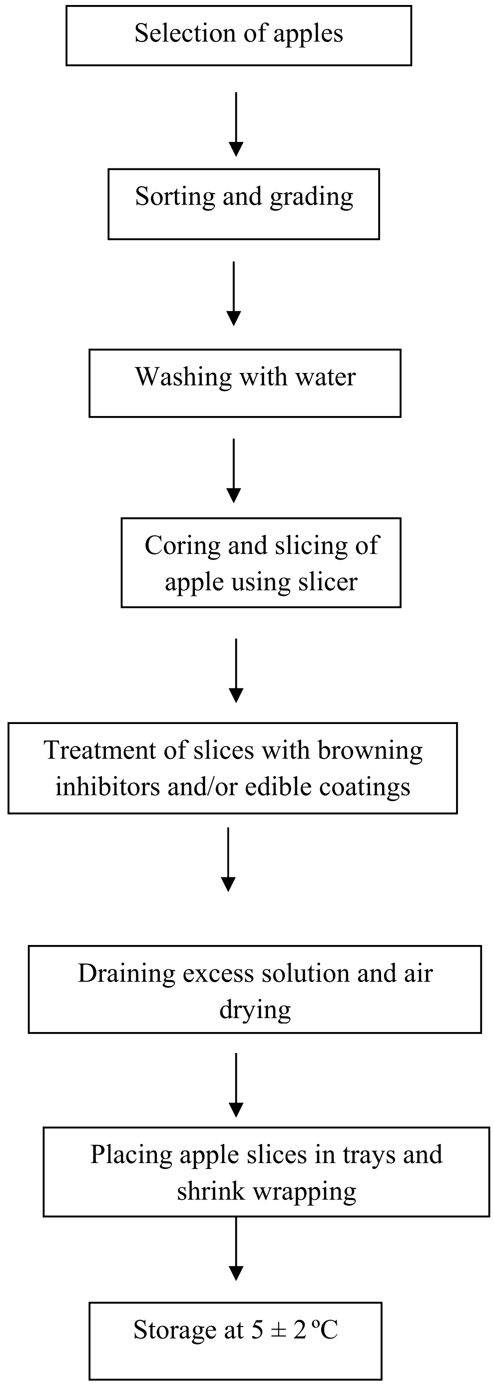

Fig. 4.2 Flow sheet for minimal processing of apple slices

Plate 12. Dipping of apple slices in anti-browning solution

Plate 13. Air drying of treated apple slices

Plate 14. Apple slices in shrink wrapped trays

Plate 15. Modified atmosphere packed apple slices

3.6.2 Physiological loss in weight (PLW)

For the measurement of physiological loss in weight, the samples were weighed during storage at regular intervals with the help of an electronic balance. Physiological loss in weight percentage was calculated by recording the difference in weight from the initial value as follows:

$$WL = \frac{(IW - FW)}{(IW)} \text{ x } 100$$

where, WL is the weight loss (%), IW, is the initial weight (g) and FW is the final weight (g) on sampling.

3.6.3 Fruit firmness

Fruit firmness was determined using a texture analyzer (model: TA+Di, Stable micro systems, UK) and was defined as maximum force during puncture by 2 mm probe, which was expressed in Newtons (N).

3.6.4 Peel colour

Peel colour was determined using portable colorTec PCM. The colour value was expressed as L*, a* and b* values where L* is a measure of lightness, positive values of a* indicate redness and negative values complement green. Positive values of b* are the vector for yellowness and negative for blueness. The browning index (BI), used as an indicator of intensity of brown color, was calculated by the following formula:

$$BI = [100(x - 0.31)]/0.172$$

where, $x = (a* + 1.75\, L*) \,/\, (5.646\, L* + a* - 3.012\, b*)$

Numerical values of L*, a* and b* were also used to calculate whiteness index (WI) as follows:

$$WI = 100 - [(100 - L*)^2 + a*^2 + b*^2]½$$

3.6.5 Respiration rate

Respiration rate (expressed as ml CO_2 kg^{-1} h^{-1}) in the intact/sliced apples was measured by using auto gas analyzer (Model: Checkmate 9900 O_2/ CO_2, PBI Dansensor, Denmark). For intact apple, two fruits were placed in 1000 ml airtight containers having twist-top lid fitted with a silicone rubber septum at the center of the lid while for the sliced apples the respiration rate was directly measured in the packaging trays. The containers were kept at 25 °C for 1h for accumulation of respiratory gases in the headspace. After specified time, the headspace gas was sucked to the sensor of analyzer through the hypodermic hollow needle and the revealed value of evolution rate of CO_2 concentration (%) was noted. Rate of respiration was calculated on the basis of evolution of carbon dioxide from the sample per unit weight per unit time using the following formula:

$$\text{Respiration rate (ml } CO_2 \text{ kg}^{-1} \text{ h}^{-1}) = \frac{CO_2 \text{ (\%) x Head space}}{\text{100 x Weight (kg) x Time (h)}}$$

3.6.6 Total soluble solids

The total soluble solids of apples were measured using ATAGO hand refractometer (0 - 93ºB). The results were expressed as % at 20ºC (AOAC, 1990).

3.6.7. Titratable acidity

For estimation of titratable acidity, five gram of apple sample was weighed, crushed and diluted to final volume of 100 ml by distilled water. The sample was filtered and the filtrate was titrated with 0.1 N sodium hydroxide using phenolphthalein indicator. The obtained titre value was used for the calculating the values as percent acidity (as malic acid) by using the following formula Ranganna (1999):

$$\text{Titratable acidity (\%)} = \frac{\text{Titre value x N of alkali x Vol. made up x Eq. wt. of acid x 100}}{\text{Vol. of sample taken for estimation x Wt. of sample taken x 1000}}$$

3.6.8 Total antioxidant activity

Total antioxidant activity in the apple samples was estimated by following the cupric reducing antioxidant capacity method (Apak *et al.,* 2004). It determined the copper (II) or cupric ion reducing ability of polyphenols. For determining the antioxidant activity, copper (II) chloride solution, a neocuproine alcoholic solution and an ammonium acetate aqueous buffer (pH 7.0) were mixed together and then measurement of the developed color was taken after thirty minutes in a spectrophotometer at absorbance of 450 nm. The antioxidant activity of the samples was expressed as μmol Trolox g^{-1}.

3.6.9 Ascorbic acid content

Ascorbic acid content was determined as per method of Ranganna (1999). Five gram of homogenized apple sample was taken and final volume was made up to 100 ml with 3 % meta-phosphoric acid followed by filteration. Ten milliliter of the aliquot was taken in a conical flask and titrated against standardized dye. The ascorbic acid content of the apple samples was calculated with the following formula:

$$\text{Ascorbic acid (mg/100 g)} = \frac{\text{Titre value x Dye factor x Vol. made up x 100}}{\text{Aliquot of extract x Volume of sample taken}}$$

3.6.10 Total phenols

Two gram of apple slice sample was crushed in ten ml of 80 % ethanol. The homogenate was centrifuged at 15,000 rpm for twenty min at 4°C and supernatant collected which was used for assay of total phenols. An aliquot (0.1 ml) of the sample was added to 2.9 mL distilled water and 0.5 ml of 1N folin-ciocalteau reagent followed by addition of 2 ml of 20 % of sodium carbonate. The mixture was kept in incubation for 30 min and absorbance was recorded at 760 nm. The total phenolic content of the sample was expressed in mg of gallic acid equivalents (GAE) / 100 g of extract (Singleton and Rossi, 1965).

3.6.11 Sugars and organic acids

The sugars and organic acids were estimated by high performance liquid chromatography method of Kelebek *et al.* (2009). Standards of sugars and organic acids were purchased from Sigma-Aldrich. Waters high performance liquid chromatography consisting of binary pump model 515, 2414 refractive index and 2998 photodiode array (PDA) detector was used for all analysis. Sugars and organic acids in aqueous phase were quantified by using Aminex HPX-87H (Bio-Rad Laboratories, Hercules, CA) column operated with 5 mM H_2SO_4 as mobile phase at a flow rate of 0.5 ml/min and the oven temperature was kept at 50°C using both detectors in series (PDA @ 210 nm). The concentration of sugars and organic acids in the apple cultivars were expressed in g/L.

3.6.12 Phenolic compound profiling

Extraction of phenolics from fresh apples was carried out as per the procedure described by Wu *et al.* (2007). A 20 µl volume of each sample was manually injected into the Water Alliance HPLC System (Waters Chromatography, Milford, MA) attached with a photodiode array detector (PDA). C18 column (5 µm, 4.6 × 250 mm) was used to estimate the individual phenolic components. The HPLC components include e2695 separation module and the 2996 photodiode array detector and the system was operated with Empower 2 Software (Waters Corporation). The mobile phase consisted of solvent A (water 0.1 % formic acid), solvent B (acetonitrile 0.1 % formic acid) with gradient programming of 100 % A to 100 % B with total run time of 55 min. with the flow rate of 0.5 ml/min. The phenolic acid peaks were detected at 280 nm and expressed as ml/L.

3.6.13 Macro and micro elements

For estimation of macro and micro elements, fruit sample (1 g) was digested in a microwave digestion system (Anton Par: Multiwave ECO) with concentrated nitric acid (Suprapur grade, Merck, Germany). The digested samples were transferred to 100 ml volumetric flask to make up the dilution volume. The element concentrations were analyzed using ICP-MS platform with auto-sampling protocol (Perkin Elmer, Model: NexION 300 ICP-MS) and computed as mg/100g for macro elements and µg/g for micro elements.

3.6.14 Fatty acid characterization

FAMEs (fatty acid methyl esters) were developed according to transesterification method. The fatty acids were methylated after dissolving the sample in methanol (2 ml) followed by the addition of few drops of conc. H_2SO_4. The corresponding FAMEs were extracted with hexane by adding salt solution (10 ml) for complete recovery. GC-MS analysis of the prepared sample was carried out using 7890A GC (Agilent Technologies) equipped with a HP-5MS column (30 m × 0.25 mm 0.25 µm, Agilent Co., USA) which was directly connected to a triple axis HED-EM 5975C mass spectrometer (Agilent Co., USA) as per the method described by Wu *et al.* (2007). The injection volume was 1 µl with flow mode in split control. The carrier gas flow was set at 1 ml/min helium (High purity, New Delhi, India) at a head pressure of 10 psi. The oven temperature was initially held at 40°C for 1 min., hereafter which the temperature was raised with a gradient of 3°C/min until the temperature reached to 60°C and held for 10 min. Again the temperature was raised with a gradient of 2°C/min up to 220°C and held for 1 min. Finally, the temperature was raised up to 280°C with increment of 5°C/min. Total run-time was 111 min. Other settings included were 250°C interface temperature, 200°C ion source temperature and electron impact ionization at 70 eV. The fatty acid profile was represented as % relative area.

3.3.15 Polyphenol oxidase (PPO) activity

Polyphenol oxidase activity was determined according to the method of Augustin (1985) with minor modifications. Two grams of apple sample were taken in pre-chilled mortar and homogenised thoroughly with 10 mL of ice-cold extraction medium which consist of phosphate buffer (0.2 M; pH 6.8), PVP (15 mg g^{-1}) and triton X-100 (5 µg g^{-1}). The homogenate was centrifuged at 11000 rpm for 20 min at 2 ºC. Then, the supernatant was collected for enzyme activity determination.

The reaction mixture for the assay of polyphenol oxidase contains 0.5 ml of 0.05 M catechol, 0.1 ml enzyme extracts and 2.9 ml of 0.2 M phosphate buffer (pH 6.8). The rate of increase in absorbance at 410 nm was measured against the blank (prepared in the absence of enzyme) at every one minute up to 30 min. The enzyme activity was expressed as change in absorbance/min/g. The one unit of enzyme activity equals to 0.001 absorbance unit/min/g.

3.3.16 Peroxidase (POD) activity

The activity of peroxidase enzyme was spectrophotometrically measured according to the method of Gonzalez *et al.* (2000) with minor modifications. One gram of sample was homogenised with 3 mL of 0.1 M phosphate buffer (pH 7). The extracted homogenate was transferred to the ice-cold centrifuge tubes. The mixture was centrifuged at 11000 rpm at 5ºC for 30 min. Then, the extract was collected for enzyme activity determination. The peroxidase activity was determined spectrophotometrically at 436 nm.The assay mixture contained 0.1 mL of enzyme extracts, 3 mL of 0.1 M phosphate buffer (pH 7), 0.03 mL of hydrogen peroxide

(0.042 %) and 0.05 ml of guaiacol. The enzyme activity was defined as the change in absorbance per minute per gram of sample.

3.3.17 Microbiological evaluation

Microbial growth on the minimally processed apple slices was determined initially and after seven days of storage. Ten gram samples were blended with 90 mL of sterile peptone buffered water for 1min in a sterile stomacher bag using a masticator. Appropriate dilutions were prepared. Plate Count Agar medium was used for total plate count. Incubation was done at 30 ºC for 48 h (Plate 16). Microbial counts were reported as log_{10} colony forming units per gram of sample (log cfu g^{-1}).

3.3.18 Sensory evaluation

Sensory evaluation of the minimally processed apple slices (Plate 17) was done by score card method which ranges from 0 to 9 scales. The colour/ appearance, texture, taste, aroma, visual browning and overall acceptability were done by this method and the average values were taken on 9 point Hedonic scale (Ranganna, 1999).

3.4 Statistical analysis

Two-way analysis of variance was performed on the data sets using SAS 9.3 software and significant effects ($p < 0.05$) were noted. Significant difference amongst the means was determined by Tukey's HSD.

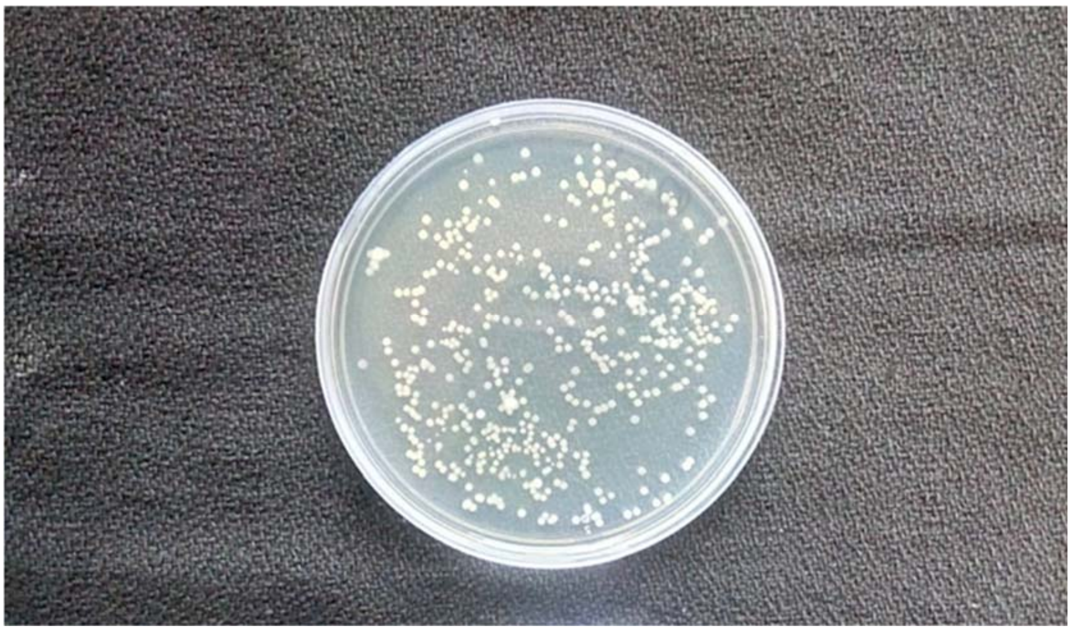

Plate 16. Total plate count of the shrink wrapped apple slices at end of storage

Plate 17. Sensory evaluation of whole and cut apples

4. RESULTS

Four experiments were planned and executed in the present study entitled '**Development of minimally processed apple slices by hurdle technology**'. The results obtained under each experiment are presented below under different subheads.

4.1 Evaluation of Nutritional Composition of Different Apple Cultivars Grown under Indian Conditions

4.1.1 Fruit weight and dimensions

A summary of the fruit weight and dimensions of twenty two apple cultivars studied is shown in Table 4.1. Among the twenty two apple cultivars red cultivar 'Oregon Spur II' recorded more than 200 g fruit weight (202.68 g). It also had maximum geometric mean (77.23 mm) and arithmetic mean diameter (77.46 mm). 'Starkrimson' cultivar had the least fruit weight (107.41 g), geometric mean (61.33 mm) and arithmetic mean diameter (61.74 mm).

4.1.2 Fruit Firmness

A variation in fruit firmness of the twenty two apple cultivars was recorded which ranged from 10.32 N in red coloured big sized fruits of 'Silver Spur' to 14.0 N in non-red coloured fruits of apple cultivar 'Winter Banana' (Table 4.1). The average fruit firmness of the twenty two apple cultivars was recorded to be 11.47 N.

4.1.3 Peel colour

Table 4.1 showed that the Gala series of cultivars recorded a higher intensity of red colour (a* values lies between 27.45 to 30.59), giving them an edge over sensory appeal in comparison to other cultivars. Few non-red apple cultivars such as 'Winter Banana' (L* = 74.87, b* = 39.70), 'Golden Delicious' (L* = 68.24, b* = 40.06) and 'Granny Smith' (L* = 61.72, = b* value = 41.17) revealed higher L* and b* values in comparison to the red coloured cultivars. Other varieties have shown varied Hunter a*/b* values, giving different shades of colour.

4.1.4 Soluble solid content

The SSC in the apple cultivars ranged from 10 °Brix ('Red Delicious') to 16.1 % ('Gale Gala'). The most popular and widely grown apple cultivars in India, 'Golden Delicious' (13.5 %) and 'Royal Delicious' (13.2 %) recorded good soluble solid content (Table 4.2).

4.1.5 Titratable acidity

A wide variation in titratable acidity was observed with higher values in all non-red cultivars. The maximum titratable acidity was recorded in non-red cultivars, 'Winter Banana' and 'Stark Spur Golden' (0.67 %) while minimum was observed in red cultivars, 'Royal Delicious', 'Red Gold', 'Early Red-I', 'Spartan' and 'Red Delicious' (0.27 %) (Table 4.2). Average titratable acidity of the twenty two cultivars was 0.39 %.

4.1.6 Ascorbic acid

Ascorbic acid varied significantly with the apple cultivar studied. The highest ascorbic acid content was recorded in red cultivars, 'Starkrimson' (32.08 mg $100g^{-1}$) and 'Oregon Spur II' (31.76 mg $100g^{-1}$) whereas the least ascorbic acid was recorded in 'Well Spur' (19.38 mg $100g^{-1}$) (Table 4.2).

4.1.7 Antioxidant (AOX) activity

There were significant differences in antioxidant activity of different apple cultivars, the highest being in red cultivar 'Silver Spur' (13.20 μmole Trolox equivalent g^{-1}) and the least in non-red cultivar, 'Granny Smith' (2.64 μmole Trolox equivalent g^{-1}) (Table 4.2).

4.1.8 Total carotenoid

The apple cultivar significantly varied in total carotenoid content, the maximum being in red cultivars, 'Red Chief' (147.06 mg kg^{-1}) and 'Oregon Spur II' (139.32 mg kg^{-1}). Amongst the non-red cultivars 'Golden Delicious' recorded the maximum total carotenoids (100.62 mg kg^{-1}) followed by cv. 'Winter Banana' (79.34 mg kg^{-1}) (Table 4.2).

4.1.9 Sugar profiling

Individual sugars varied significantly in twenty two apple cultivars studied (Table 4.3). The predominant monosaccharide was found to be fructose that ranged between 10.85 to 67.55 g L^{-1}. All analyzed apple cultivars showed a higher concentration of fructose as compared to sucrose and glucose. Sucrose was observed to be present in small amount with an average of 20.40 g L^{-1}. The highest content was

Table 4.1 Physical attributes of apple cultivars

Cultivar	Fruit weight (g)	Fruit dimension (mm)				Firmness (N)	Colour		
		Width	Length	Geometric mean diameter	Arithmetic mean diameter		L*	a*	b*
Non-red cultivars									
‘Golden Delicious’	133.40±1.84^{k}	69.71±1.51^{ih}	59.63±0.34^{g}	66.17±0.71^{hij}	66.35±1.22^{ihj}	11.25±0.08^{edf}	68.24±1.10^{c}	-8.76±0.03^{n}	40.06±0.48^{c}
‘Granny Smith’	175.00±3.07^{d}	73.86±0.80^{ed}	65.76±0.17^{d}	71.05±0.79^{c}	71.16±0.80^{d}	12.69±0.10^{b}	61.72±0.66^{fe}	11.40±0.05^{o}	41.17±0.37^{b}
‘Winter Banana’	131.24±0.21^{l}	68.24±1.08^{ijk}	59.08±0.60^{g}	65.04±1.17^{ikj}	65.19±0.63^{ikj}	14.00±0.24^{a}	74.87±1.09^{a}	-9.22±0.07^{n}	39.70±0.14^{c}
‘Goldspur’	131.42±1.38^{lk}	70.49±0.78^{ihg}	60.56±0.93^{feg}	67.01±0.76^{hifgj}	67.18±1.17^{igh}	10.90±0.08^{hgf}	69.36±0.94^{c}	-8.03±0.08^{m}	41.27±0.26^{b}
‘Starkspur Golden’	134.04±2.25^{k}	71.79±0.29^{ehgf}	62.48±0.61^{fe}	68.54±0.73^{dfge}	68.69±0.85^{eghf}	10.53±0.06^{hi}	72.15±0.82^{b}	-9.21±0.09^{n}	42.66±0.52^{a}
Red cultivars									
‘Royal Delicious’	165.32±2.02^{feg}	76.42±0.51^{cb}	68.21±0.82^{cb}	73.58±0.86^{b}	73.68±0.40^{c}	10.72±0.16^{hgi}	54.94±0.06^{h}	15.20±0.13^{i}	26.15±0.07^{i}
‘Top Red’	170.30±2.48^{d}	78.50±0.77^{b}	72.02±0.46^{a}	76.28±0.52^{a}	76.34±1.27^{ba}	12.64±0.05^{b}	49.46±0.39^{ji}	18.24±0.26^{hg}	21.79±0.27^{kj}
‘Oregon Spur II’	202.68±1.23^{a}	81.61±0.15^{a}	69.17±0.68^{b}	77.23±1.27^{a}	77.46±0.24^{a}	11.63±0.13^{d}	45.69±0.81^{k}	21.32±0.21^{f}	18.30±0.16^{nm}
‘Starkrimson’	107.41±1.70^{n}	66.61±0.51^{jk}	51.99±0.54^{h}	61.33±0.56^{l}	61.74±0.55^{l}	10.37±0.15^{i}	54.31±0.75^{h}	15.56±0.29^{i}	22.60±0.17^{j}
‘Well Spur’	168.82±1.99^{e}	72.50±0.25^{egf}	65.57±0.60^{d}	70.11±0.91^{dce}	70.19±1.42^{edf}	12.08±0.22^{c}	55.52±0.06^{h}	11.25±0.06^{j}	27.06±0.45^{h}
‘Red Chief’	145.72±0.84^{j}	73.09±1.69^{edf}	65.39±1.53^{d}	70.43±0.71^{dc}	70.52±0.44^{ed}	12.33±0.18^{cb}	47.89±0.85^{j}	18.00±0.12^{h}	20.79±0.06^{l}
‘Super Chief’	151.06±2.05^{i}	70.93±0.74^{hgf}	62.73±0.48^{e}	68.08±1.31^{hdfge}	68.20±0.26^{eghf}	11.09±0.15^{egf}	45.81±0.13^{k}	22.08±0.27^{e}	17.67±0.25^{n}
‘Red Gold’	159.66±1.30^{g}	73.17±0.95^{edf}	62.29±1.36^{fe}	69.35±0.78^{dfce}	69.54±0.39^{egdf}	10.92±0.17^{hgf}	62.95±1.12^{e}	7.32±0.09^{k}	32.02±0.25^{f}
‘Royal Gala’	195.82±4.10^{b}	72.86±0.72^{egf}	65.49±0.45^{d}	70.32±0.72^{dc}	70.40±1.55^{ed}	11.11±0.01^{egf}	63.19±0.32^{e}	27.45±0.19^{b}	29.83±0.21^{g}
‘Scarlet Spur-II’	163.52±1.43^{fg}	71.25±0.56^{hgf}	62.56±0.49^{fe}	68.23±0.68^{hdfge}	68.35±0.87^{eghf}	11.45±0.13^{ed}	44.33±0.64^{k}	24.39±0.39^{c}	18.89±0.14^{m}
‘Scarlet Gala’	134.96±1.36^{k}	69.77±0.51^{ih}	61.98±0.40^{fe}	67.07±0.72^{hifg}	67.17±0.78^{igh}	10.52±0.23^{hi}	60.87±0.83^{f}	27.94±0.34^{b}	33.42±0.21^{e}
‘Early Red-I’	120.68±1.01^{m}	65.92±0.39^{lk}	62.28±0.25^{fe}	64.68±0.69^{kj}	64.71±0.63^{kj}	11.04±0.13^{gf}	47.89±0.69^{j}	18.00±0.34^{h}	20.79±0.20^{l}
‘Gale Gala’	141.01±2.21^{k}	70.60±1.14^{ihg}	58.53±0.87^{g}	66.32±1.25^{higj}	66.58±1.07^{ihj}	11.46±0.09^{ed}	38.52±0.69^{m}	30.59±0.42^{a}	15.76±0.16^{o}
‘Spartan’	157.35±1.84^{h}	75.40±0.67^{cd}	60.64±0.95^{feg}	70.12±0.52^{dce}	70.48±0.16^{ed}	12.39±0.05^{cb}	65.33±0.51^{d}	-2.51±0.04^{l}	36.78±0.17^{d}
‘Vance Delicious’	166.48±1.96^{fe}	68.42±0.81^{ij}	66.94±1.38^{cd}	67.92±0.87^{hfge}	67.93±0.68^{ghf}	10.44±0.12^{i}	50.78±0.45^{i}	18.70±0.28^{g}	21.71±0.07^{k}
‘Silver Spur’	185.47±1.91^{c}	80.23±0.70^{b}	70.12±0.76^{d}	76.71±0.60^{b}	76.86±0.26^{bc}	10.32±0.12^{i}	41.8±0.61^{l}	23.55±0.21^{d}	13.79±0.29^{p}
‘Red Delicious’	142.00±2.99^{k}	64.13±0.95^{l}	60.37±0.60^{fg}	62.85±0.52^{lk}	62.88±0.55^{lk}	12.35±0.11^{cb}	58.17±0.63^{g}	11.57±0.19^{j}	30.16±0.66^{g}
Average	**154.04±1.11**	**71.98±0.48**	**63.14±0.45**	**68.88±0.48**	**69.03±0.48**	**11.47±0.08**	**56.08±0.41**	**11.91±0.13**	**27.83±0.17**

Results as mean ± SE of triplicate measurements. Means with same superscript are not significantly different

Table 4.2 Nutritional composition of apple cultivars

Cultivar	SSC (%)	Titratable acidity (% malic acid)	Ascorbic acid (mg 100 g^{-1})	Total antioxidant (µmole Trolox g^{-1})	Total carotenoids (mg kg^{-1})
Non-red cultivars					
'Golden Delicious'	13.50±0.20^{c}	0.54±0.00^{fe}	21.60±0.33^{h}	6.45±0.06^{h}	100.62±0.75^{d}
'Granny Smith'	12.00±0.08^{g}	0.54±0.00^{b}	20.00±0.17^{i}	2.64±0.02^{n}	32.90±0.11^{p}
'Winter Banana'	11.00±0.11^{h}	0.67±0.00^{a}	21.39±0.27^{h}	10.57±0.19^{c}	79.34±0.46^{h}
'Goldspur'	11.00±0.09^{h}	0.54±0.00^{c}	20.19±0.24^{i}	6.64±0.08^{g}	42.57±0.66^{n}
'Starkspur Golden'	12.50±0.22^{f}	0.67±0.00^{ba}	21.18±0.25^{h}	6.01±0.10^{ij}	44.51±0.15^{m}
Red cultivars					
'Royal Delicious'	13.20±0.17^{dc}	0.27±0.00^{j}	21.18±0.28^{h}	5.56±0.08^{kl}	46.44±0.60^{l}
'Top Red'	12.70±0.08^{fe}	0.54±0.00^{e}	25.37±0.08^{ed}	7.11±0.03^{g}	90.95±0.91^{e}
'Oregon Spur II'	12.60±0.02^{fe}	0.47±0.00^{hi}	31.76±0.70^{a}	7.35±0.12^{e}	139.32±1.24^{b}
'Starkrimson'	12.50±0.12^{f}	0.56±0.00^{i}	32.08±0.38^{a}	5.71±0.06^{kl}	46.44±0.96^{l}
'Well Spur'	12.50±0.09^{f}	0.59±0.00^{d}	19.38±0.28^{i}	5.97±0.09^{kj}	83.21±0.31^{g}
'Red Chief'	14.40±0.12^{b}	0.42±0.00^{k}	24.92±0.31^{e}	9.33±0.11^{d}	147.06±0.75^{a}
'Super Chief'	13.20±0.03^{dc}	0.52±0.00^{fe}	23.77±0.05^{f}	7.27±0.08^{f}	38.70±0.01^{o}
'Red Gold'	12.50±0.14^{f}	0.27±0.00^{hi}	19.82±0.32^{i}	2.77±0.02^{m}	48.38±0.46^{k}
'Royal Gala'	14.30±0.03^{b}	0.64±0.00^{j}	26.18±0.32^{d}	5.76±0.07^{kj}	52.25±0.32^{j}
'Scarlet Spur-II'	11.90±0.04^{g}	0.48±0.00^{h}	22.59±0.43^{g}	6.21±0.09^{ih}	108.36±1.15^{c}
'Scarlet Gala'	13.20±0.16^{dc}	0.51±0.00^{l}	25.20±0.20^{e}	9.40±0.06^{c}	87.08±0.88^{f}
'Early Red-I'	11.10±0.01^{h}	0.27±0.00^{hg}	21.60±0.40^{h}	11.58±0.14^{b}	29.03±0.29^{o}
'Gale Gala'	16.10±0.11^{a}	0.62±0.00^{m}	27.17±0.26^{c}	5.11±0.06^{l}	38.70±0.52^{o}
'Spartan'	11.90±0.07^{g}	0.27±0.00^{h}	28.80±0.41^{b}	6.69±0.08^{g}	59.99±0.90^{i}
'Vance Delicious'	12.90±0.16^{de}	0.54±0.00^{f}	28.24±0.29^{b}	6.43±0.07^{h}	79.34±0.36^{h}
'Silver Spur'	12.10±0.09^{g}	0.47±0.00^{i}	27.09±0.08^{c}	13.20±0.17^{a}	38.70±0.56^{o}
'Red Delicious'	10.00±0.08i	0.27±0.00^{g}	21.60±0.08^{h}	8.24±0.11^{e}	42.57±0.23^{n}
Average	**12.60±0.06**	**0.39±0.00**	**24.14±0.18**	**7.09±0.05**	**67.11±0.382**

Results as mean ± SE of triplicate measurements. Means with same superscript are not significantly different

recorded in non-red cultivar ‘Granny Smith’ (48.90 g L^{-1}) and lowest in red cultivar ‘Royal Delicious’ (9.85 g L^{-1}).

4.1.10 Organic acid profiling

Of the twenty two studied apple cultivars, all the non-red cultivars recorded higher quantity of organic acids, with malic acid being the most predominant, followed by succinic acid and traces of citric and acetic acid (Table 4.3). In the cultivars we studied, malic acid ranged from 4.40 g L^{-1} (‘Red Gold’) to 9.50 g L^{-1} (‘Stark Spur Golden’) whereas succinic acid was found to be absent in cultivar ‘Stark Spur Golden’ and maximum (5.10 g L^{-1}) in ‘Red Delicious’.

4.1.11 Mineral analysis

Table 4.4 presented the mineral content of twenty two apple cultivars. Among the major elements studied in different cultivars of apple fruits, K was the most abundant with an overall mean concentration of 795.14 mg $100g^{-1}$. Maximum potassium concentration was observed in ‘Scarlet Gala’ (1142 mg $100g^{-1}$) and minimum in ‘Winter Banana’ (550 mg $100g^{-1}$). The sodium, calcium and magnesium concentrations among different apple cultivars fell in the range of 9.70-70.20 mg $100g^{-1}$, 14.20-90.37 mg $100g^{-1}$ and 15.98-31.12 mg $100g^{-1}$, respectively (Table 4.4). The average values for Na, Ca and Mg recorded were 23.70 mg$100g^{-1}$, 14.20-90.37 mg $100g^{-1}$ and15.98-29.54 mg $100g^{-1}$, respectively in the apple cultivars.

The average concentrations of investigated micro elements in the 22 apple cultivars were: Fe (2.04 µg g^{-1}), Zn (0.64 µg g^{-1}), Mn (0.16 µg g^{-1}) and Cu (0.12 µg g^{-1}) (Table 4.4). Among different apple cultivars studied, maximum Fe (6.14 µg g^{-1}), Zn (2 µg g^{-1}), Mn (0.28 µg g^{-1}) and Cu (0.22 µg g^{-1}) concentrations were recorded in ‘Red Chief’, ‘Scarlet Spur-II’, ‘Starkrimson’ and ‘Early Red-I’, respectively (Table 4.4).

4.1.12 Profiling of phenolic compounds

In the present investigation, chlorogenic acid was found to be the predominant phenolic compound with an average concentration of 28.42 mg L^{-1} (Table 4.5). The highest concentration of chlorogenic acid was found in non-red cultivar ‘Winter Banana’ (163.97 mg L^{-1}) and lowest in cultivar ‘Granny Smith’ (3.69 mg L^{-1}). Phloridzindihydrate, the second highest phenolic compound in the apple cultivars averaged to 26.71 mg L^{-1}, with the highest concentration being in ‘Silver Spur’ (67.40 mg L^{-1}). In addition, traces of rutin, catechin and 3-hydroxy cinnamic acid were also found in some cultivars (Table 4.5).

4.1.13 Fatty acids profiling

Seven fatty acids were found to be present in the studied apple cultivars. Valeric acid (C5:0) was found to be the dominant fatty acid and its maximum percent relative area was recorded in ‘Silver Spur’ (58.45 %). Other fatty acids detected in minor amounts were palmitic, stearic, oleic and linoleic (Table 4.6).

4.2 Evaluation of Nutritional Composition of Apple Cultivars Grown at Different Altitudes

4.2.1 Fruit dimensions and weight

Table 4.7 describes the fruit dimensions and weight of the three apple cultivars, namely, ‘Royal Delicious’, ‘Golden Delicious’ and ‘Red Gold’ grown at two elevations. Red cultivar ‘Royal Delicious’ possessed the maximum fruit weight (186 g) and also exhibited maximum geometric mean diameter (76.46 mm) and arithmetic mean diameter (76.50 mm). The non-red cultivar ‘Golden Delicious’ had the minimum fruit weight (133.40 g), geometric mean diameter (66.17 mm) and arithmetic mean diameter (66.35 mm). Lower fruit weight was recorded in fruits grown at lower altitude. However, elevation did not affect the fruit weight of ‘Golden Delicious’ cultivar.

4.2.2 Fruit firmness

Fruit firmness of three apple cultivars from different growing sites is listed in Table 4.7. Average fruit firmness of apples differed significantly with the cultivar and sites of production. The maximum fruit firmness was recorded for ‘Golden Delicious’ cultivar, while the minimum fruit firmness was observed for ‘Royal Delicious’. Fruits grown at higher altitude recorded higher (average 11.85 N) fruit firmness as compared to those grown at lower elevation (average 11.13 N).

4.2.3 Peel colour analysis

Table 4.7 depicts the peel colour of the different apple cultivars grown at the two elevations viz., 1000-1400 msl and 1500-1800 msl. Fruits of ‘Golden Delicious’ (L^* = 69.29) and ‘Red Gold’ (L^* = 60.04) grown at 1500-1800 msl altitude showed the maximum L^* values, whereas fruits of the ‘Royal Delicious’ (L^* = 55.59) showed

Table 4.3 Sugars and organic acids (g L^{-1}) profiling in apple cultivars

Cultivar	Sugars			Organic acids			
	Glucose	**Fructose**	**Sucrose**	**Malic Acid**	**Succinic Acid**	**Citric Acid**	**Acetic Acid**
Non-red cultivars							
'Golden Delicious'	9.50±0.11^{j}	49.10±0.41^{f}	26.00±0.18^{e}	6.50±0.03^{d}	0.90±0.01^{h}	-	-
'Granny Smith'	10.50±0.14^{h}	10.85±0.18^{k}	48.90±0.59^{a}	6.60±0.05^{d}	3.35±0.03^{b}	5.10±0.08^{b}	0.65±0.00^{a}
'Winter Banana'	10.05±0.08^{i}	52.30±0.40^{e}	14.05±0.09^{g}	8.25±0.06^{b}	1.10±0.01^{gf}	-	-
'Goldspur'	22.50±0.47^{b}	54.60±0.13^{d}	12.75±0.06^{g}	7.20±0.08^{c}	0.85±0.01^{ih}	-	-
'Starkspur Golden'	9.85±0.09^{ij}	65.55±1.17^{b}	22.30±0.27^{f}	9.50±0.11^{a}	0.00±0.00^{l}	-	-
Red cultivars							
'Royal Delicious'	17.00±0.15^{c}	48.05±0.41^{f}	9.85±0.10^{l}	5.50±0.06^{hgi}	0.15±0.00^{k}	-	-
'Top Red'	24.40±0.49^{g}	56.60±0.43^{c}	17.80±0.34^{ih}	6.10±0.04^{e}	1.10±0.02^{gf}	-	-
'Oregon Spur II'	25.15±0.44^{ih}	58.15±1.06^{h}	17.90±0.16^{ih}	5.90±0.10^{f}	1.45±0.01^{e}	-	-
'Starkrimson'	21.35±0.26^{fe}	51.40±0.30^{h}	16.75±0.36^{ihj}	5.35±0.08^{i}	1.15±0.01^{f}	-	-
'Well Spur'	16.80±0.17^{d}	47.85±0.94^{g}	17.15±0.16^{ihj}	5.50±0.06^{hgi}	1.55±0.01^{d}	-	-
'Red Chief'	20.45±0.17^{d}	57.55±1.09^{h}	24.80±0.31^{e}	5.70±0.03^{g}	1.45±0.02^{e}	-	-
'Super Chief'	26.70±0.26^{m}	57.05±0.73^{c}	17.05±0.23^{ihj}	4.90±0.04^{j}	1.40±0.02^{e}	-	-
'Red Gold'	12.50±0.11^{e}	52.20±0.40^{e}	29.90±0.29^{c}	4.40±0.05^{l}	0.55±0.00^{j}	-	-
'Royal Gala'	16.85±0.09^{k}	52.05±0.52^{h}	19.55±0.17^{k}	5.55±0.06^{hg}	1.40±0.01^{e}	-	-
'Scarlet Spur-II'	20.40±0.12^{c}	48.00±0.41^{g}	23.15±0.27^{f}	4.65±0.06^{k}	1.60±0.02^{d}	-	-
'Scarlet Gala'	25.90±0.25^{e}	67.55±0.20^{a}	10.95±0.08^{h}	6.05±0.11^{fe}	1.55±0.03^{d}	-	-
'Early Red-I'	13.00±0.02^{d}	54.30±0.62^{d}	14.10±0.20^{g}	6.10±0.04^{e}	1.05±0.01^{g}	-	-
'Gale Gala'	11.40±0.12^{l}	51.65±0.87^{h}	23.90±0.17^{f}	5.90±0.02^{f}	0.80±0.00^{i}	-	-
'Spartan'	10.00±0.15^{i}	48.10±0.34^{f}	14.65±0.09^{g}	6.00±0.07^{fe}	1.85±0.03^{c}	-	-
'Vance Delicious'	24.50±0.21^{d}	59.90±0.78^{h}	22.20±0.23^{f}	5.40±0.05^{hi}	1.85±0.00^{c}	-	-
'Silver Spur'	23.25±0.28^{f}	54.85±0.59^{dc}	19.85±0.19^{k}	6.05±0.02^{fe}	1.90±0.00^{c}	-	-
'Red Delicious'	23.00±0.18^{a}	19.65±0.24^{j}	25.20±0.36^{d}	5.50±0.02^{hgi}	5.10±0.05^{a}	11.00±0.10^{a}	-
Average	**17.96±0.13**	**50.79±0.36**	**20.40±0.14**	**6.03±0.03**	**1.46±0.01**	**0.73±0.01**	**0.03±0.00**

Results as mean ± SE of triplicate measurements. Means with same superscript are not significantly different

Table 4.4 Comparison of minerals content in apple cultivars

Cultivar	Macro-elements (mg 100 g^{-1})				Micro-elements (µg g^{-1})			
	Na	K	Ca	Mg	Fe	Zn	Mn	Cu
Non-red cultivars								
'Golden Delicious'	54.50±0.59^{c}	936.00±11.00^{c}	15.74±0.14^{n}	17.37±0.34^{kj}	1.27±0.01^{i}	0.51±0.02^{jk}	0.13±0.00^{k}	0.07±0.00^{p}
'Granny Smith'	11.40±0.14^{k}	674.00±12.26^{k}	14.94±0.24^{o}	21.22±0.32^{gf}	1.10±0.00^{j}	0.77±0.00^{d}	0.12±0.00^{lm}	0.11±0.00^{i}
'Winter Banana'	16.30±0.18^{i}	550.00±4.39^{m}	18.07±0.17^{l}	17.23±0.21^{k}	0.93±0.02^{k}	0.25±0.00^{n}	0.08±0.00^{o}	0.09±0.01^{m}
'Goldspur'	9.90±0.11^{l}	1047.00±16.44^{b}	15.94±0.17^{n}	18.13±0.21^{ij}	1.13±0.00^{j}	0.50±0.00^{k}	0.20±0.01^{d}	0.14±0.00^{e}
'Starkspur Golden'	70.20±0.58^{a}	753.00±8.69^{ijh}	21.01±0.44^{i}	21.82±0.25^{f}	1.12±0.00^{j}	0.61±0.01^{g}	0.15±0.00^{i}	0.13±0.00^{g}
Red cultivars								
'Royal Delicious'	19.30±0.30^{g}	618.00±5.31^{l}	19.94±0.11^{k}	21.41±0.28^{f}	1.26±0.00^{i}	0.86±0.00^{b}	0.16±0.00^{ih}	0.08±0.00^{n}
'Top Red'	57.00±0.53^{b}	868.00±5.62^{d}	26.39±0.14^{f}	23.86±0.29^{d}	1.55±0.00^{h}	0.82±0.00^{c}	0.13±0.00^{lk}	0.12±0.00^{h}
'Oregon Spur II'	19.40±0.12^{g}	810.00±4.14^{e}	19.55±0.05^{k}	21.89±0.25^{f}	1.31± 0.01^{i}	0.53±0.00^{ji}	0.16±0.00^{h}	0.09±0.00^{k}
'Starkrimson'	41.20±0.30^{d}	696.00±12.75^{k}	42.21±0.20^{c}	31.12±0.24^{a}	2.56±0.02^{d}	0.80±0.01^{c}	0.28±0.00^{a}	0.09±0.00^{lk}
'Well Spur'	19.60±0.17^{g}	739.00±7.90^{j}	22.37±0.18^{h}	20.47±0.25^{g}	1.28±0.01^{i}	0.72±0.00^{e}	0.17±0.00^{g}	0.16±0.00^{c}
'Red Chief'	17.60±0.01^{h}	769.00±3.38^{igh}	17.55±0.17^{ml}	21.23±0.17^{gf}	6.14±0.12^{a}	0.13±0.00^{o}	0.17±0.01^{g}	0.15±0.00^{d}
'Super Chief'	11.70±0.12^{k}	887.00±12.09^{d}	23.01±0.04^{g}	23.30±0.28^{ed}	1.80±0.02^{gf}	0.54±0.00^{i}	0.14±0.00^{j}	0.11±0.02^{j}
'Red Gold'	19.80±0.29^{g}	782.00±2.87^{fg}	28.95±0.15^{e}	26.42±0.35^{c}	2.53±0.01^{d}	0.51±0.00^{jk}	0.23±0.00^{b}	0.12±0.00^{h}
'Royal Gala'	10.30±0.08^{l}	754.00±8.01^{ijh}	55.17±0.25^{b}	15.98±0.14^{l}	1.54±0.00^{h}	0.58±0.00^{h}	0.12±0.00^{lm}	0.09±0.00^{lm}
'Scarlet Spur-II'	11.30±0.10^{k}	614.00±6.96^{l}	31.98±0.40^{d}	29.54±0.50^{b}	3.52±0.04^{c}	2.00±0.01^{a}	0.22±0.00^{c}	0.18±0.00^{b}
'Scarlet Gala'	9.70±0.09^{l}	1142.00±19.10^{a}	14.20±0.05^{p}	17.73±0.17^{kj}	1.82±0.00^{gf}	0.66±0.00^{f}	0.11±0.00^{n}	0.09±0.00^{m}
'Early Red-I'	23.30±0.21^{f}	778.00±10.82^{fgh}	16.99±0.28^{m}	19.46±0.17^{h}	2.31±0.01^{e}	0.65±0.00^{f}	0.14±0.00^{j}	0.22±0.00^{a}
'Gale Gala'	11.70±0.10^{k}	748.00±1.63^{ij}	21.04±0.21^{i}	17.55±0.25^{kj}	1.87±0.00^{f}	0.51±0.00^{jk}	0.22±0.00^{c}	0.14±0.00^{f}
'Spartan'	33.10±0.49^{e}	795.00±9.14^{fe}	22.38±0.13^{h}	18.71±0.26^{ih}	1.78±0.02^{g}	0.38±0.00^{m}	0.19±0.00^{f}	0.15±0.00^{d}
'Vance Delicious'	22.80±0.27^{f}	886.00±4.36^{d}	90.37±0.15^{a}	29.25±0.43^{b}	4.33±0.02^{b}	0.87± 0.01^{b}	0.19±0.00^{e}	0.11±0.00^{i}
'Silver Spur'	18.10±0.24^{h}	872.00±7.75^{d}	21.74±0.07^{i}	23.40±0.24^{ed}	2.33±0.03^{e}	0.40±0.00^{m}	0.12±0.00^{lm}	0.12±0.00^{i}
'Red Delicious'	13.30±0.10^{j}	775.00±10.56^{fgh}	20.94±0.16^{i}	22.93±0.26^{e}	1.32±0.00^{i}	0.45±0.00^{l}	0.16±0.00^{ih}	0.08±0.00^{o}
Average	**23.70±0.16**	**795.14±5.47**	**26.39±0.11**	**21.82±0.16**	**2.04±0.01**	**0.64±0.00**	**0.16±0.00**	**0.12±0.00**

Results as mean ± SE of triplicate measurements. Means with same superscript are not significantly different

Table 4.5 HPLC profile of phenolic compounds (mg L^{-1}) in apple cultivars

Cultivar	Chlorogenic acid	Coumaric acid	Phloridzindihydrate	Rutin	Catechin	3-hydroxy cinnamic acid	Total
Non-red cultivars							
'Golden Delicious'	53.29±0.47^{d}	3.58±0.03^{j}	4.72±0.00^{p}	TR	TR	0.53±0.00^{c}	62.11
'Granny Smith'	3.69±0.06^{m}	2.06±0.02^{m}	22.11±0.12^{k}	TR	TR	TR	27.89
'Winter Banana'	163.97±1.79^{a}	9.32±0.10^{a}	18.68±0.24^{l}	TR	TR	0.74±0.00^{a}	192.70
'Goldspur'	8.00±0.10^{k}	1.11±0.01^{o}	2.05±0.00^{q}	0.46±0.00^{c}	TR	TR	11.60
'Starkspur Golden'	67.76±0.36^{b}	5.11±0.04^{e}	13.23±0.20^{n}	0.30±0.00^{f}	TR	0.66±0.00^{b}	87.06
Red cultivars							
'Royal Delicious'	17.88±0.40^{h}	5.76±0.06^{c}	46.21±0.12^{c}	TR	TR	TR	69.84
'Top Red'	8.89±0.04^{k}	3.39±0.04^{k}	24.94±0.31^{j}	TR	TR	TR	37.22
'Oregon Spur II'	7.75±0.09^{k}	9.32±0.01^{a}	30.46±0.40^{g}	TR	TR	TR	
'Starkrimson'	40.48±0.49^{e}	6.08±0.06^{b}	38.39±0.26^{d}	0.50±0.00^{b}	TR	TR	85.45
'Well Spur'	15.17±0.18^{i}	4.03±0.04^{h}	35.23±0.24^{f}	TR	TR	TR	54.43
'Red Chief'	27.23±0.28^{f}	5.70±0.07^{c}	65.34±0.26^{b}	0.29±0.00^{g}	42.44±0.51^{b}	TR	140.99
'Super Chief'	15.44±0.02^{i}	3.82±0.04^{i}	35.60±0.40^{f}	0.21±0.00^{h}	TR	TR	55.07
'Red Gold'	10.34±0.09^{j}	1.01±0.00^{o}	16.85±0.19^{m}	TR	TR	TR	
'Royal Gala'	17.99±0.09^{h}	4.92±0.06^{f}	5.67±0.01^{o}	TR	TR	0.25±0.00^{e}	28.81
'Scarlet Spur-II'	18.83±0.13^{h}	4.74±0.05^{g}	29.87±0.17^{hg}	0.32±0.00^{e}	TR	0.18±0.00^{f}	53.93
'Scarlet Gala'	5.44±0.06^{l}	1.90±0.00^{n}	4.40±0.04^{p}	TR	TR	0.11±0.00^{h}	11.85
'Early Red-I'	7.75±0.09^{k}	3.00±0.03^{l}	37.48±0.46^{e}	0.43±0.00^{d}	45.71±0.70^{a}	TR	
'Gale Gala'	7.74±0.09^{k}	1.99±0.04^{nm}	2.37±0.04^{q}	TR	TR	0.12±0.00^{g}	12.22
'Spartan'	54.82±0.27^{c}	3.90±0.04^{ih}	26.79±0.13^{i}	0.20±0.00^{i}	TR	TR	85.71
'Vance Delicious'	22.84±0.54^{g}	5.14±0.05^{e}	29.38±0.35^{h}	0.10±0.00^{j}	TR	TR	57.46
'Silver Spur'	23.30±0.28^{g}	5.53±0.06^{d}	67.40±0.73^{a}	0.65±0.00^{a}	TR	TR	96.87
'Red Delicious'	26.68±0.24^{f}	4.79±0.04^{gf}	30.54±0.40^{g}	TR	TR	0.36±0.00^{d}	62.37
Average	**28.42±0.26**	**4.37±0.03**	**26.71±0.17**	**0.16±0.00**	**4.01±0.10**	**0.13±0.00**	

Results as mean ± SEM of triplicate measurements. Means with same superscript are not significantly different
TR= Traces

Table 4.6 Fatty acids (% relative area) profiling of apple cultivars

Cultivar	Valeric acid (C5:0)	Levulinic acid (C5:0)	Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)	13-Docosenoic acid (C22:0)
Non-red cultivars							
'Golden Delicious'	TR	TR	TR	TR	TR	TR	TR
'Granny Smith'	55.56	0.89	0.01	TR	TR	0.05	TR
'Winter Banana'	TR	0.4	0.25	TR	TR	0.88	TR
'Goldspur'	TR	TR	TR	TR	TR	TR	TR
'Starkspur Golden'	TR	1.62	TR	TR	TR	ND	TR
Red cultivars							
'Royal Delicious'	TR	TR	TR	TR	TR	TR	TR
'Top Red'	TR	TR	TR	TR	TR	TR	TR
'Oregon Spur II'	TR	TR	TR	TR	TR	TR	TR
'Starkrimson'	TR	TR	TR	TR	TR	TR	TR
'Well Spur'	TR	TR	TR	TR	TR	TR	TR
'Red Chief'	TR	TR	TR	TR	TR	TR	TR
'Super Chief'	TR	TR	TR	TR	TR	TR	TR
'Red Gold'	TR	TR	TR	TR	TR	TR	TR
'Royal Gala'	11.74	0.01	TR	TR	TR	0.35	TR
'Scarlet Spur-II'	TR	0.08	TR	TR	TR	TR	TR
'Scarlet Gala'	TR	0.16	TR	TR	TR	ND	TR
'Early Red-I'	TR	0.95	TR	TR	TR	0.07	TR
'Gale Gala'	6.85	0.08	TR	TR	TR	ND	TR
'Spartan'	4.73	0.03	1.87	0.88	3.39	TR	0.05
'Vance Delicious'	4.73	0.24	1.97	0.88	3.39	TR	0.05
'Silver Spur'	58.45	TR	TR	TR	TR	TR	TR
'Red Delicious'	0.08	50.76	2.56	0.18	0.68	2.54	TR

Means with same superscript are not significantly different
TR: Traces

Table 4.7 Influence of cultivar and orchard elevation on physical attributes of apples

Cultivar	Elevation (msl)	Fruit weight (g)	Fruit dimensions (mm)				Firmness (N)	Peel Colour		
			Width	Length	Geometric mean diameter	Arithmetic mean diameter		L*	a*	b*
'Golden Delicious'	1000-1400	133.40[d]	69.71[b]	59.63	66.17[d]	66.35[d]	11.63[a]	68.24[a]	-8.76[f]	40.06[a]
	1500-1800	152.00[b]	70.63[b]	63.70	68.24[dc]	68.32[dc]	11.87[a]	69.29[a]	10.37[b]	42.00[a]
'Royal Delicious'	1000-1400	135.31[d]	76.42[a]	68.21	73.58[ba]	73.68[ba]	10.32[b]	54.94[d]	15.20[a]	26.15[d]
	1500-1800	186.00[a]	78.40[a]	72.71	76.46[a]	76.50[a]	11.84[a]	55.59[d]	8.88[c]	31.19[c]
'Red Gold'	1000-1400	142.00[cd]	70.74[b]	58.07	66.24[d]	66.52[d]	11.45[a]	62.95[b]	7.32[d]	32.02[c]
	1500-1800	148.48[cb]	75.56[a]	64.00	71.49[bc]	71.71[bc]	11.83[a]	60.04[c]	1.34[e]	36.28[b]
Cultivar (G)		<.0001	0.0003	<.0001	0.0001	<.0001	0.0001	<.0001	<.0001	<.0001
Elevation (E)		<.0001	0.0037	<.0001	0.0122	0.0061	<.0001	<.0001	0.0039	<.0001
G * E		<.0001	0.0003	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Values in rows with the same letters in superscript do not differ significantly ($p < 0.05$) according to Tukey's HSD.

lower L* values. Cultivars 'Royal Delicious' (a* = 15.20) and 'Red Gold' (L* = 7.32) grown at lower elevation (1000-1400 msl) displayed higher a* values with dark red coloured fruits.

4.2.4 Soluble solid content

Soluble solid content of different apple cultivars grown at different altitudes is shown in Table 4.8. Soluble solid content ranged between 12.50 and 14.02 %. The cultivar 'Golden Delicious' (14.02 %) displayed higher concentrations of soluble solid content followed by the 'Royal Delicious' (13.08 %) cultivar whereas the 'Red Gold' (13.01 %) cultivar reported the lowest content. Further, we observed that the elevation did not influence the soluble solids content significantly.

4.2.5 Titratable acidity

Titratable acidity was also affected by the elevation of growing sites for all the three cultivars (Table 4.8). Royal Delicious' and 'Red Gold' apples grown in orchard situated at 1500-1800 msl presented significantly higher titratable acidity (0.34 %) as compared to those grown at an altitude of 1000-1400 msl (0.26 %).

4.2.6 Ascorbic acid

In case of ascorbic acid content, significant difference was observed between 1000-1400 msl and 1500-1800 msl elevations of cultivation. The vitamin C content varied from 14.40 ('Red Gold') to 28.80 ('Golden Delicious') (Table 4.8). It was evident that the apples grown at higher elevation showed higher ascorbic acid content.

4.2.7 Total phenolic content

Apples grown at 1500-1800 msl presented significantly higher total phenolic content as compared to the lower elevation irrespective of cultivar with average value of 147.75, 157.50 and 231 mg GAE 100 g^{-1} in 'Golden Delicious', 'Royal Delicious' and 'Red Gold', respectively (Table 4.8).

4.2.8 Total antioxidant activity

Data pertaining to total antioxidant activity of apples grown at different elevations is given in Table 4.8. Antioxidant activity in the apple cultivars ranged from 2.77 ('Red Gold') to 9.98 μmol Trolox g^{-1} ('Golden Delicious'). Apples grown at 1500-1800 msl exhibited the maximum antioxidant activity for all cultivars. The antioxidant activity was the highest (9.98 μmol Trolox g^{-1}) in 'Golden Delicious' fruits growing a higher elevation. The cultivar ‘Red Gold’ showed the lowest antioxidant activity in both the elevations studied.

4.2.9 Total carotenoid content

Apples cultivated at higher elevation (1500-1800 msl) showed higher total carotenoid content compared with those grown at lower elevation (1000-1400 msl) (Table 4.8). Carotenoids content varied between 46.44 and 104.50 mg kg^{-1}.

4.2.10 Sugar profiling

The profile of sugars had a differential expression depending on the cultivar and elevation considered. The major sugar for apple cultivars was fructose followed by glucose and sucrose. Significant differences ($p < 0.05$) in all sugars were found within the cultivars and elevation (Table 4.9). Apples grown at a lower elevation was found to contain low levels of glucose in comparison to the apples grown at a higher elevation. However, fructose and sucrose contain in the three cultivars studied did not show a similar pattern. The cultivar ‘Royal Delicious’ showed the highest glucose content (18.50 g L^{-1}) at higher elevation, while ‘Golden Delicious’ cultivar had the lowest value (9.50 g L^{-1}) at the lowest elevation. Fructose ranged from 15.55 g L^{-1} in ‘Golden Delicious’ to 56.65 g L^{-1} in ‘Royal Delicious’ and sucrose was present in lower amount in all cultivars, ranging from 9.85 g L^{-1} in ‘Royal Delicious’ to 29.90 g L^{-1} in ‘Red Gold’.

4.2.11 Organic acid composition

Results exhibited that the organic acid composition (i.e., malic, citric and succinic acid) differed between the apple cultivars and was influenced by the altitude of the production site. It was observed that the principal organic acid in apple cultivars was malic acid whereas succinic and citric acid were found at lower levels (Table 4.9). Cultivars ‘Golden Delicious’ (6.50 g L^{-1} & 3.85 g L^{-1}), ‘Royal Delicious’ (5.80 g L^{-1} & 1.10 g L^{-1}) and ‘Red Gold’ (7.10 g L^{-1} & 5.90 g L^{-1}) grown at 1500-1800 msl exhibited the maximum amount of malic and succinic acid in comparison to the apples grown at lower elevation (1000-1400 msl). Fruits grown at 1500-1800 msl (higher elevation) developed maximum organic acids.

Table 4.8 Nutritional attributes of apples as affected by cultivar and orchard elevation

Cultivar	Elevation (msl)	TSS (°B)	Titratable acidity (%)	Total phenol (mg 100 g^{-1})	Ascorbic acid (mg 100 g^{-1})	Total antioxidant activity (µmol Trolox g^{-1})	Total carotenoids (mg kg^{-1})
‘Golden Delicious’	1000-1400	13.49^{bac}	0.53^{b}	119.25^{d}	21.59^{b}	6.45^{b}	100.62^{b}
	1500-1800	14.02^{a}	0.57^{a}	147.75^{c}	28.80^{a}	9.98^{a}	104.50^{a}
‘Royal Delicious’	1000-1400	13.19^{bdc}	0.26^{d}	82.50^{e}	21.18^{b}	5.55^{c}	46.44^{d}
	1500-1800	13.80^{ba}	0.34^{c}	157.50^{b}	21.59^{b}	9.95^{a}	47.40^{dc}
‘Red Gold’	1000-1400	12.50^{d}	0.26^{d}	117.00^{d}	19.82^{c}	2.77^{d}	48.37^{dc}
	1500-1800	13.01^{dc}	0.34^{c}	231.00^{a}	14.40^{d}	5.79^{c}	50.23^{c}
Cultivar (G)		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Elevation (E)		0.0010	<.0001	<.0001	0.0023	<.0001	0.0038
G * E		0.9488	0.0037	<.0001	<.0001	<.0001	0.1919

Values in rows with the same letters in superscript do not differ significantly ($p < 0.05$) according to Tukey's HSD.

4.2.12 Mineral content

The mineral composition of the apple fruits of different cultivars as a function of elevation is given in Table 4.10. Potassium (K) was the most abundant element in the fruits of the different cultivars with an average of 803.5 mg 100 g^{-1}. The K content for the 'Golden Delicious' cultivar grown at 1500-1800 msl was 994 mg 100 g^{-1} which was superior to that reported for other cultivars. 'Royal Delicious' recorded the maximum sodium (Na) concentration at 1500-1800 msl and the least amount was also reported in the 'Royal Delicious' cultivar at 1000-1400 msl altitude. The maximum calcium (Ca) amount was found in 'Royal Delicious' and 'Golden Delicious' cultivars with 39.15 and 35.06 mg 100 g^{-1}, respectively grown at 1500-1800 msl. Differences were also seen among the cultivars with respect to the magnesium (Mg) content with a difference from 17.37 ('Golden Delicious') to 35.48 mg 100 g^{-1} ('Royal Delicious') at 1500-1800 msl with the mean concentration being 24.64 mg 100 g^{-1}. The Cu, Mn, Zn and Fe micronutrient varied among the apple cultivars with averages of 0.182, 0.203, 0.657, 2.45 μg g^{-1}, respectively.

4.2.13 Phenolic acid profiling

Table 4.11 shows the phenolic acid composition at 1000-1400 msl and 1500-1800 msl in apple fruits as affected by growing site elevation and cultivar. Chlorogenic and phloridzindihydrate acid was the main compound detected and its concentration in apple was found significantly influenced by cultivar, site and their interaction. Chlorogenic acid varied between traces in 'Red Gold' (1000-1400 msl) and 53.29mg L^{-1} in 'Golden Delicious' (1500-1800 msl). Fruit from higher elevation (1800 msl) had significantly higher phloridzindihydrate concentration averaging 24.35 mg L^{-1}. Coumaric acid and hydroxycinnamic acid did not vary much among all the elevations and cultivars. Rutin and catechin were identified in traces. Fruit harvested from higher altitude (1500-1800 msl) had higher phenolics than those from lower elevation.

4.2.14 Fatty acid composition

The contents of fatty acids for each apple cultivar at each site were evaluated. Among fatty acids, linoleic, linolenic, stearic, oleic, palmitic, palmitoleic and arachidic acids were identified only in the cultivars grown at higher altitude (1500-1800 msl) (Table 4.12). Linoleic, oleic, palmitic and stearic acids were found as the major components. For these acids, the highest valeric acid and the highest Linoleic acid were found in the 'Golden Delicious' cultivar at higher altitude (15.36 and 0.89 % relative area, respectively) and the highest amount of levulinic acid in the 'Red Gold' cultivar at 1500-1800 msl (0.69 % relative area). The palmitic acid was 0.11 % relative area ('Golden Delicious') and oleic acid 0.12 % relative area ('Red Gold'), whereas Stearic acid and 13-docosenoic acid was not detected in any apple samples.

4.3 Synergistic Effect of Edible Coatings and Anti-Browning Agents on Quality of Minimally Processed Apple Slices

The apple slices were treated with edible coatings and anti-browning agents singly or in combination as discussed in section 3.4. The results obtained are discussed below.

4.3.1 Fruit firmness

Firmness of the apple slices continuously decreased during storage in all samples except those that were dipped in calcium chloride (Table 4.13). CMC and AVG coatings effectively retarded softening of the apple slices. CMC (1 %) + HR (0.01 %) + AA (0.5 %) + $CaCl_2$ (0.2 %), AVG (15 %) + HR (0.01 %) + AA (0.5 %) + $CaCl_2$ (0.2 %) and CMC (1 %) + AA (1 %) + $CaCl_2$ (1 %) treated apple slices had the highest fruit firmness. Fruits coated with CMC and AVG in combination with HR (0.01 %) + AA (0.5 %) + $CaCl_2$ (0.2 %) retained ~95 and ~93 % of firmness, respectively, after 7 days of storage. Among anti-browning treatment, HR (0.01 %) + AA (0.5 %) + $CaCl_2$ (0.2 %) combination was most effective in retention of firmness while CMC coating alone or in combination with HR (0.01 %) + AA (0.5 %) + $CaCl_2$ (0.2 %) was found better to retain the firmness.

4.3.2 Browning index (BI)

Fig. 4.3 shows the variation in BI of the minimally processed apple slices as a function of storage time. We observed that the untreated apple slices exhibited maximum BI. All treated slices had significantly reduced BI with the least values recorded for slices coated with CMC and AVG in combination with anti-browning agents. The most effective combination was CMC (1 %) + HR (0.01 %) + AA (0.5 %) + $CaCl_2$ (0.2 %), followed by AVG (15 %) + HR (0.01 %) + AA (0.5 %) + $CaCl_2$ (0.2 %) and CMC (1 %) + AA (1 %) + $CaCl_2$ (1 %). The effect of CMC and AVG in

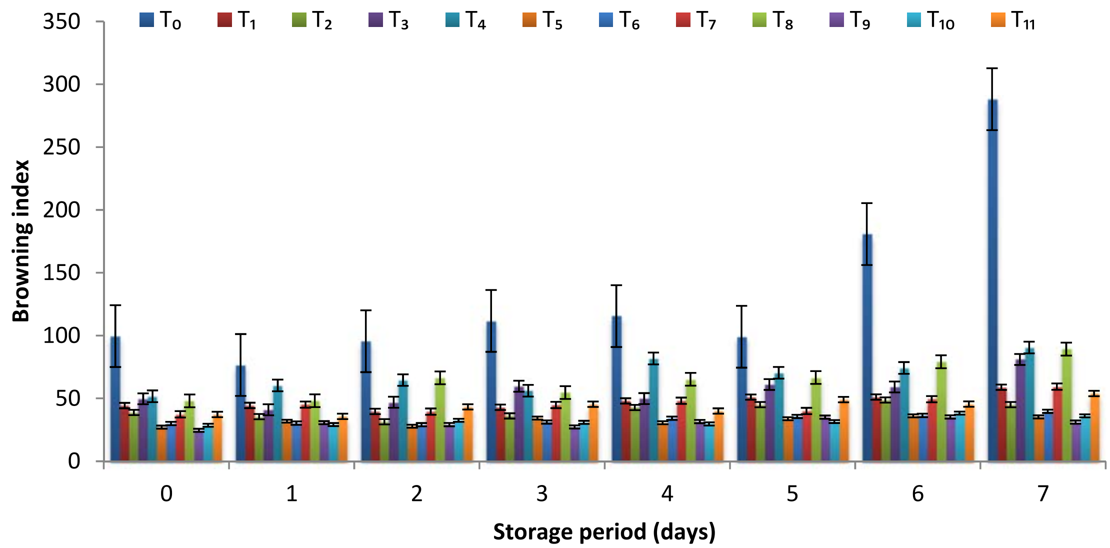

Fig. 4.3 Browning index (BI) of minimally processed apple slices affected by the anti-browning agents and edible coatings alone or in combination on under cold storage (5 ± 2ºC)

T_0: Distilled water (control), **T_1:** 1% ascorbic acid + 1% $CaCl_2$, **T_2:** 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_3:** 0.1% cysteine, **T_4:** 15% AVG, **T_5:** 15% AVG + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_6:** 15% AVG + 1% ascorbic acid + 1% $CaCl_2$, **T_7:** 15% AVG + 0.1% cysteine, **T_8:** 1% CMC, **T_9:** 1% CMC + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_{10}:** 1% CMC + 1% ascorbic acid + 1% $CaCl_2$, **T_{11}:** 1% CMC + 0.1% cysteine.

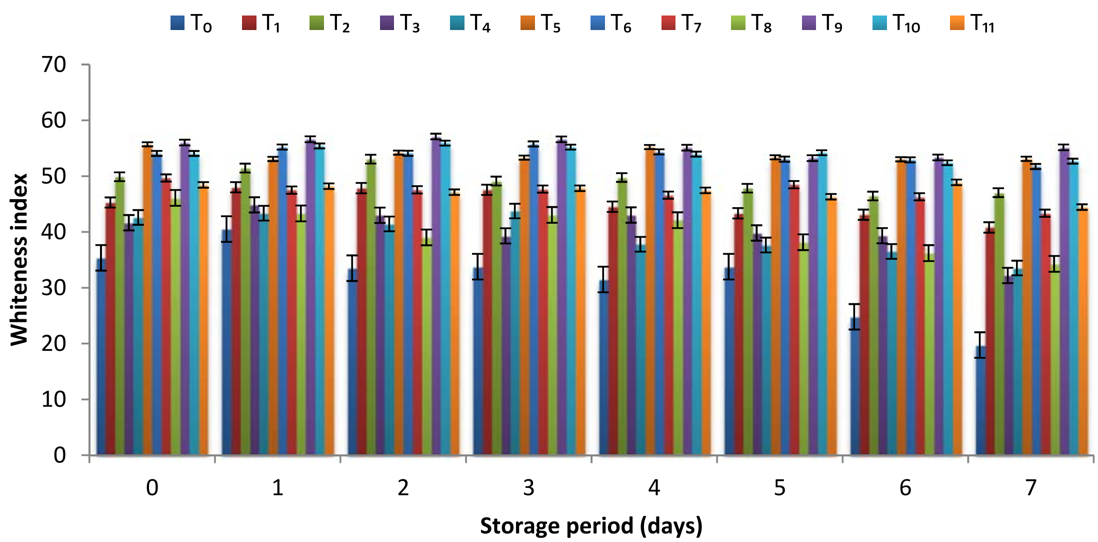

Fig.4.4 Whiteness index (WI) of minimally processed apple slices dipped in edible coatings alone or in combination under cold storage (5 ± 2ºC)

T_0: Distilled water (control), **T_1:** 1% ascorbic acid + 1% $CaCl_2$, **T_2:** 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_3:** 0.1% cysteine, **T_4:** 15% AVG, **T_5:** 15% AVG + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_6:** 15% AVG + 1% ascorbic acid + 1% $CaCl_2$, **T_7:** 15% AVG + 0.1% cysteine, **T_8:** 1% CMC, **T_9:** 1% CMC + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_{10}:** 1% CMC + 1% ascorbic acid + 1% $CaCl_2$, **T_{11}:** 1% CMC + 0.1% cysteine.

Table 4.10 Variation of mineral content in apples with respect to cultivar and orchard elevation

Cultivar	Elevation (msl)	Macro-elements (mg 100 g^{-1})				Micro-elements (µg g^{-1})			
		Na	K	Ca	Mg	Fe	Zn	Mn	Cu
'Golden Delicious'	1000-1400	54.50^{b}	936.00^{b}	15.74^{f}	17.37^{e}	1.27^{e}	0.51^{d}	0.13^{d}	0.07^{c}
	1500-1800	60.20^{a}	994.00^{a}	35.06^{b}	24.52^{c}	2.10^{d}	0.40^{e}	0.12^{d}	0.08^{c}
'Royal Delicious'	1000-1400	19.30^{c}	618.00^{f}	19.93^{e}	21.40^{d}	1.26^{e}	0.86^{b}	0.16^{c}	0.08^{c}
	1500-1800	61.60^{a}	664.00^{e}	39.15^{a}	35.48^{a}	2.85^{b}	0.98^{a}	0.16^{c}	0.63^{a}
'Red Gold'	1000-1400	19.80^{c}	782.00^{d}	28.95^{c}	26.42^{b}	2.53^{c}	0.51^{d}	0.23^{b}	0.12^{b}
	1500-1800	20.00^{c}	827.00^{c}	26.24^{d}	22.65^{d}	4.70^{a}	0.68^{c}	0.42^{a}	0.11^{b}
Cultivar (G)		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Elevation (E)		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
G * E		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Values in rows with the same letters in superscript do not differ significantly ($p < 0.05$) according to Tukey's HSD.

Table 4.11 Changes in phenolic acid composition of apples grown at different orchard elevations

Cultivar	Elevation (msl)	Phenolic acids (mg L^{-1})					
		Chlorogenic acid	Coumaric acid	Phloridzindihydrate	Rutin	Catechin	3-hydroxy cinnamic acid
'Golden Delicious'	1000-1400	17.74^{b}	3.58^{b}	4.72^{e}	TR	TR	0.53
	1500-1800	53.29^{a}	2.89^{c}	16.33^{d}	TR	TR	0.53
'Royal Delicious'	1000-1400	17.88^{b}	5.76^{a}	18.96^{c}	TR	TR	TR
	1500-1800	17.66^{b}	2.60^{d}	46.21^{a}	TR	TR	0.92
'Red Gold'	1000-1400	TR	1.01^{f}	16.85^{d}	TR	TR	TR
	1500-1800	3.69^{c}	2.06^{e}	22.11^{b}	TR	TR	0.03

Values in rows with the same letters in superscript do not differ significantly ($p < 0.05$) according to Tukey's HSD.

TR: Traces

Table 4.12 Fatty acid content of different apple cultivars cultivated at varied orchard elevations

Cultivar	Elevation (msl)	Fatty acids (% relative area)						
		Valeric acid (C5:0)	Levulinic acid (C5:0)	Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)	13-Docosenoic acid (C22:0)
'Golden Delicious'	1000-1400	TR	TR	TR	TR	TR	TR	TR
	1500-1800	15.36	0.25	0.11	TR	TR	0.89	TR
'Royal Delicious'	1000-1400	TR	TR	TR	TR	TR	TR	TR
	1500-1800	11.23	0.56	TR	TR	TR	0.26	TR
'Red Gold'	1000-1400	TR	TR	TR	TR	TR	TR	TR
	1500-1800	9.56	0.69	TR	TR	0.12	TR	TR

TR: Traces

combination with anti-browning agents on BI was greater than that for anti-browning, CMC and AVG alone. Apple slices treated with CMC (1 %) + HR (0.01 %) + AA (0.5 %) + $CaCl_2$ (0.2 %) showed ~89 % lower BI on day 7 than control.

4.3.3 Whiteness Index (WI)

Fig. 4.4 shows the effect of anti-browning agents and coatings as well as their combination on WI values of the apple slices during storage at 5 ± 2 ºC. Whiteness index of untreated apple slices decreased from 35.37 to 19.73 during 7 days of storage while treated apple slices with CMC and AVG in combination with anti-browning agents had a significant lower loss of whiteness than the uncoated slices.

4.3.4 Headspace gas composition

Package headspace oxygen for all treatments declined at a slow rate initially and then at a slightly higher rate over the remaining storage period while carbon dioxide in the shrink wrapped packs continuously increased during the storage (Fig. 4.5 and 4.6). Apple slices given a combined treatment of edible coatings and anti-browning agents showed lower O_2 consumption and carbon dioxide production while the untreated apple slices showed highest oxygen consumption and CO_2 production during storage. Apple slices dipped in CMC containing 4-HR, AA, $CaCl_2$ showed the lowest CO_2 production and O_2 consumption during storage whereas apple slices given only cysteine treatment showed highest CO_2 production and O_2 consumption.

4.3.5 Weight loss

The physiological loss in weight of apple slices from each treatment group was monitored during storage of 7 days. The treated as well untreated minimally processed apple slices lost weight during the entire storage period. Apple slices coated with CMC and AVG alongwith anti-browning agents showed lowest weight loss amongst all the treatments. After 7 days of storage, untreated samples lost 3.67 % of their weight while treated apple slices had a significantly lower loss in weight in different combinations of anti-browning solutions (Fig. 4.7). The three best coating formulations CMC (1 %) + HR (0.01 %) + AA (0.5 %) + $CaCl_2$ (0.2 %), AVG (15 %) + HR (0.01 %) + AA (0.5 %) + $CaCl_2$ (0.2 %) and CMC (1 %) + AA (1 %) + $CaCl_2$ (1 %) prevented the weight loss significantly, resulting in only 0.532, 0.668 and 0.676 % loss, respectively. Among anti-browning treatments, HR (0.01 %) + AA (0.5 %) +

$CaCl_2$ (0.2 %) treatment was most effective in reducing weight loss while among coatings alone and their combinations with anti-browning agents, 1 % CMC alone and CMC (1 %) + HR (0.01 %) + AA (0.5 %) + $CaCl_2$ (0.2 %) were found best, respectively, resulting in 1.632, 1.001 and 0.532 % weight loss on 7 days of storage.

4.3.6 Browning potential

Minimally processed apple slices treated with anti-browning agents and edible coatings as well as their combination recorded the minimum browning potential during the storage of 7 days (Figs. 4.8). At the termination of experiment, lowest browning potential was recorded in apple slices treated with CMC and aloe vera in combination with 4-HR+AA+$CaCl_2$. Control (water dipped) apple slices showed the highest browning potential. Apple slices treated with edible coatings and anti-browning agents singly showed the lowest browning potential.

4.3.7 Soluble solid content (SSC) and titratable acidity (TA)

Tables 4.14 and 4.15 show the changes in total soluble solid content and titratable acidity of cut apple slices during the 7 day storage period under cold conditions. In general, the total soluble solid content initially increased then declined after achieving a peak. In untreated samples, the maximum value (15.70 %) was obtained on second day of storage but in case of 1 % CMC + 0.01 % 4-hexylresorcinol + 0.5 % ascorbic acid + 0.2 % $CaCl_2$ treated samples (give treatment number) there was a delayed peak till end of storage period. Titratable acidity declined as storage period progressed regardless of the treatment used but remained higher in apple slices treated with anti-browning agents and edible coatings especially of those treated with carboxymethyl cellulose. At the end of experiment, for all the treated apple slices, soluble solid content and titratable acidity was higher than that of the control apple slices. The most effective treatment was T_9 (1 % CMC + 0.01 % 4-hexylresorcinol + 0.5 % ascorbic acid + 0.2 % $CaCl_2$) followed by T_5 (15 % AVG + 0.01 % 4-hexylresorcinol + 0.5 % ascorbic acid + 0.2 % $CaCl_2$) and T_{10} (1 % CMC + 1 % ascorbic acid + 1 % $CaCl_2$) for retention of SSC and TA in apple samples.

4.3.8 Ascorbic acid content

Regarding the ascorbic acid changes, it was observed that samples treated with either CMC or AVG coating + browning inhibitors had higher retention of ascorbic acid (Table 4.16). CMC coating in combination with anti-browning agents performed

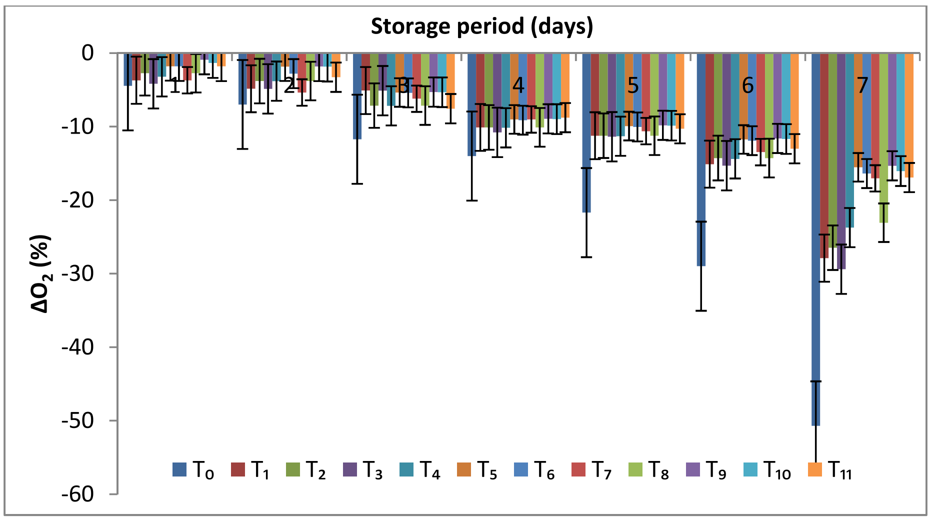

Fig. 4.5 Changes in ΔO_2 of minimally processed apple slices treated with edible coatings and chemical dips alone or in combinations under cold storage (5 ± 2^oC)

T_0: Distilled water (control), **T_1:** 1% ascorbic acid + 1% $CaCl_2$, **T_2:** 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_3:** 0.1% cysteine, **T_4:** 15% AVG, **T_5:** 15% AVG + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_6:** 15% AVG + 1% ascorbic acid + 1% $CaCl_2$, **T_7:** 15% AVG + 0.1% cysteine, **T_8:** 1% CMC, **T_9:** 1% CMC + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_{10}:** 1% CMC + 1% ascorbic acid + 1% $CaCl_2$, **T_{11}:** 1% CMC + 0.1% cysteine.

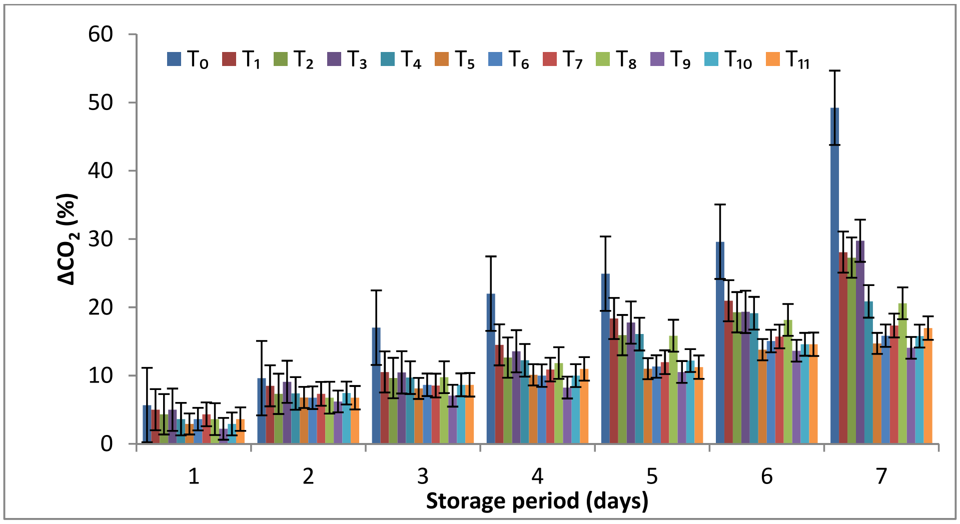

Fig.4.6 Effect of edible coatings and anti-browning agents on ΔCO_2 of minimally processed apple slices under cold storage (5 ± 2°C)

T_0: Distilled water (control), **T_1:** 1% ascorbic acid + 1% $CaCl_2$, **T_2:** 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_3:** 0.1% cysteine, **T_4:** 15% AVG, **T_5:** 15% AVG + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_6:** 15% AVG + 1% ascorbic acid + 1% $CaCl_2$, **T_7:** 15% AVG + 0.1% cysteine, **T_8:** 1% CMC, **T_9:** 1% CMC + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_{10}:** 1% CMC + 1% ascorbic acid + 1% $CaCl_2$, **T_{11}:** 1% CMC + 0.1% cysteine.

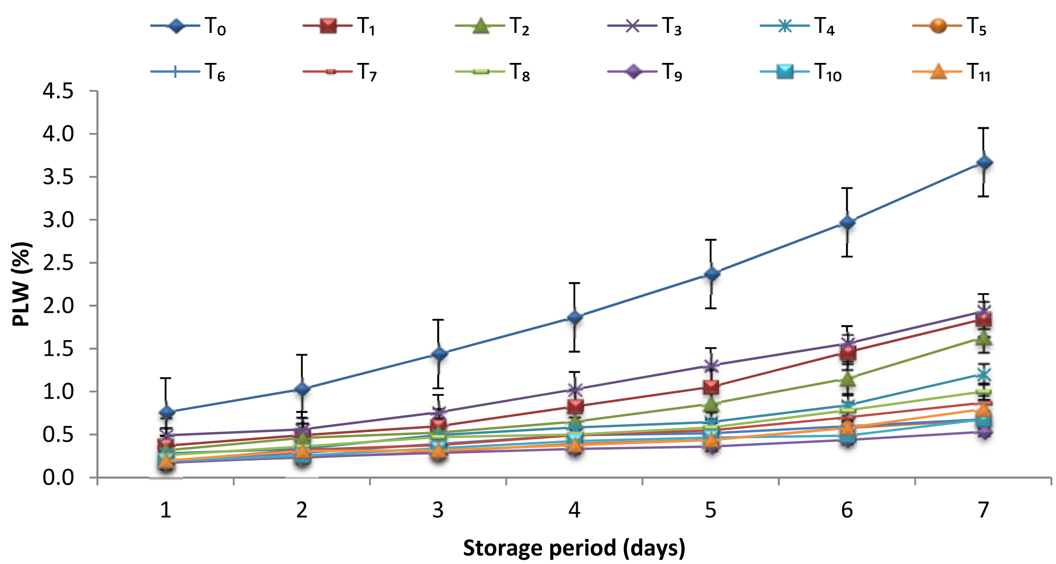

Fig. 4.7 Influence of anti-browning agents and edible coatings alone or in combination on physiological loss in weight (PLW) of minimally processed apple slices under cold storage (5 ± 2ºC)

T_0: Distilled water (control), **T_1:** 1% ascorbic acid + 1% $CaCl_2$, **T_2:** 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_3:** 0.1% cysteine, **T_4:** 15% AVG, **T_5:** 15% AVG + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_6:** 15% AVG + 1% ascorbic acid + 1% $CaCl_2$, **T_7:** 15% AVG + 0.1% cysteine, **T_8:** 1% CMC, **T_9:** 1% CMC + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_{10}:** 1% CMC + 1% ascorbic acid + 1% $CaCl_2$, **T_{11}:** 1% CMC + 0.1% cysteine.

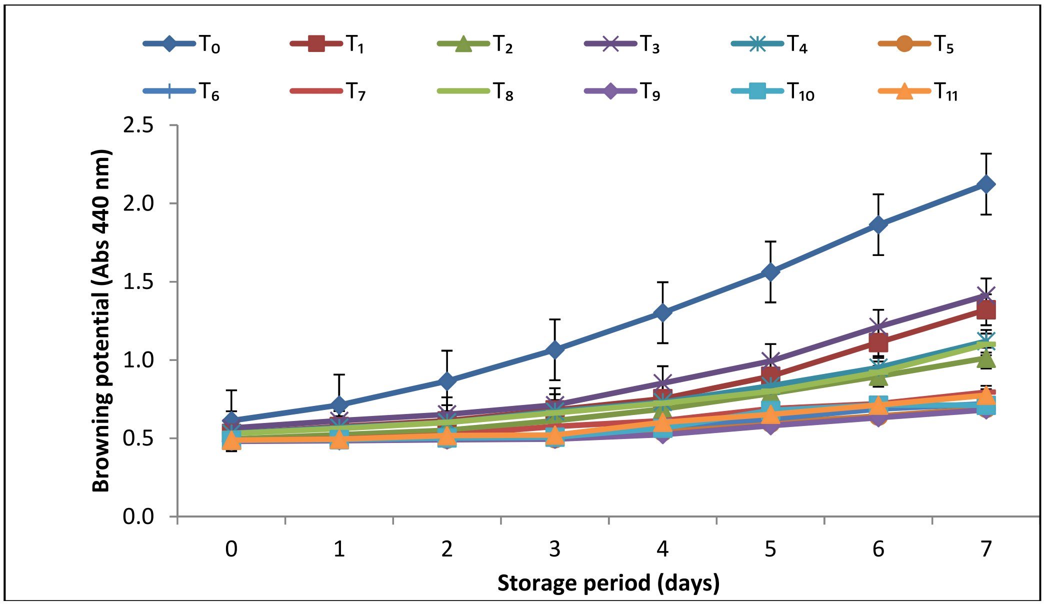

Fig. 4.8 Impact of edible coatings and chemical dips on browning potential of minimally processed apple slices under cold storage (5 ± 2ºC)

T_0: Distilled water (control), **T_1:** 1% ascorbic acid + 1% $CaCl_2$, **T_2:** 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_3:** 0.1% cysteine, **T_4:** 15% AVG, **T_5:** 15% AVG + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_6:** 15% AVG + 1% ascorbic acid + 1% $CaCl_2$, **T_7:** 15% AVG + 0.1% cysteine, **T_8:** 1% CMC, **T_9:** 1% CMC + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_{10}:** 1% CMC + 1% ascorbic acid + 1% $CaCl_2$, **T_{11}:** 1% CMC + 0.1% cysteine.

Table 4.14 Soluble solids content (SSC) of apple slices dipped in anti-browning agents and edible coatings alone or in combination during storage at 5 ± 2°C

Treatment	SSC (%)								
	Storage period (days)								
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Mean**
T_0	13.60^{n-o}	14.50^{e-f}	15.70^{a}	13.20^{q-p}	11.50^{ut}	10.60^{u}	9.30^{v}	8.10^{w}	12.06^{d}
T_1	13.30^{q-o}	13.60^{n-o}	13.90^{n-o}	14.60^{e-f}	15.50^{b-c}	14.20^{n-o}	13.40^{n-o}	12.10^{st}	13.83^{b}
T_2	13.30^{q-o}	13.50^{n-o}	13.80^{n-o}	14.50^{e-f}	15.30^{e-c}	14.40^{e-f}	13.50^{n-o}	12.50^{sr}	13.85^{b}
T_3	13.40^{n-o}	13.70^{n-o}	14.00^{n-o}	14.80^{e-f}	15.70^{a}	14.10^{n-o}	13.10^{qr}	11.80^{st}	13.83^{b}
T_4	13.20^{q-p}	13.60^{n-o}	14.10^{n-o}	14.70^{e-f}	15.60^{ba}	14.20^{n-o}	13.20^{q-p}	11.90^{st}	13.81^{b}
T_5	13.10^{qr}	13.20^{q-p}	13.30^{q-o}	13.30^{q-o}	13.70^{nm}	14.00^{n-o}	14.40^{e-f}	14.50^{e-f}	13.69^{cb}
T_6	13.10^{qr}	13.20^{q-p}	13.50^{n-o}	13.40^{n-o}	14.00^{n-o}	14.30^{n-f}	14.70^{e-f}	15.10^{e-f}	13.91^{b}
T_7	13.10^{qr}	13.40^{n-o}	13.80^{n-o}	14.10^{n-o}	14.60^{e-f}	14.90^{e-f}	15.20^{e-f}	15.60^{ba}	14.34^{a}
T_8	13.20^{q-p}	13.60^{n-o}	14.00^{n-o}	14.60^{e-f}	15.40^{b-c}	14.30^{n-f}	13.30^{q-o}	12.00^{st}	13.80^{b}
T_9	13.10^{qr}	13.10^{qr}	13.10^{qr}	13.20^{q-p}	13.40^{n-o}	13.60^{n-o}	13.80^{n-o}	14.10^{n-o}	13.43^{c}
T_{10}	13.10^{qr}	13.20^{q-p}	13.40^{n-o}	13.40^{n-o}	13.90^{n-o}	14.10^{n-o}	14.60^{e-f}	14.80^{e-f}	13.81^{b}
T_{11}	13.10^{qr}	13.30^{q-o}	13.70^{n-o}	13.90^{n-o}	14.40^{e-f}	14.80^{e-f}	15.10^{e-f}	15.40^{b-c}	14.21^{a}
Mean	**13.22^{d}**	**13.49^{c}**	**13.86^{b}**	**13.98^{b}**	**14.42^{a}**	**13.96^{b}**	**13.63^{c}**	**13.22^{d}**	

Means with same superscript are not significantly different

T_0: Distilled water (control), **T_1:** 1% ascorbic acid + 1% $CaCl_2$, **T_2:** 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_3:** 0.1% cysteine, **T_4:** 15% AVG, **T_5:** 15% AVG + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_6:** 15% AVG + 1% ascorbic acid + 1% $CaCl_2$, **T_7:** 15% AVG + 0.1% cysteine, **T_8:** 1% CMC, **T_9:** 1% CMC + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_{10}:** 1% CMC + 1% ascorbic acid + 1% $CaCl_2$, **T_{11}:** 1% CMC + 0.1% cysteine.

Table 4.15 Titratable acidity of minimally processed apple slices treated with edible coatings and anti-browning agents alone or in combination stored at 5 ± 2ºC

Treatment	Titratable acidity (%)								
	Storage period (days)								
	0	1	2	3	4	5	6	7	Mean
T_0	0.536^{b-c}	0.496^{h-g}	0.469^{k-i}	0.429^{ml}	0.348^{q-p}	0.268^{v}	0.214^{xw}	0.121^{z}	0.360^{i}
T_1	0.536^{b-c}	0.509^{e-g}	0.496^{h-g}	0.482^{h-i}	0.375^{n-p}	0.322^{t-s}	0.295^{t-v}	0.174^{y}	0.399^{g}
T_2	0.563^{a}	0.536^{b-c}	0.523^{e-c}	0.521^{e-g}	0.383^{no}	0.335^{q-s}	0.322^{t-s}	0.188^{xy}	0.421^{f}
T_3	0.563^{a}	0.523^{e-c}	0.469^{k-i}	0.440^{kl}	0.360^{q-p}	0.308^{t-s}	0.281^{uv}	0.161^{y}	0.388^{h}
T_4	0.563^{a}	0.524^{e-c}	0.509^{e-g}	0.507^{e-g}	0.387^{no}	0.348^{q-p}	0.322^{t-s}	0.214^{xw}	0.422^{f}
T_5	0.549^{ba}	0.548^{ba}	0.533^{e-c}	0.521^{e-g}	0.482^{h-i}	0.348^{q-p}	0.335^{q-s}	0.322^{t-s}	0.455^{b}
T_6	0.549^{ba}	0.543^{b-c}	0.509^{e-g}	0.507^{e-g}	0.469^{k-i}	0.347^{q-p}	0.322^{t-s}	0.295^{t-v}	0.443^{cd}
T_7	0.549^{ba}	0.548^{ba}	0.511^{e-g}	0.503^{e-g}	0.456^{k-l}	0.334^{q-s}	0.308^{t-s}	0.268^{v}	0.434^{ed}
T_8	0.536^{b-c}	0.531^{e-c}	0.509^{e-g}	0.501^{e-g}	0.456^{k-l}	0.360^{q-p}	0.335^{q-s}	0.228^{w}	0.432^{e}
T_9	0.549^{ba}	0.545^{ba}	0.533^{e-c}	0.512^{e-g}	0.494^{h-g}	0.402^{nm}	0.375^{n-p}	0.348^{q-p}	0.470^{a}
T_{10}	0.549^{ba}	0.543^{b-c}	0.521^{e-g}	0.496^{h-g}	0.469^{k-i}	0.389^{no}	0.362^{q-p}	0.308^{t-s}	0.455^{b}
T_{11}	0.536^{b-c}	0.529^{e-c}	0.519^{e-g}	0.508^{e-g}	0.489^{h-g}	0.375^{n-p}	0.348^{q-p}	0.281^{uv}	0.448^{cb}
Mean	**0.548^{a}**	**0.531^{b}**	**0.508^{c}**	**0.494^{d}**	**0.431^{e}**	**0.345^{f}**	**0.318^{g}**	**0.242^{h}**	

Means with same superscript are not significantly different

T_0: Distilled water (control), **T_1:** 1% ascorbic acid + 1% $CaCl_2$, **T_2:** 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_3:** 0.1% cysteine, **T_4:** 15% AVG, **T_5:** 15% AVG + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_6:** 15% AVG + 1% ascorbic acid + 1% $CaCl_2$, **T_7:** 15% AVG + 0.1% cysteine, **T_8:** 1% CMC, **T_9:** 1% CMC + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_{10}:** 1% CMC + 1% ascorbic acid + 1% $CaCl_2$, **T_{11}:** 1% CMC + 0.1% cysteine.

Table 4.16 Effect of anti-browning agents and edible coatings alone or in combination on ascorbic acid content of apple slices stored at 5 ± 2°C

Treatment	Ascorbic acid content (mg 100 g^{-1})								
	Storage period (days)								
	0	1	2	3	4	5	6	7	Mean
T_0	22.70^{n-j}	19.25^{c-a}	17.11^{h-f}	16.24^{ij}	15.12^{j}	13.54^{k}	10.38^{n}	7.92^{o}	15.28^{h}
T_1	24.69^{f-c}	24.00^{g-h}	23.29^{g-h}	22.63^{n-j}	22.32^{n-o}	18.51^{e-f}	17.83^{e-f}	11.52^{l-m}	20.60^{d}
T_2	24.24^{g-h}	23.14^{g-h}	22.81^{n-h}	21.60^{n-o}	20.88^{v-r}	18.00^{e-f}	17.82^{e-f}	12.24^{l-m}	20.09^{e}
T_3	21.91^{n-o}	20.84^{v-w}	20.77^{v-w}	19.93^{v-w}	18.72^{e-a}	16.42^{h-j}	16.28^{ij}	10.80^{nm}	18.21^{g}
T_4	22.34^{n-o}	21.53^{n-o}	21.41^{n-o}	20.77^{v-w}	19.44^{v-w}	16.82^{h-g}	16.76^{h-g}	12.24^{l-m}	18.91^{f}
T_5	25.41^{b-c}	25.33^{b-c}	24.43^{g-c}	24.22^{g-h}	23.04^{g-h}	20.67^{v-w}	18.76^{c-a}	18.00^{e-f}	22.48^{b}
T_6	26.18^{ba}	24.95^{b-c}	24.22^{g-h}	23.78^{g-h}	22.32^{n-o}	19.96^{v-w}	18.45^{e-f}	16.56^{h-j}	22.05^{c}
T_7	23.14^{g-h}	22.63^{n-j}	22.46^{n-o}	21.86^{n-o}	21.60^{n-o}	19.20^{c-a}	18.64^{e-b}	15.12^{j}	20.58^{d}
T_8	23.07^{g-h}	22.85^{n-h}	22.81^{n-h}	22.37^{n-o}	22.32^{n-o}	19.38^{c-w}	18.87^{c-a}	12.96^{lk}	20.58^{d}
T_9	25.41^{b-c}	25.13^{b-c}	24.47^{g-c}	24.21^{g-h}	23.76^{g-h}	21.06^{t-o}	20.47^{v-w}	18.72^{e-a}	22.90^{a}
T_{10}	26.42^{a}	24.95^{b-c}	24.23^{g-h}	23.52^{g-h}	23.04^{g-h}	20.67^{v-w}	19.16^{c-a}	17.28^{e-f}	22.41^{cb}
T_{11}	23.12^{g-h}	21.67^{n-o}	21.06^{t-o}	20.86^{v-r}	20.16^{v-w}	18.87^{c-a}	18.50^{e-f}	15.84^{ij}	20.01^{e}
Mean	**24.05^{a}**	**23.02^{b}**	**22.42^{c}**	**21.83^{d}**	**21.06^{e}**	**18.59^{f}**	**17.66^{g}**	**14.10^{h}**	

Means with same superscript are not significantly different

T_0: Distilled water (control), **T_1:** 1% ascorbic acid + 1% $CaCl_2$, **T_2:** 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_3:** 0.1% cysteine, **T_4:** 15% AVG, **T_5:** 15% AVG + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_6:** 15% AVG + 1% ascorbic acid + 1% $CaCl_2$, **T_7:** 15% AVG + 0.1% cysteine, **T_8:** 1% CMC, **T_9:** 1% CMC + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_{10}:** 1% CMC + 1% ascorbic acid + 1% $CaCl_2$, **T_{11}:** 1% CMC + 0.1% cysteine.

best. Among the anti-browning agents, HR (0.01 %) + AA (0.5 %) + $CaCl_2$ (0.2 %) resulted in highest retention of ascorbic acid (~54 %) in comparison to the control at end of storage. Among the coatings alone and coatings combination with anti-browning agents, CMC and CMC (1 %) + HR (0.01 %) + AA (0.5 %) + $CaCl_2$ (0.2 %) showed the lowest decrease in ascorbic acid (12 % and 35 %, respectively).

4.3.9 Total phenols and antioxidant activity

Tables 4.17 show the effects of anti-browning agents and edible coatings (CMC and AVG) on antioxidant activity of the apple slices, respectively during low temperature storage. A rapid decrease in antioxidant activity was observed in control apple slices during cold storage. Treatment with edible coatings, CMC and AVG, were significantly more effective in preventing reduction of antioxidant activity during cold storage of slices. At the end of storage period the maximum retention (~43 %) of total antioxidant activity was observed in HR (0.01 %) + AA (0.5 %) + $CaCl_2$ (0.2 %) among anti-browning treatment alone, whereas for coating application alone and the combinations with anti-browning agents CMC (~50 %) and CMC (1 %) + HR (0.01 %) + AA (0.5 %) + $CaCl_2$ (0.2 %) (~51 %) were found to be best.

Tables 4.18 show the effect of anti-browning agents and edible coatings alone and their combinations on total phenols during storage at 5 ± 2 °C. Anti-browning agents + edible coatings resulted in the lowest loss of total phenols among all treatments during storage. The control showed a large decrease in total phenolic (~169 %) after 7 days of storage, while CMC (1 %) + HR (0.01 %) + AA (0.5 %) + $CaCl_2$ (0.2 %) showed the lowest decrease (~38 %). Among edible coatings, CMC alone treatments effectively retarded loss of total phenols on minimally processed apples during storage. HR (0.01 %) + AA (0.5 %) + $CaCl_2$ (0.2 %) presented the lowest decrease in total phenolic content among anti-browning agents alone treatments during storage.

4.3.10 Polyphenol oxidase (PPO) and peroxidase (POD) activity

Activity of polyphenol oxidase and peroxidase enzymes increased continuously in all apple slices with advancement in storage (Figs. 4.9 and 4.10). CMC and AVG coated apple slices used alongwith browning inhibitors showed relatively lower polyphenol oxidase and peroxidase activity as compared to the other samples. Maximum suppression of polyphenol oxidase and peroxidase enzymes activity was observed in CMC + HR (0.01 %) + AA (0.5 %) + $CaCl_2$ (0.2 %) treatment. At the end of storage period the PPO and POD activities in control sample was 48 and 77 U min^{-1} g^{-1}, respectively while in CMC (1 %) + HR (0.01 %) + AA (0.5 %) + $CaCl_2$ (0.2 %) treated apple slices the activity of PPO and POD was 36 and 61 units g^{-1} min^{-1}, respectively.

4.3.11 Microbiological analysis

The total aerobic plate count on minimally processed apple slices was monitored on day 0 and 7th day (Table 4.19). It was found that the total aerobic plate count in all treated as well as untreated apple samples was below 3 log_{10} CFU g^{-1} on day 0. The microbial population was found to increase significantly till the last day of the experiment. At the end of storage period, control slices showed the highest total aerobic plate count (6.40 log_{10} CFU g^{-1}) while all other samples recorded counts <4 log_{10} CFU g^{-1}. In the present study, it was found that AVG and CMC with anti-browning agents showed reduced microbial growth in comparison to the other treatments.

4.3.12 Sensory quality

The sensory evaluation results revealed that there was a significant variation between treated apple slices and untreated apples up to 7 days of storage (Fig. 4.11). After 7 days of storage, the treated apple slices had good scores for appearance, firmness, flavour and sourness. In addition, the quality of the untreated slices rapidly deteriorated in one day of storage. The sensory panel scored all the treated samples within the limit of marketability on basis of colour up to 7 days of storage at 5 ± 2°C, whereas the control samples became unmarketable within a day of storage. By day 7, the apple slices coated with the CMC and AVG containing anti-browning agents, were better than those which were treated either with anti-browning agents and coatings alone. CMC coating maintained fruit flavour of the slices till last day of storage. The lowest rated samples were those treated with cysteine, CMC and AVG alone due to loss of fruit flavour.

4.4 Effect of Modified Atmospheres on Quality of Minimally Processed Apple Slices

4.4.1 Firmness

Fig. 4.12 shows the changes in firmness of minimally processed apple slices

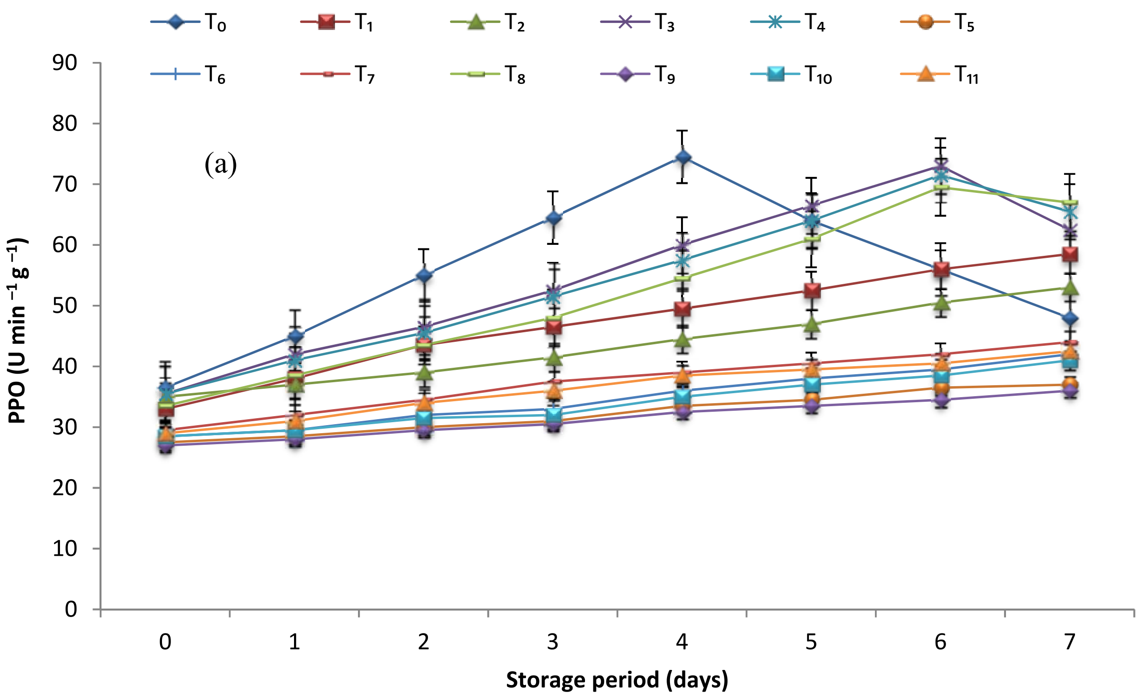

Fig. 4.9 Changes in polyphenol oxidase (PPO) activity of treated minimally processed apple slices under cold storage (5 ± 2°C)

T_0: Distilled water (control), **T_1:** 1% ascorbic acid + 1% $CaCl_2$, **T_2:** 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_3:** 0.1% cysteine, **T_4:** 15% AVG, **T_5:** 15% AVG + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_6:** 15% AVG + 1% ascorbic acid + 1% $CaCl_2$, **T_7:** 15% AVG + 0.1% cysteine, **T_8:** 1% CMC, **T_9:** 1% CMC + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_{10}:** 1% CMC + 1% ascorbic acid + 1% $CaCl_2$, **T_{11}:** 1% CMC + 0.1% cysteine.

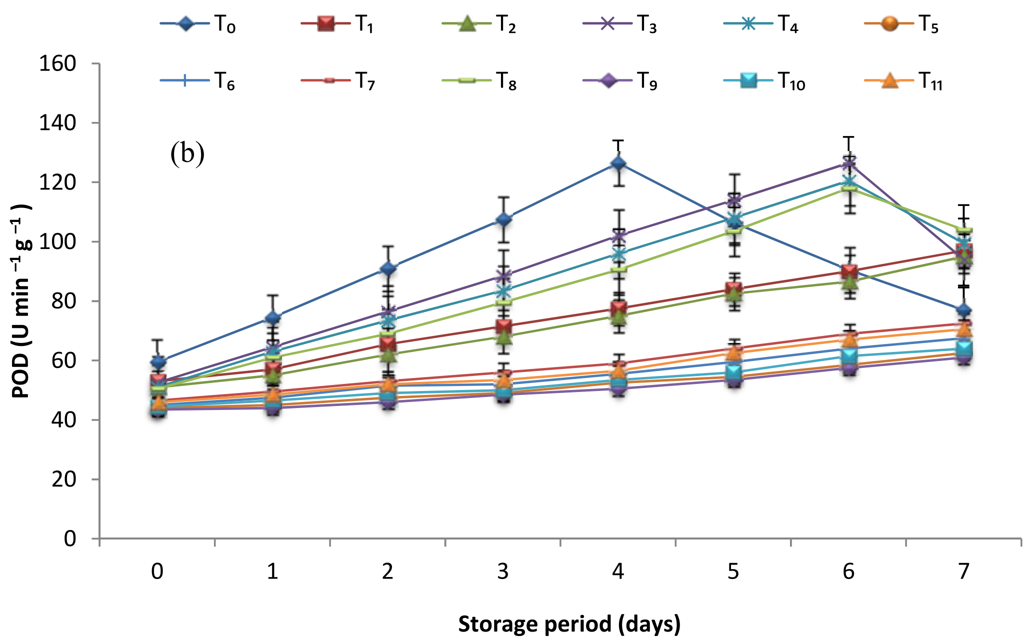

Fig.4.10 Effect of anti-browning agents and edible coatings alone or in combination on peroxidase (POD) of minimally processed apple slices under cold storage (5 ± 2ºC)

T_0: Distilled water (control), **T_1:** 1% ascorbic acid + 1% $CaCl_2$, **T_2:** 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_3:** 0.1% cysteine, **T_4:** 15% AVG, **T_5:** 15% AVG + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_6:** 15% AVG + 1% ascorbic acid + 1% $CaCl_2$, **T_7:** 15% AVG + 0.1% cysteine, **T_8:** 1% CMC, **T_9:** 1% CMC + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_{10}:** 1% CMC + 1% ascorbic acid + 1% $CaCl_2$, **T_{11}:** 1% CMC + 0.1% cysteine.

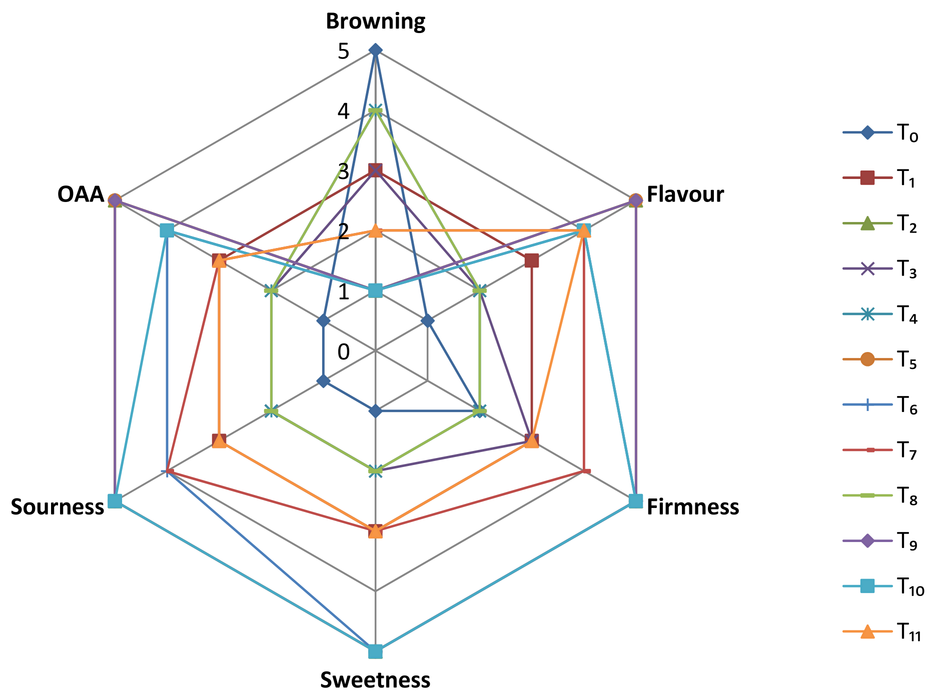

Fig. 4.11 Scores for sensory analysis of control and treated minimally processed apple slices on 7th day under cold storage (5 ± 2°C)

T_0: Distilled water (control), **T_1:** 1% ascorbic acid + 1% $CaCl_2$, **T_2:** 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_3:** 0.1% cysteine, **T_4:** 15% AVG, **T_5:** 15% AVG + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_6:** 15% AVG + 1% ascorbic acid + 1% $CaCl_2$, **T_7:** 15% AVG + 0.1% cysteine, **T_8:** 1% CMC, **T_9:** 1% CMC + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_{10}:** 1% CMC + 1% ascorbic acid + 1% $CaCl_2$, **T_{11}:** 1% CMC + 0.1% cysteine.

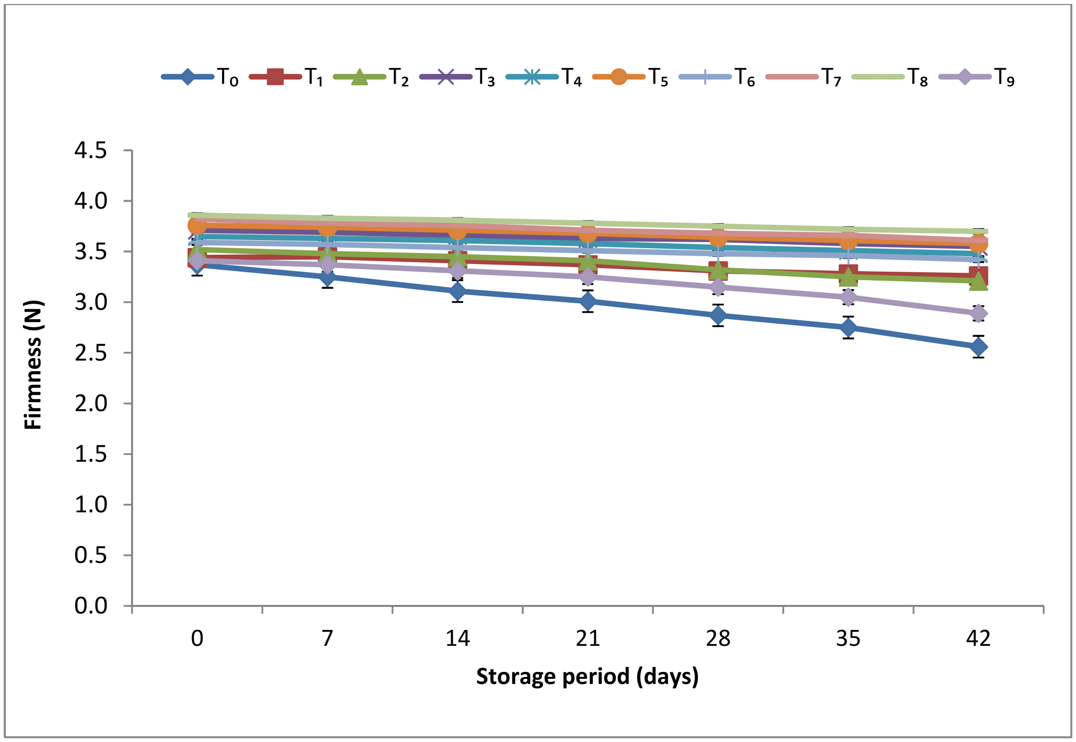

Fig. 4.12 Effect of modified atmospheric packaging on the firmness of minimally processed apple slices during cold storage (5 ± 2°C)

T_0: air, **T_1:** 1.5 % O_2 + 2.5 % CO_2, **T_2:** 1.5 % O_2 + 5 % CO_2, **T_3:** 1.5 % O_2 + 7.5 % CO_2, **T_4:** 3 % O_2 + 2.5 % CO_2, **T_5:** 3 %O_2 + 5 % CO_2, **T_6:** 3 % O_2 + 7.5 % CO_2, **T_7:** 5 % O_2 + 2.5 % CO_2, **T_8:** 5 % O_2 + 5 % CO_2, **T_9:** 5 % O_2 + 7.5 % CO_2

Table 4.17 Changes in total antioxidant activity of minimally processed apple slices treated with browning inhibitors and edible coatings alone or in combination

Treatment	Total antioxidant (µmol Trolox g^{-1})								
	Storage period (days)								
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Mean**
T_0	14.93^{hf}	12.04^{vs}	10.28^{ae}	9.09^{h-i}	8.10^{kl}	7.09^{no}	5.59^{p}	4.76^{q}	8.98^{i}
T_1	15.53^{ef}	15.00^{hf}	13.35^{no}	12.23^{rs}	11.48^{vx}	10.59^{ac}	9.13^{h-i}	8.10^{kl}	11.93^{f}
T_2	15.60^{ef}	15.38^{hf}	14.66^{hi}	13.35^{no}	12.34^{rs}	11.04^{ax}	9.81^{g-e}	8.89^{k-i}	12.63^{d}
T_3	15.49^{ef}	14.25^{jk}	11.70^{vx}	10.56^{ae}	9.60^{g-i}	8.34^{k-l}	7.01^{no}	6.68^{o}	10.45^{h}
T_4	15.56^{ef}	14.44^{jk}	11.21^{wx}	10.74^{ac}	9.75^{g-e}	8.91^{k-i}	7.93^{ml}	7.26^{n-o}	10.73^{g}
T_5	17.25^{a}	16.91^{ba}	15.04^{hf}	14.23^{jk}	13.58^{no}	13.24^{no}	12.19^{rs}	11.08^{ax}	14.19^{ba}
T_6	17.06^{a}	16.58^{bc}	14.44^{j-k}	13.09^{no}	12.66^{rs}	11.68^{vx}	10.91^{ax}	10.09^{gc}	13.31^{c}
T_7	15.90^{dc}	15.83^{ec}	14.51^{j-k}	12.09^{vs}	11.04^{ax}	10.50^{ae}	9.13^{h-i}	8.12^{kl}	12.14^{f}
T_8	15.56^{ef}	13.80^{nk}	11.29^{vx}	10.82^{az}	9.98^{g-e}	8.91^{k-i}	7.97^{ml}	7.73^{n-l}	10.76^{g}
T_9	17.33^{a}	16.58^{bc}	15.45^{e-f}	14.25^{jk}	13.78^{nk}	13.59^{no}	12.23^{rs}	11.44^{vx}	14.33^{a}
T_{10}	17.18^{a}	16.91^{ba}	14.93^{h-f}	14.06^{jk}	13.35^{no}	12.84^{ro}	11.85^{vs}	10.97^{ax}	14.01^{b}
T_{11}	16.13^{bc}	15.94^{dc}	14.59^{hk}	12.23^{rs}	11.64^{vx}	10.59^{ac}	9.39^{h-i}	8.46^{k-l}	12.37^{e}
Mean	**16.13^{a}**	**15.30^{b}**	**13.45^{c}**	**12.23^{d}**	**11.44^{e}**	**10.61^{f}**	**9.43^{g}**	**8.63^{h}**	

Means with same superscript are not significantly different

T_0: Distilled water (control), **T_1:** 1% ascorbic acid + 1% $CaCl_2$, **T_2:** 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_3:** 0.1% cysteine, **T_4:** 15% AVG, **T_5:** 15% AVG + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_6:** 15% AVG + 1% ascorbic acid + 1% $CaCl_2$, **T_7:** 15% AVG + 0.1% cysteine, **T_8:** 1% CMC, **T_9:** 1% CMC + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_{10}:** 1% CMC + 1% ascorbic acid + 1% $CaCl_2$, **T_{11}:** 1% CMC + 0.1% cysteine.

Table 4.18 Impact of anti-browning inhibitors and edible coatings on total phenols of apple slices during storage under 5 ± 2ºC

Treatment	Total phenols (mg 100 g^{-1})								
	Storage period (days)								
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Mean**
T_0	203.18^{km}	184.67^{zx}	171.53^{jh}	154.78^{on}	139.23^{qp}	117.00^{s}	94.13^{t}	75.38^{u}	142.49^{h}
T_1	205.23^{km}	202.21^{km}	197.06^{qm}	192.33^{qr}	182.40^{za}	167.25^{jk}	150.38^{op}	129.38^{qr}	178.28^{f}
T_2	207.00^{km}	204.29^{km}	200.24^{km}	194.93^{qm}	184.29^{za}	171.00^{jh}	157.50^{qm}	133.88^{q}	181.64^{e}
T_3	204.00^{km}	187.13^{wr}	183.00^{z-a}	181.16^{z-a}	169.50^{jk}	157.88^{ok}	134.25^{q}	112.88^{s}	166.22^{g}
T_4	204.86^{km}	187.50^{q-r}	184.03^{z-a}	181.25^{z-a}	169.85^{jk}	158.63^{ok}	135.38^{q}	117.00^{s}	167.31^{g}
T_5	218.13^{ba}	215.53^{b-c}	210.71^{e-f}	204.29^{km}	190.00^{qr}	182.25^{za}	172.88^{z-a}	159.38^{j-k}	194.14^{b}
T_6	215.00^{e-c}	212.38^{e-f}	207.18^{km}	200.12^{km}	187.69^{qr}	178.88^{za}	163.13^{j-k}	156.75^{onp}	190.14^{c}
T_7	209.43^{k-f}	206.40^{km}	202.50^{km}	196.92^{qm}	185.47^{wx}	172.13^{fa}	160.13^{j-k}	134.25^{q}	183.40^{ed}
T_8	205.71^{km}	188.24^{q-r}	186.50^{wr}	181.99^{z-a}	174.64^{z-a}	162.38^{jk}	135.75^{q}	119.63^{sr}	169.35^{g}
T_9	221.59^{a}	218.69^{ba}	214.60^{e-f}	209.85^{k-f}	198.13^{km}	184.50^{zx}	173.25^{z-a}	160.13^{j-k}	197.59^{a}
T_{10}	216.59^{b-c}	213.82^{e-f}	208.66^{k-f}	201.22^{km}	188.79^{q-r}	179.25^{za}	166.88^{j-k}	157.88^{o-k}	191.64^{cb}
T_{11}	212.50^{e-f}	209.50^{k-f}	205.15^{km}	199.77^{km}	186.41^{wr}	173.25^{za}	162.00^{j-k}	140.63^{qp}	186.15^{d}
Mean	**210.27^{a}**	**202.53^{b}**	**197.60^{c}**	**191.55^{d}**	**179.70^{e}**	**167.03^{f}**	**150.47^{g}**	**133.09^{h}**	

Means with same superscript are not significantly different

T_0: Distilled water (control), **T_1:** 1% ascorbic acid + 1% $CaCl_2$, **T_2:** 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_3:** 0.1% cysteine, **T_4:** 15% AVG, **T_5:** 15% AVG + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_6:** 15% AVG + 1% ascorbic acid + 1% $CaCl_2$, **T_7:** 15% AVG + 0.1% cysteine, **T_8:** 1% CMC, **T_9:** 1% CMC + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_{10}:** 1% CMC + 1% ascorbic acid + 1% $CaCl_2$, **T_{11}:** 1% CMC + 0.1% cysteine.

Table 4.19 Effect of anti-browning agents and edible coatings alone or in combination on total microbial count (log CFU g^{-1}) of minimally processed apple slices under cold storage (5 ± 2°C)

Storage period	Treatment												
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	Mean
Initial (0 day)	2.95^{g}	2.90^{hg}	2.85^{h-i}	2.90^{hg}	2.78^{hi}	2.48^{k}	2.60^{kj}	2.85^{h-i}	2.70^{ji}	2.48^{k}	2.60^{kj}	2.85^{h-i}	2.75^{b}
At end (7 day)	6.40^{a}	3.85^{cb}	3.84^{cb}	3.89^{b}	3.80^{c-d}	3.49^{fe}	3.65^{ed}	3.72^{cd}	3.78^{c-d}	3.48^{f}	3.64^{ed}	3.70^{cd}	3.77^{a}
Mean	**3.68^{a}**	**3.38^{cb}**	**3.35^{cb}**	**3.40^{b}**	**3.29^{cd}**	**2.99^{f}**	**3.13^{e}**	**3.29^{cd}**	**3.24^{d}**	**2.98^{f}**	**3.12^{e}**	**3.28^{cd}**	

Means with same superscript are not significantly different

T_0: Distilled water (control), **T_1:** 1% ascorbic acid + 1% $CaCl_2$, **T_2:** 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_3:** 0.1% cysteine, **T_4:** 15% AVG, **T_5:** 15% AVG + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_6:** 15% AVG + 1% ascorbic acid + 1% $CaCl_2$, **T_7:** 15% AVG + 0.1% cysteine, **T_8:** 1% CMC, **T_9:** 1% CMC + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% $CaCl_2$, **T_{10}:** 1% CMC + 1% ascorbic acid + 1% $CaCl_2$, **T_{11}:** 1% CMC + 0.1% cysteine.

stored under different modified atmospheric composition. During storage, firmness decreased in the range of 4.14–15.24 % in case of MAP samples while control samples showed significantly higher loss (24.03 %) of firmness under atmospheric condition during storage of 42 days. At the termination of experiment, samples stored in gas composition containing 5 % O_2 + 5 % CO_2 showed significantly ($P < 0.05$) higher firmness value (3.78 N) compared to other gas mixtures up to 42 days of cold storage (5 ± 2 ºC).

4.4.2 Browning index (BI)

The effect of modified atmospheric packaging conditions on the BI of minimally processed apple slices during storage at 5 ± 2 ºC is presented in Fig. 4.13. The increase in enzymatic browning during storage was accompanied by an increase in the BI values. The statistical analysis shows that applying the different atmospheric conditions had a significant effect on the BI. We observed that the apple slices stored under 5 % O_2 + 5 % CO_2 (T_8) exhibited a significantly lower BI than those stored under atmospheric (T_0) condition.

4.4.3 Whiteness index (WI)

The effect of varied gas compositions on the WI of apple slices during storage at 5 ± 2 ºC is presented in Fig. 4.14. The increase in browning during storage was accompanied by a drop in the whiteness index values. It was reported that the apple slices packed in different modified atmospheric composition inhibit the loss of colour. The whiteness index decreased in all samples with the advancement of storage but apple slices stored under 5 % O_2 + 5 CO_2 (T_8) and3 % O_2 + 2.5 CO_2 (T_4) conditions were decreased at a slower rate.

4.4.4 Headspace gas composition

A continuous decrease in the oxygen levels was observed in the control packs and modified atmosphere packs with a simultaneous increase in the CO_2 levels during storage at 5 ± 2 ºC (Tables 4.20 and 4.21). Initially, CO_2 concentration increased sharply in air packs compared to a relatively gradual increase in MAP samples. The values of ΔO_2 reached around -10.82 (%) for treatment T_8 (5 % O_2 + 5 CO_2) at the end of 42 days of storage. However, the values of ΔCO_2 reached to 12.24 % and 12.50 % for T_8 (5 % O_2 + 5 CO_2) and T_7 (5 % O_2 + 2.5 CO_2), respectively and 13.74 and 29.55

% for T_5 (3 % O_2 + 5 CO_2) and T_0 (normal atmospheric condition), respectively. On day 21 the off-flavors and off-odors were developed in the package of control samples. However, no off-flavors and off-odours were observed in MAP samples.

4.4.5 Weight loss

Fig. 4.15 shows the effect of modified Atmospheres on the weight loss of minimally processed apple slices during 42 days of storage at 5 ± 2 ºC. In general, weight loss for all samples increased continuously during storage period. A significant ($p < 0.05$) difference in weight loss was observed among the MAP samples. The control (T_0) samples (packed in normal atmospheric composition) showed 3.03 % of weight loss followed by 5 % O_2 + 5 % CO_2 (T_8) (0.114 %) and 5 % O_2 + 2.5 % CO_2 (T_7) (0.160 %) samples.

4.4.6 Browning potential

Enzymatic browning in terms of browning potential as A_{420} values has been presented in Fig. 4.16 for samples under different modified atmospheric conditions during storage. The difference in browning intensity was found to be significant ($p < 0.05$) amongst the different MA conditions. After 42 days of storage, air pack samples recorded nearly 1.81 browning potential values whilst MAP samples showed a restricted rise in browning potential.

4.4.7 Soluble solids content (SSC)

SSC content of air packs and modified atmosphere packs minimally processed apple slices during 42 days of storage is shown in Table 4.22. In air packs, the soluble solid content increased initially and declined again after achieving a peak on 14 days of storage. In case of MAP samples, there was a delayed peak. The average initial SSC content of minimally processed apple slices was 13.86 %.

4.4.8 Titratable acidity (TA)

Titratable acidity of the minimally processed apple slices during 42 day of storage is shown in Table 4.23. In general, the titratable acidity for all the samples decreased during storage. Apple slices stored under an atmosphere of 5 % O_2 + 5 % CO_2 (T_8) and 5 % O_2 + 2.5 % CO_2 (T_7) had higher retention of titratable acidity during the entire storage period. At the end of storage period, titratable acidity showed a reduction of 0.18 % for the control and 0.26, 0.34, 0.38, 0.37, 0.40, 0.36, 0.44, 0.49

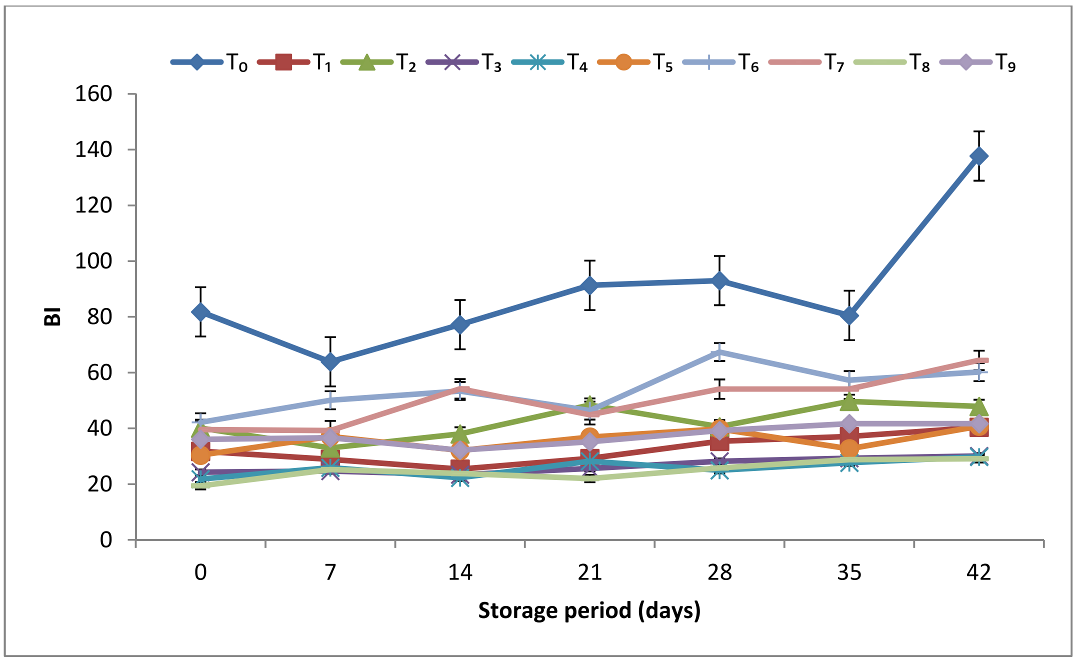

Fig. 4.13 Browning index (BI) of minimally processed apple slices stored under modified atmospheric conditions at low temperature (5 ± 2ºC)

T_0: air, **T_1:** 1.5 % O_2 + 2.5 % CO_2, **T_2:** 1.5 % O_2 + 5 % CO_2, **T_3:** 1.5 % O_2 + 7.5 % CO_2, **T_4:** 3 % O_2 + 2.5 % CO_2, **T_5:** 3 %O_2 + 5 % CO_2, **T_6:** 3 % O_2 + 7.5 % CO_2, **T_7:** 5 % O_2 + 2.5 % CO_2, **T_8:** 5 % O_2 + 5 % CO_2, **T_9:** 5 % O_2 + 7.5 % CO_2

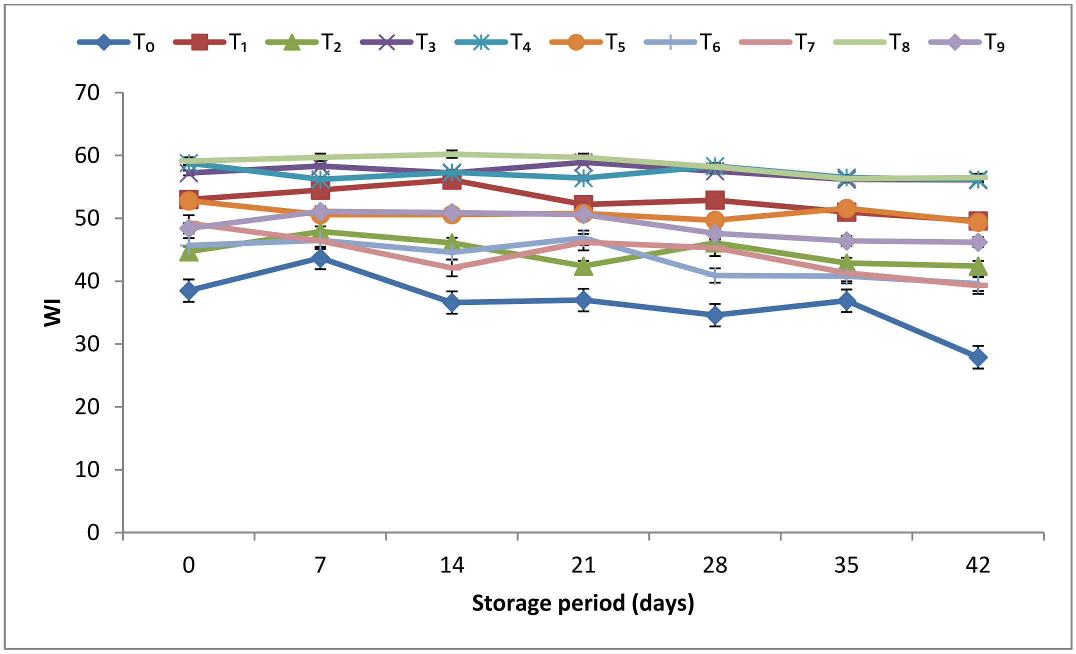

Fig. 4.14 Influence of modified atmospheres on whiteness index (WI) of minimally processed apple slices during storage at 5 ± 2°C

T_0: air, **T_1:** 1.5 % O_2 + 2.5 % CO_2, **T_2:** 1.5 % O_2 + 5 % CO_2, **T_3:** 1.5 % O_2 + 7.5 % CO_2, **T_4:** 3 % O_2 + 2.5 % CO_2, **T_5:** 3 %O_2 + 5 % CO_2, **T_6:** 3 % O_2 + 7.5 % CO_2, **T_7:** 5 % O_2 + 2.5 % CO_2, **T_8:** 5 % O_2 + 5 % CO_2, **T_9:** 5 % O_2 + 7.5 % CO_2

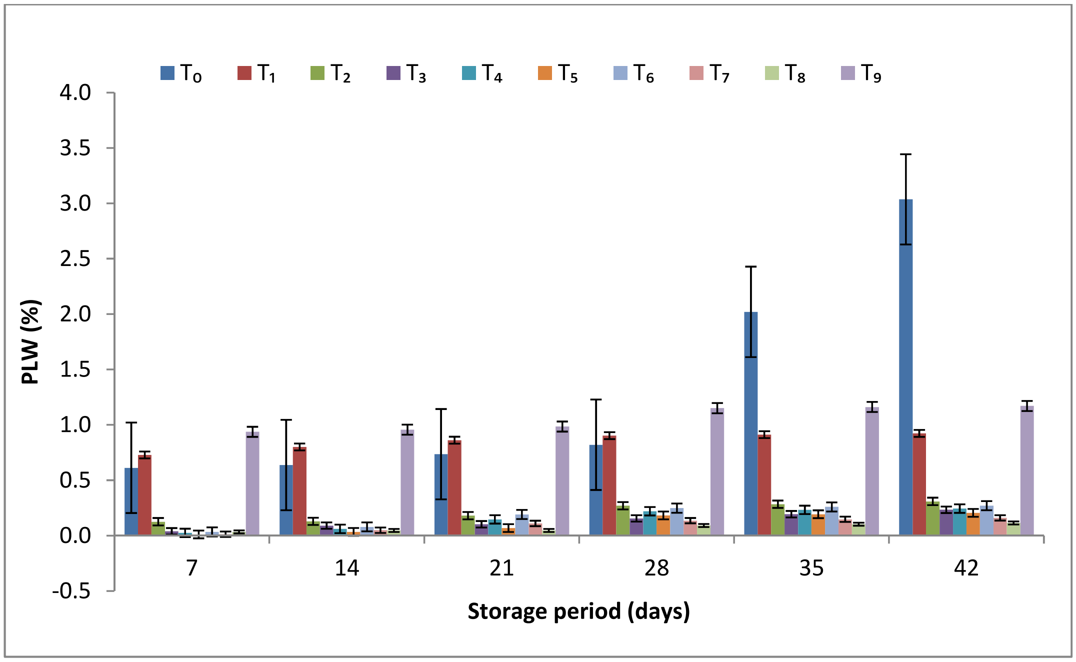

Fig. 4.15 Influence of modified atmospheric packaging on physiological loss in weight of minimally processed apple slices during storage at 5 ± 2ºC

T_0: air, **T_1:** 1.5 % O_2 + 2.5 % CO_2, **T_2:** 1.5 % O_2 + 5 % CO_2, **T_3:** 1.5 % O_2 + 7.5 % CO_2, **T_4:** 3 % O_2 + 2.5 % CO_2, **T_5:** 3 %O_2 + 5 % CO_2, **T_6:** 3 % O_2 + 7.5 % CO_2, **T_7:** 5 % O_2 + 2.5 % CO_2, **T_8:** 5 % O_2 + 5 % CO_2, **T_9:** 5 % O_2 + 7.5 % CO_2

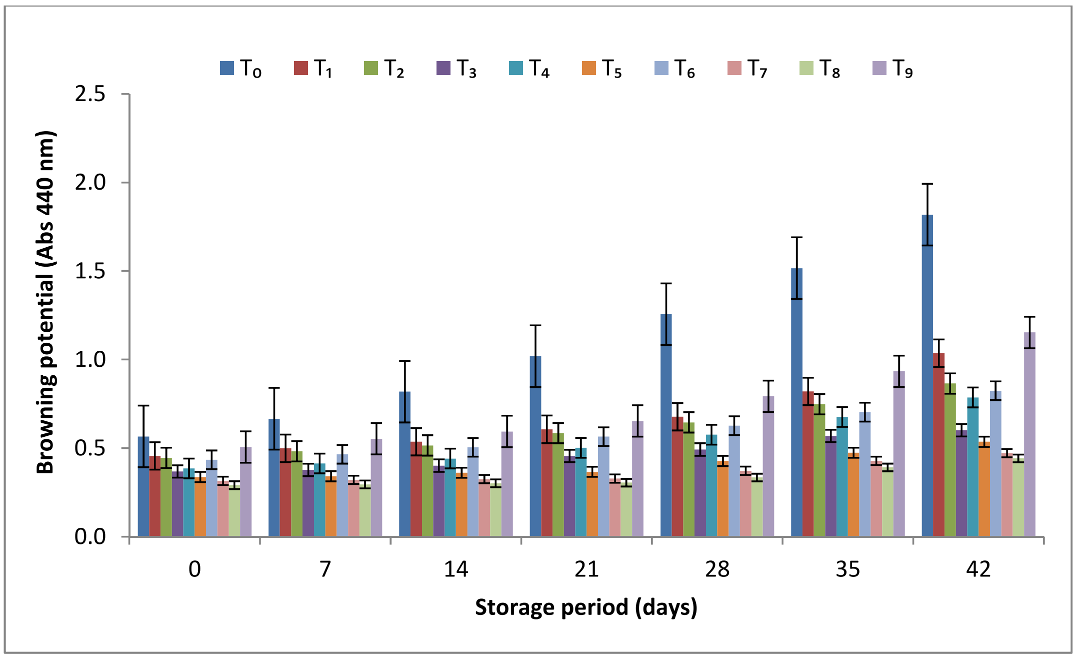

Fig. 4.16 Browning potential of apple slices packed under modified atmospheric conditions at 5 ± 2ºC
T_0: air, **T_1:** 1.5 % O_2 + 2.5 % CO_2, **T_2:** 1.5 % O_2 + 5 % CO_2, **T_3:** 1.5 % O_2 + 7.5 % CO_2, **T_4:** 3 % O_2 + 2.5 % CO_2, **T_5:** 3 %O_2 + 5 % CO_2, **T_6:** 3 % O_2 + 7.5 % CO_2, **T_7:** 5 % O_2 + 2.5 % CO_2, **T_8:** 5 % O_2 + 5 % CO_2, **T_9:** 5 % O_2 + 7.5 % CO_2

Table 4.20 Oxygen concentration in modified atmosphere packs stored at 5 ± 2ºC

Treatment	O₂ (%)						
	Storage period (days)						
	7	**14**	**21**	**28**	**35**	**42**	**Mean**
T_0	-4.15[g-f]	-6.46	-10.61[p]	-14.49[ts]	-17.10[u]	-25.17[v]	-11.14[h]
T_1	-3.56[de]	-4.66[gh]	-4.89[h]	-9.65[nm]	-10.68[p]	-14.23[s]	-6.81[f]
T_2	-3.09[dc]	-3.65[d-f]	-6.83[j]	-9.63[nm]	-10.66[p]	-13.43[r]	-6.76[ef]
T_3	-3.53[de]	-5.10[h]	-5.89[i]	-8.52[k]	-9.96[no]	-12.52[q]	-6.50[d]
T_4	-2.63[c]	-3.63[d-f]	-6.78[j]	-9.55[nm]	-10.88[p]	-12.96[rq]	-6.63[ed]
T_5	-1.73[b]	-2.67[c]	-5.14[h]	-8.63[lk]	-9.45[nm]	-11.12[p]	-5.53[c]
T_6	-2.63[c]	-3.63[d-f]	-6.79[j]	-9.56[nm]	-10.57[p]	-13.31[r]	-6.64[ed]
T_7	-1.73[b]	-1.76[b]	-5.09[h]	-8.53[k]	-9.33[m]	-10.96[p]	-5.34[b]
T_8	-0.86[a]	-1.74[b]	-5.04[h]	-8.44[k]	-9.22[lm]	-10.82[p]	-5.16[a]
T_9	-4.10[g-f]	-4.78[h]	-5.02[h]	-10.54[po]	-11.09[p]	-14.86[t]	-7.20[g]
Mean	**-2.80[a]**	**-3.81[b]**	**-6.21[c]**	**-9.75[d]**	**-10.89[e]**	**-13.94[f]**	

Means with same superscript are not significantly different.

T_0: air, **T_1:** 1.5 % O_2 + 2.5 % CO_2, **T_2:** 1.5 % O_2 + 5 % CO_2, **T_3:** 1.5 % O_2 + 7.5 % CO_2, **T_4:** 3 % O_2 + 2.5 % CO_2, **T_5:** 3 %O_2 + 5 % CO_2, **T_6:** 3 % O_2 + 7.5 % CO_2, **T_7:** 5 % O_2 + 2.5 % CO_2, **T_8:** 5 % O_2 + 5 % CO_2, **T_9:** 5 % O_2 + 7.5 % CO_2

Table 4.21 Carbon dioxide concentration in modified atmosphere packs stored at 5 ± 2°C

Treatment	CO_2 (%)						
	Storage period (days)						
	7	**14**	**21**	**28**	**35**	**42**	**Mean**
T_0	4.73^{cd}	8.15^{xw}	13.21	18.77^{d}	23.46^{b}	29.55^{a}	13.98^{a}
T_1	4.55^{ce}	8.33^{xw}	9.68^{r-s}	12.68^{kl}	16.80^{g}	18.47^{ed}	10.07^{b}
T_2	3.80^{fe}	6.51^{az}	8.65^{vw}	11.48^{mn}	14.69^{h}	18.06^{ed}	9.03^{c}
T_3	3.77^{fe}	6.47^{az}	7.61^{yx}	9.80^{rs}	10.92^{o-p}	14.55^{h}	7.59^{e}
T_4	3.11^{gf}	5.85^{ab}	8.56^{vw}	10.53^{op}	14.34^{ih}	16.72^{g}	8.44^{d}
T_5	3.11^{gf}	5.85^{ab}	7.57^{yx}	8.87^{vw}	10.18^{r-p}	13.74^{ij}	7.04^{f}
T_6	3.14^{gf}	6.47^{az}	8.60^{vw}	11.00^{on}	14.69^{h}	17.79^{ef}	8.81^{c}
T_7	2.48^{gh}	5.85^{ab}	7.07^{yz}	8.91^{vw}	9.82^{r-s}	12.50^{kl}	6.66^{g}
T_8	1.86^{h}	5.29^{cb}	6.08^{ab}	7.18^{yz}	9.30^{v-s}	12.24^{ml}	5.99^{h}
T_9	4.40^{de}	7.56^{yx}	9.47^{r-s}	13.24^{kj}	17.05^{gf}	19.76^{c}	10.21^{b}
Mean	$\mathbf{3.50^{f}}$	$\mathbf{6.63^{e}}$	$\mathbf{8.65^{d}}$	$\mathbf{11.25^{c}}$	$\mathbf{14.13^{b}}$	$\mathbf{17.34^{a}}$	

Means with same superscript are not significantly different

T_0: air, **T_1:** 1.5 % O_2 + 2.5 % CO_2, **T_2:** 1.5 % O_2 + 5 % CO_2, **T_3:** 1.5 % O_2 + 7.5 % CO_2, **T_4:** 3 % O_2 + 2.5 % CO_2, **T_5:** 3 %O_2 + 5 % CO_2, **T_6:** 3 % O_2 + 7.5 % CO_2, **T_7:** 5 % O_2 + 2.5 % CO_2, **T_8:** 5 % O_2 + 5 % CO_2, **T_9:** 5 % O_2 + 7.5 % CO_2

Table 4.22 Effect of modified atmospheric packaging on soluble solids content (SSC) of minimally processed apple slices during storage at 5 ± 2°C

Treatment	SSC (%)							
	Storage period (days)							
	0	7	14	21	28	35	42	Mean
T_0	14.0^{j-l}	14.9^{e-f}	16.1^{ba}	13.6^{l}	11.9^{m}	11.0^{m}	9.7^{n}	13.0^{d}
T_1	13.7^{kl}	14.1^{j-l}	14.6^{e-f}	15.2^{e-f}	16.1^{ba}	14.7^{e-f}	13.7^{kl}	14.5^{ba}
T_2	13.7^{kl}	14.1^{j-l}	14.5^{e-f}	15.1^{e-f}	15.9^{b-c}	14.8^{e-f}	13.8^{j-l}	14.5^{ba}
T_3	13.8^{j-l}	14.1^{j-l}	14.5^{e-f}	14.8^{e-f}	15.3^{e-f}	15.6^{b-c}	15.9^{b-c}	14.8^{a}
T_4	13.9^{j-l}	14.1^{j-l}	14.4^{e-f}	15.1^{e-f}	15.9^{b-c}	15.0^{e-f}	14.1^{j-l}	14.6^{ba}
T_5	13.8^{j-l}	13.9^{j-l}	14.2^{j-f}	14.1^{j-l}	14.7^{e-f}	15.0^{e-f}	15.4^{e-c}	14.4^{bc}
T_6	13.9^{j-l}	14.2^{j-f}	14.5^{e-f}	15.2^{e-f}	16.1^{ba}	14.8^{e-f}	14.0^{j-l}	14.6^{ba}
T_7	13.9^{j-l}	13.9^{j-l}	13.9^{j-l}	14.0^{j-l}	14.2^{j-f}	14.4^{e-f}	14.6^{e-f}	14.1^{c}
T_8	14.0^{j-l}	14.1^{j-l}	14.2^{j-f}	14.2^{j-f}	14.6^{e-f}	14.9^{e-f}	15.3^{e-f}	14.4^{b}
T_9	13.9^{j-l}	14.2^{j-f}	14.5^{e-f}	15.3^{e-f}	16.2^{a}	14.6^{e-f}	13.6^{l}	14.6^{ba}
Mean	**13.8^{d}**	**14.1^{c}**	**14.5^{b}**	**14.6^{b}**	**15.0^{a}**	**14.4^{b}**	**14.0^{dc}**	

Means with same superscript are not significantly different.

T_0: air, **T_1:** 1.5 % O_2 + 2.5 % CO_2, **T_2:** 1.5 % O_2 + 5 % CO_2, **T_3:** 1.5 % O_2 + 7.5 % CO_2, **T_4:** 3 % O_2 + 2.5 % CO_2, **T_5:** 3 %O_2 + 5 % CO_2, **T_6:** 3 % O_2 + 7.5 % CO_2, **T_7:** 5 % O_2 + 2.5 % CO_2, **T_8:** 5 % O_2 + 5 % CO_2, **T_9:** 5 % O_2 + 7.5 % CO_2

Table 4.23 Titratable acidity of modified atmosphere packed minimally processed apple slices during storage at 5 ± 2°C

Treatment	Titratable acidity (%)							
	Storage period (days)							
	0	**7**	**14**	**21**	**28**	**35**	**42**	**Mean**
T_0	0.53^{b-c}	0.48^{e-f}	0.42^{k-i}	0.37^{n-o}	0.30^{q}	0.24^{s}	0.18^{t}	0.36^{h}
T_1	0.55^{ba}	0.53^{b-c}	0.52^{b-c}	0.50^{e-c}	0.40^{k-l}	0.34^{po}	0.26^{sr}	0.44^{f}
T_2	0.55^{ba}	0.52^{b-c}	0.50^{e-c}	0.49^{e-f}	0.41^{k-l}	0.37^{n-o}	0.34^{po}	0.46^{e}
T_3	0.55^{ba}	0.54^{ba}	0.53^{b-c}	0.52^{b-c}	0.49^{e-f}	0.45^{h-i}	0.38^{n-l}	0.50^{c}
T_4	0.55^{a}	0.54^{ba}	0.53^{b-c}	0.52^{b-c}	0.48^{e-f}	0.41^{k-l}	0.37^{n-o}	0.49^{c}
T_5	0.56^{a}	0.55^{ba}	0.54^{ba}	0.53^{b-c}	0.52^{b-c}	0.46^{h-f}	0.40^{k-l}	0.51^{b}
T_6	0.55^{ba}	0.53^{b-c}	0.52^{b-c}	0.50^{e-c}	0.48^{e-f}	0.38^{n-l}	0.36^{no}	0.47^{d}
T_7	0.56^{a}	0.55^{ba}	0.54^{ba}	0.53^{b-c}	0.52^{b-c}	0.48^{e-f}	0.44^{h-i}	0.52^{b}
T_8	0.56^{a}	0.55^{ba}	0.54^{ba}	0.53^{b-c}	0.52^{b-c}	0.50^{e-c}	0.49^{e-f}	0.53^{a}
T_9	0.54^{ba}	0.53^{b-c}	0.48^{e-f}	0.45^{h-i}	0.37^{n-o}	0.32^{qp}	0.29^{qr}	0.43^{g}
Mean	**0.55^{a}**	**0.53^{b}**	**0.51^{c}**	**0.50^{d}**	**0.45^{e}**	**0.40^{f}**	**0.35^{g}**	

Means with same superscript are not significantly different

T_0: air, **T_1:** 1.5 % O_2 + 2.5 % CO_2, **T_2:** 1.5 % O_2 + 5 % CO_2, **T_3:** 1.5 % O_2 + 7.5 % CO_2, **T_4:** 3 % O_2 + 2.5 % CO_2, **T_5:** 3 %O_2 + 5 % CO_2, **T_6:** 3 % O_2 + 7.5 % CO_2, **T_7:** 5 % O_2 + 2.5 % CO_2, **T_8:** 5 % O_2 + 5 % CO_2, **T_9:** 5 % O_2 + 7.5 % CO_2

and 0.29 % for T_1 to T_9, respectively.

4.4.9 Ascorbic acid content

Table 4.24 shows the influence of modified atmospheric conditions on the ascorbic acid content of minimally processed apple slices stored at 5 ± 2 ºC for 42 days. Initially, the average ascorbic acid content of minimally processed apple slices was 26.0 mg 100 g^{-1} FW. Ascorbic acid value of air pack (T_0) samples was always lower than MAP samples (T_1 to T_9) during storage. A higher reduction in ascorbic acid content was observed in air pack samples compared to MAP samples. Ascorbic acid values at the end of the storage period for air pack samples was 11.0 g^{-1} FW on day 42 and while a content of 24.4 g^{-1} FW ascorbic acid was recorded for slices packed under 5 % O_2 + 2.5 % CO_2 (T_7).

4.4.10 Antioxidant activity

Antioxidant activity of the minimally processed apple slices decreased for all the samples during storage of 42 days at 5 ± 2 ºC (Table 4.25). Air packed apple slices (T_0) exhibited lower antioxidant activity than active MAP samples throughout storage period. Antioxidant activity values for air pack samples (T_0) at the end of the storage period was 5.63 µmol Trolox 100 g^{-1} whereas for MAP samples it was 12.3 and 7.0 µmol Trolox 100 g^{-1} for T_8 (5 % O_2 + 5 % CO_2), and T_9 (5 % O_2 + 7.5 % CO_2) treatments, respectively.

4.4.11 Total phenolic content

In the present study, it was observed that the total phenolic content decreased during storage of minimally processed apple slices in modified atmospheres as well as in normal atmospheres slices (Table 4.26). Minimally processed apple slices under different modified atmospheric conditions showed a significantly ($p < 0.05$) lower loss in total phenols as compared to normal atmospheres packed samples which recorded a higher degree of reduction in phenolics after just 7 days of storage. In the present study, the gas flushed polypropylene trays with 5 % O_2 + 5 % CO_2 (T_8) showed maximum retention of total phenolics followed by 5 % O_2 + 2.5 % CO_2 (T_7) and 3 % O_2 + 5 % CO_2 (T_5) during 42 days of storage.

4.4.12 Polyphenol oxidase (PPO) activity

Table 4.27 shows that PPO activity increased substantially in air packs slices for the first 21 days, before decreasing during the later period of storage. Similarly, for apple slices under different modified atmospheres, the PPO activity was retarded and was lower than the normal atmospheres samples. The PPO activity at the end of the storage period for control samples (air packs) was 50 U/min/g on day 42 while for MAP samples were 36.50, 50.0, 39.0, 45.0 and 48.0 units/min/g for T_8 (5 % O_2 + 5 % CO_2), T_7 (5 % O_2 + 2.5 % CO_2), T_5 (3 % O_2 + 5 % CO_2) and T_3 (1.5 % O_2 + 7.5 % CO_2) on day 42, respectively. The treatment 5 % O_2 + 5 % CO_2 (T_8) was found to be more efficient in inhibiting the polyphenol oxidase activity.

4.4.13 Peroxidase (POD) activity

POD activity increased sharply during the initial stage of storage in air packed samples compared to a relatively gradual increase in modified atmospheres packed apple samples (Table 4.28). POD activity reached a maximum value of around 130.0 units/min/g for control samples at the 21 days followed by a sharp decline. POD values at the end of the storage period for control samples was 95.50 units/min/g on day 42 and 65.50 and 141.5 U/min/g for T_8 (5 % O_2 + 5 % CO_2) and T_9 (5 % O_2 + 7.5 % CO_2) samples on day 42, respectively. A significant difference was recorded between all treatments for each gas composition ($p < 0.05$).

4.4.14 Microbial growth

The microbiological quality of the apple slices packed under different gas compositions with respect to total aerobic plate count is given in Table 4.29. An increase in microbial count was observed for all the samples. Modified atmospheric packaged apple slices tend to reduce the microbial counts. Air packs apple samples show the highest microbial growth. The initial microbial counts gradually increased from 3.38 log CFU to 6.10 log CFU in the air pack samples during 42 days of storage. Minimally processed apple slices packed under different modified atmospheres (T_1 to T_9) showed better control over the microbial count compared with the samples under normal atmospheres (T_0).

4.4.15 Sensory characteristics

Fig. 4.17 represents the browning, flavour, texture, sweetness, sourness and overall acceptability scores for all minimally processed apple slices. A wide variation in the selected sensory parameters was observed among the MAP (T_0 to T_9) and air pack samples. Colour, flavour and texture were considered as the most important

Table 4.24 Impact of modified atmospheres on ascorbic acid content of minimally processed apple slices during storage at 5 ± 2°C

Treatment	Ascorbic acid content (mg 100 g^{-1} FW)							
	Storage period (days)							
	0	7	14	21	28	35	42	Mean
T_0	23.3^{q-t}	20.6^{a-b}	18.5^{fg}	16.2^{j}	14.4^{k}	12.8^{l}	11.0^{m}	16.7^{g}
T_1	24.9^{l-n}	23.7^{q-t}	22.8^{r-t}	22.2^{y-t}	21.6^{a-b}	19.2^{f-e}	17.8^{h-i}	21.7^{e}
T_2	25.0^{l-n}	24.0^{q-s}	23.5^{q-t}	21.2^{a-b}	20.8^{a-b}	18.3^{hg}	16.9^{h-i}	21.4^{e}
T_3	27.3^{e-c}	26.3^{l-f}	26.2^{l-f}	25.1^{l-n}	24.4^{q-n}	23.5^{q-t}	22.0^{a-b}	25.0^{c}
T_4	27.2^{e-f}	26.0^{l-f}	25.0^{l-n}	24.2^{q-n}	23.0^{q-t}	22.0^{a-b}	20.5^{d-b}	24.0^{d}
T_5	27.0^{e-f}	26.7^{e-f}	26.4^{l-f}	25.7^{l-f}	25.2^{l-n}	24.0^{q-s}	23.7^{q-t}	25.5^{b}
T_6	26.0^{l-f}	25.5^{l-n}	25.3^{l-n}	24.2^{q-n}	23.7^{q-t}	22.2^{a-z}	19.8^{d-e}	23.8^{d}
T_7	27.9^{ba}	27.6^{b-c}	27.0^{e-f}	26.5^{e-f}	25.9^{l-f}	24.9^{l-n}	24.4^{q-n}	26.3^{a}
T_8	28.2^{a}	27.8^{b-c}	27.2^{e-f}	26.6^{e-f}	26.6^{e-f}	25.1^{l-n}	24.0^{q-s}	26.5^{a}
T_9	23.3^{q-t}	22.6^{y-t}	21.8^{a-b}	20.5^{d-b}	19.4^{d-e}	17.8^{h-i}	16.4^{ji}	20.3^{f}
Mean	**26.0^{a}**	**25.1^{b}**	**24.4^{c}**	**23.2^{d}**	**22.5^{e}**	**21.0^{f}**	**19.6^{g}**	

Means with same superscript are not significantly different

T_0: air, **T_1:** 1.5 % O_2 + 2.5 % CO_2, **T_2:** 1.5 % O_2 + 5 % CO_2, **T_3:** 1.5 % O_2 + 7.5 % CO_2, **T_4:** 3 % O_2 + 2.5 % CO_2, **T_5:** 3 %O_2 + 5 % CO_2, **T_6:** 3 % O_2 + 7.5 % CO_2, **T_7:** 5 % O_2 + 2.5 % CO_2, **T_8:** 5 % O_2 + 5 % CO_2, **T_9:** 5 % O_2 + 7.5 % CO_2

Table 4.25 Changes in total antioxidant activity of apple slices stored under modified atmospheres at 5 ± 2ºC

Treatment	Total antioxidant activity (µmol Trolox g^{-1} FW)							
	Storage period (days)							
	0	**7**	**14**	**21**	**28**	**35**	**42**	**Mean**
T_0	$15.0^{g\text{-}h}$	$12.1^{v\text{-}u}$	$10.3^{a\text{-}c}$	$9.1^{f\text{-}e}$	8.1^{hi}	7.1^{kj}	5.6^{l}	9.6^{g}
T_1	$15.6^{e\text{-}d}$	$14.5^{k\text{-}l}$	$11.3^{y\text{-}z}$	$10.8^{a\text{-}z}$	$9.8^{d\text{-}e}$	$8.9^{h\text{-}g}$	7.9^{ji}	11.3^{e}
T_2	$15.6^{e\text{-}d}$	$13.9^{n\text{-}l}$	$11.4^{v\text{-}z}$	$10.8^{a\text{-}z}$	$10.0^{d\text{-}e}$	$8.9^{h\text{-}g}$	8.0^{ji}	11.2^{e}
T_3	16.0^{bc}	$16.0^{b\text{-}d}$	$14.7^{g\text{-}h}$	$13.5^{n\text{-}o}$	$12.4^{t\text{-}q}$	$11.2^{a\text{-}z}$	$9.9^{d\text{-}e}$	13.4^{c}
T_4	$15.7^{e\text{-}d}$	$15.5^{g\text{-}h}$	$14.8^{g\text{-}h}$	13.4^{po}	$12.4^{t\text{-}u}$	$11.1^{a\text{-}z}$	$9.8^{d\text{-}e}$	13.2^{c}
T_5	17.2^{a}	16.7^{ba}	$14.6^{k\text{-}h}$	$13.1^{p\text{-}q}$	$12.7^{p\text{-}q}$	$11.7^{v\text{-}u}$	$11.0^{a\text{-}z}$	13.9^{b}
T_6	$15.6^{e\text{-}d}$	$15.1^{g\text{-}h}$	$13.5^{n\text{-}o}$	$12.3^{v\text{-}u}$	$11.5^{v\text{-}u}$	$10.6^{a\text{-}z}$	$9.2^{f\text{-}e}$	12.5^{d}
T_7	17.4^{a}	17.1^{a}	$15.2^{g\text{-}h}$	$14.3^{n\text{-}l}$	$13.6^{n\text{-}o}$	$13.3^{p\text{-}q}$	12.3^{vu}	14.7^{a}
T_8	17.5^{a}	16.8^{ba}	$15.7^{e\text{-}d}$	$14.3^{n\text{-}l}$	$13.9^{n\text{-}l}$	$13.7^{n\text{-}l}$	12.3^{tu}	14.9^{a}
T_9	$15.5^{g\text{-}d}$	$13.6^{n\text{-}o}$	$11.7^{v\text{-}u}$	$10.^{a\text{-}z}$	$9.6^{d\text{-}e}$	$8.3^{h\text{-}i}$	7.0^{k}	10.9^{f}
Mean	$\mathbf{16.1^{a}}$	$\mathbf{15.1^{b}}$	$\mathbf{13.3^{c}}$	$\mathbf{12.2^{d}}$	$\mathbf{11.4^{e}}$	$\mathbf{10.5^{f}}$	$\mathbf{9.3^{g}}$	

Means with same superscript are not significantly different.

T_0: air, **T_1:** 1.5 % O_2 + 2.5 % CO_2, **T_2:** 1.5 % O_2 + 5 % CO_2, **T_3:** 1.5 % O_2 + 7.5 % CO_2, **T_4:** 3 % O_2 + 2.5 % CO_2, **T_5:** 3 %O_2 + 5 % CO_2, **T_6:** 3 % O_2 + 7.5 % CO_2, **T_7:** 5 % O_2 + 2.5 % CO_2, **T_8:** 5 % O_2 + 5 % CO_2, **T_9:** 5 % O_2 + 7.5 % CO_2

Table 4.26 Total phenols of minimally processed apple slices as affected by modified atmospheric packaging during storage under 5 ± 2ºC

Treatment	Total phenols (mg GAE 100 g^{-1} FW)							
	Storage period (days)							
	0	7	14	21	28	35	42	Mean
T_0	$205.9^{o\text{-}p}$	187.5^{vw}	$174.5^{c\text{-}d}$	156.2^{gf}	140.7^{ih}	118.5^{j}	95.6^{k}	154.1^{h}
T_1	$210.7^{n\text{-}k}$	$196.4^{o\text{-}p}$	191.5^{vw}	$180.4^{v\text{-}w}$	$174.6^{c\text{-}d}$	$160.5^{e\text{-}f}$	137.2^{i}	178.7^{f}
T_2	$216.7^{g\text{-}h}$	$201.2^{o\text{-}p}$	$195.0^{o\text{-}p}$	$181.1^{v\text{-}w}$	$178.1^{c\text{-}w}$	$164.2^{e\text{-}f}$	137.6^{i}	182.0^{fe}
T_3	$228.1^{e\text{-}c}$	$226.4^{e\text{-}h}$	$225.7^{e\text{-}h}$	$217.5^{g\text{-}h}$	$195.1^{o\text{-}p}$	$176.6^{c\text{-}x}$	$166.5^{e\text{-}f}$	205.1^{c}
T_4	$224.3^{e\text{-}h}$	$212.6^{l\text{-}h}$	$200.7^{o\text{-}p}$	$194.2^{v\text{-}p}$	$186.4^{v\text{-}w}$	$173.2^{e\text{-}d}$	$159.7^{e\text{-}f}$	193.0^{d}
T_5	$235.6^{b\text{-}c}$	$228.7^{e\text{-}c}$	$227.0^{e\text{-}c}$	$221.0^{e\text{-}h}$	$208.7^{o\text{-}k}$	$181.5^{v\text{-}w}$	$169.5^{e\text{-}f}$	210.3^{b}
T_6	$221.5^{e\text{-}h}$	$195.1^{o\text{-}p}$	190.2^{vw}	$182.5^{v\text{-}w}$	$178.3^{c\text{-}w}$	$169.5^{e\text{-}f}$	152.6^{gh}	184.2^{e}
T_7	$237.8^{b\text{-}c}$	$235.2^{e\text{-}c}$	$233.6^{e\text{-}c}$	$225.0^{e\text{-}h}$	$211.7^{l\text{-}k}$	$187.5^{v\text{-}w}$	$176.2^{c\text{-}x}$	215.3^{a}
T_8	244.0^{a}	241.8^{ba}	$238.3^{b\text{-}c}$	$229.4^{e\text{-}c}$	$213.4^{g\text{-}h}$	$188.2^{v\text{-}w}$	$178.5^{c\text{-}w}$	219.1^{a}
T_9	$207.2^{o\text{-}p}$	191.1^{vw}	182.3^{vw}	$176.3^{c\text{-}x}$	$166.0^{e\text{-}f}$	155.6^{gf}	135.7^{i}	173.5^{g}
Mean	**223.2^{a}**	**211.6^{b}**	**205.9^{c}**	**196.4^{d}**	**185.3^{e}**	**167.5^{f}**	**150.9^{g}**	

Means with same superscript are not significantly different

T_0: air, **T_1:** 1.5 % O_2 + 2.5 % CO_2, **T_2:** 1.5 % O_2 + 5 % CO_2, **T_3:** 1.5 % O_2 + 7.5 % CO_2, **T_4:** 3 % O_2 + 2.5 % CO_2, **T_5:** 3 %O_2 + 5 % CO_2, **T_6:** 3 % O_2 + 7.5 % CO_2, **T_7:** 5 % O_2 + 2.5 % CO_2, **T_8:** 5 % O_2 + 5 % CO_2, **T_9:** 5 % O_2 + 7.5 % CO_2

Table 4.27 Polyphenol oxidase (PPO) activity of minimally processed apple slices packed in modified atmospheres

Treatment	PPO activity (U min^{-1} g^{-1})							
	Storage period (days)							
	0	**7**	**14**	**21**	**28**	**35**	**42**	**Mean**
T_0	37.5^{s-q}	48.0^{j-k}	62.0^{e}	77.0^{a}	67.5^{c}	58.5^{f}	50.0^{ji}	57.2^{a}
T_1	37.5^{s-q}	41.5^{p-n}	46.0^{m-k}	50.5^{hi}	58.0^{f}	65.5^{dc}	73.0^{b}	53.1^{c}
T_2	36.5^{s-u}	39.0^{p-q}	44.0^{m-n}	47.5^{j-k}	53.5^{hg}	62.5^{de}	68.0^{c}	50.1^{d}
T_3	30.0^{yx}	33.0^{w-x}	35.5^{s-u}	39.0^{p-q}	40.0^{p-q}	42.0^{p-n}	45.5^{m-k}	37.8^{g}
T_4	32.5^{w-x}	35.0^{s-u}	37.5^{s-q}	40.0^{p-q}	43.0^{m-n}	46.0^{m-k}	48.5^{j-k}	40.3^{f}
T_5	29.0^{zy}	30.0^{yx}	32.5^{w-x}	34.0^{w-u}	35.5^{s-u}	36.5^{s-u}	39.0^{p-q}	33.7^{h}
T_6	34.0^{w-u}	39.5^{p-q}	44.0^{m-n}	45.5^{m-k}	48.5^{j-k}	50.5^{hi}	54.5^{g}	45.2^{e}
T_7	28.5^{zy}	30.0^{yx}	31.0^{w-x}	31.5^{w-x}	32.5^{w-x}	33.5^{w-u}	37.0^{s-q}	32.0^{i}
T_8	26.5^{z}	29.0^{zy}	30.0^{yx}	31.0^{w-x}	33.5^{w-u}	35.0^{s-u}	36.5^{s-u}	31.6^{i}
T_9	37.0^{s-q}	41.5^{p-n}	47.0^{j-k}	53.5^{hg}	62.0^{e}	68.0^{c}	76.0^{ba}	55.0^{b}
Mean	$\mathbf{32.9^{g}}$	$\mathbf{36.6^{f}}$	$\mathbf{40.9^{e}}$	$\mathbf{44.9^{d}}$	$\mathbf{47.4^{c}}$	$\mathbf{49.8^{b}}$	$\mathbf{52.8^{a}}$	

Means with same superscript are not significantly different.

T_0: air, **T_1:** 1.5 % O_2 + 2.5 % CO_2, **T_2:** 1.5 % O_2 + 5 % CO_2, **T_3:** 1.5 % O_2 + 7.5 % CO_2, **T_4:** 3 % O_2 + 2.5 % CO_2, **T_5:** 3 %O_2 + 5 % CO_2, **T_6:** 3 % O_2 + 7.5 % CO_2, **T_7:** 5 % O_2 + 2.5 % CO_2, **T_8:** 5 % O_2 + 5 % CO_2, **T_9:** 5 % O_2 + 7.5 % CO_2

Table 4.28 Influence of modified atmospheric packaging on the peroxidase (POD) activity of minimally processed apple slices during storage at 5 ± 2ºC

Treatment	POD activity (U min $^{-1}$ g $^{-1}$)							
	Storage period (days)							
	0	**7**	**14**	**21**	**28**	**35**	**42**	**Mean**
T_0	61.5^{y-z}	82.0^{lm}	103.5^{fg}	130.0^{b}	120.0^{c}	108.1^{fe}	95.5^{hi}	100.0^{a}
T_1	56.5^{b-z}	65.0^{t-w}	75.5^{n-o}	85.5^{lk}	99.0^{hg}	111.5^{de}	126.5^{b}	88.5^{b}
T_2	55.0^{e-f}	63.0^{y-w}	71.0^{q-s}	82.5^{lm}	93.0^{ij}	106.5^{fe}	115.0^{dc}	83.7^{c}
T_3	49.0^{i-g}	51.5^{e-f}	55.0^{e-f}	59.5^{y-z}	63.0^{y-w}	67.0^{t-s}	71.0^{q-s}	59.4^{g}
T_4	52.5^{e-f}	57.5^{y-z}	63.5^{v-w}	66.5^{t-s}	77.5^{n-o}	85.0^{lk}	88.5^{kj}	70.1^{e}
T_5	48.0^{ih}	50.0^{e-f}	53.0^{e-f}	55.5^{e-c}	61.5^{y-z}	67.5^{t-s}	72.0^{q-o}	58.2^{g}
T_6	54.5^{e-f}	59.0^{y-z}	66.5^{t-s}	73.0^{q-o}	79.0^{nm}	86.5^{lk}	94.0^{h-j}	73.2^{d}
T_7	47.5^{ih}	49.5^{i-f}	52.0^{e-f}	55.0^{e-f}	59.0^{y-z}	63.0^{y-w}	65.5^{t-s}	55.9^{h}
T_8	45.5^{i}	48.0^{ih}	51.5^{e-f}	54.0^{e-f}	57.5^{y-z}	62.0^{y-z}	65.5^{t-s}	54.8^{h}
T_9	58.0^{y-z}	70.5^{t-s}	81.5^{lm}	96.0^{hi}	114.0^{d}	128.0^{b}	141.5^{a}	98.5^{a}
Mean	**52.8^{g}**	**59.6^{f}**	**67.3^{e}**	**75.7^{d}**	**82.3^{c}**	**88.5^{b}**	**93.5^{a}**	

Means with same superscript are not significantly different

T_0: air, **T_1:** 1.5 % O_2 + 2.5 % CO_2, **T_2:** 1.5 % O_2 + 5 % CO_2, **T_3:** 1.5 % O_2 + 7.5 % CO_2, **T_4:** 3 % O_2 + 2.5 % CO_2, **T_5:** 3 %O_2 + 5 % CO_2, **T_6:** 3 % O_2 + 7.5 % CO_2, **T_7:** 5 % O_2 + 2.5 % CO_2, **T_8:** 5 % O_2 + 5 % CO_2, **T_9:** 5 % O_2 + 7.5 % CO_2

Table 4.29 Changes in total microbial counts (log_{10} CFU g^{-1}) on minimally processed apple slices stored under modified atmospheric conditions at 5 ± 2ºC

Storage period	Treatment										
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	Mean
Initial (0 day)	3.38^{g}	3.34^{hg}	3.28^{hg}	3.00^{h-i}	3.11^{kj}	$2.90^{ji\ i}$	3.23^{bi}	2.85^{k}	2.18^{k}	3.32^{b-}	3.06^{b}
At end (42 day)	6.10^{a}	3.98^{cb}	3.75^{b}	3.18^{ed}	3.72^{fe}	3.15^{c-d}	3.73^{c-d}	2.90^{f}	2.85^{ed}	3.99^{cb}	3.64^{a}
Mean	4.24^{a}	3.66^{cb}	3.52^{cb}	3.09^{b}	3.42^{cd}	3.03^{f}	3.48^{e}	2.88^{cd}	2.52^{d}	3.66^{f}	

Means with same superscript are not significantly different.

T_0: air, **T_1:** 1.5 % O_2 + 2.5 % CO_2, **T_2:** 1.5 % O_2 + 5 % CO_2, **T_3:** 1.5 % O_2 + 7.5 % CO_2, **T_4:** 3 % O_2 + 2.5 % CO_2, **T_5:** 3 %O_2 + 5 % CO_2, **T_6:** 3 % O_2 + 7.5 % CO_2, **T_7:** 5 % O_2 + 2.5 % CO_2, **T_8:** 5 % O_2 + 5 % CO_2, **T_9:** 5 % O_2 + 7.5 % CO_2

attribute by the panelists in deciding the shelf-life of apple slices. In comparison with control (T_0) samples, apple slices under different modified atmospheric conditions (T_0 to T_9) showed better retention of sensory scores during 42 days of storage ($p < 0.05$). Loss of flavour increased in both control packs and MAP apple slices. Firmness scores decreased with time in both control packs as well as in MAP apple slices during storage. MAP slices were observed to be firm after 42 days of storage whereas softening took place in control pack samples on day 7. Apple slices packed under 5 % O_2 + 5 % CO_2 (T_8) and 5 % O_2 + 2.5 % CO_2 (T_7) gas composition had better texture on day 42 than control samples. Overall acceptability varied significantly ($P < 0.05$) during storage in all MAP samples compared with each other.

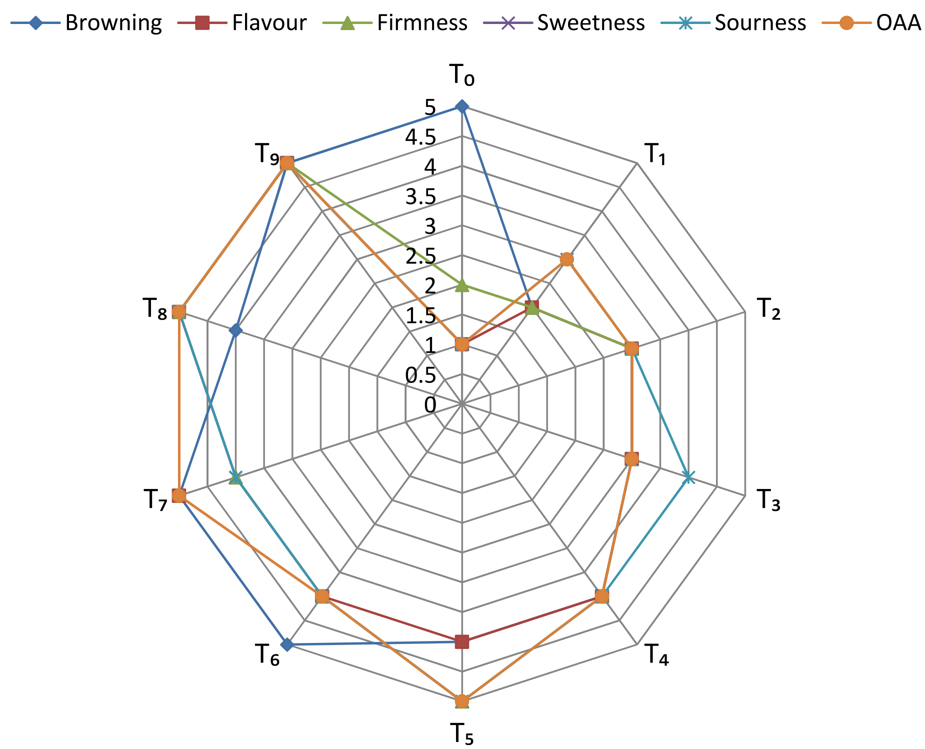

Fig. 4.17 Sensory analysis of minimally processed apple slices packed in modified atmospheres under cold storage (5 + 2 ºC)

T_0: air, **T_1:** 1.5 % O_2 + 2.5 % CO_2, **T_2:** 1.5 % O_2 + 5 % CO_2, **T_3:** 1.5 % O_2 + 7.5 % CO_2, **T_4:** 3 % O_2 + 2.5 % CO_2, **T_5:** 3 %O_2 + 5 % CO_2, **T_6:** 3 % O_2 + 7.5 % CO_2, **T_7:** 5 % O_2 + 2.5 % CO_2, **T_8:** 5 % O_2 + 5 % CO_2, **T_9:** 5 % O_2 + 7.5 % CO_2

5. DISCUSSION

In this section, an attempt has been made to evaluate and discuss the results obtained for the four experiments conducted in light of the available literature.

5.1 Evaluation of Nutritional Composition of Different Cultivars of Apples Grown in Indian Conditions

5.1.1 Fruit dimensions and weight

For design of grading, conveying, processing, and packaging systems physical characteristics of fruits and vegetables are the foremost desirable attributes (Tabatabaeefar and Rajabipour, 2005). Colour, size and shape are important characteristics of appearance which are the major purchase driving attribute of fruits that affects the consumers' choice for consumption of fruit. A genotypic variation for the fruit dimensions and weight of the apple fruit was observed in the twenty two cultivars we studied. Similar observations have been previous reported by Péroumal *et al.* (2017) who reported significant variations in the average fruit weight of six mamey apple accessions. Beyer *et al.* (2002) have also stated that the dimensional elements of different genotypes might be used in describing the fruit shape and specific descriptions of the genotypes.

5.1.2 Fruit firmness

Consumers take fruit firmness into contemplation as a prime criterion which influences the selection of fruit (Harker *et al.*, 2008). Significant variation of fruit firmness was observed among the twenty apple cultivars we studied that might be due to varied factors such as cultivar, growing location, maturity stage and climatic factors (Ornelas-Paz *et al.,* 2018; Musacchi and Serra, 2017).

5.1.3 Peel colour

Colour has been playing a major role in fruit choice, preference, and acceptability and as a sorting criterion for different cultivars. The colour of apple skin from green, yellow and red contributes to distinguish the genotypes. Commonly, the red colour is preferred while bright red apples and new alternative colours are recently receiving the consumer attention. Variation in the colour of the peel of the different varieties of apple studied was observed ranging from green to red with or without yellow streaks. Such wide differentiation in peel colour of the investigated apple cultivars may be due to genotypic variations and composition of pigments in respective cultivars (Ma *et al.,* 2017).

5.1.4 Soluble solid content (SSC)

Soluble solid content is an important index to evaluate the quality and sweetness of fruits that is decisive for consumer acceptance. Soluble solid content mainly consist of sugars, organic acids, vitamins and few minerals. The soluble solid content (SSC) of the apple cultivars studied ranged from 10 °Brix ('Red Delicious') to 16.1 °Brix ('Gale Gala'). Since cultivation of all the cultivars was done at the same location, such variation might be as a result of the difference in synthesis of sugars and organic acids during the growth of the fruits as a result of the genetic variation. Our values are higher to those reported by Jan *et al.* (2012) who reported values in the range of 11.24-11.79 %. Ma *et al.* (2017) also described genotype differences to be the main factor determining the soluble solid content in kiwifruits.

5.1.5 Titratable acidity

Generally the taste of the fruit is reined by a balance between sugars and titratable acidity. Non-red coloured cultivars were shown to possess higher titratable acidity as compared to the red cultivars. This maybe as a result of the genetic variation os the cultivars. Previously, Jan *et al.* (2012) have reported a lower range of 0.50-0.56 % titratable acidity in apples. The ratio of sweetness and acidity of apples can determine their end use. According to Wu *et al.* (2007), genotypes having higher soluble solid content and acidity can be considered good for apple juice concentrate production.

5.1.6 Ascorbic acid content

Apple fruit has antioxidant properties owing to the presence of ascorbic acid and phenolics (Lata and Tomala, 2007). In the present study, apple cultivars, namely, 'Starkrimson' (32.08 mg $100g^{-1}$) and 'Oregon Spur II' (31.76 mg $100g^{-1}$) had higher ascorbic acid content while the minimum ascorbic acid was observed in the cultivar 'Well Spur' (19.38 mg $100g^{-1}$). Such wide variation in the values of ascorbic acid might be due to genetic traits of each genotype. Similarly, Jan *et al.* (2012) and Joshi *et al.* (2007) have reported variation in ascorbic acid content in different apple cultivars.

5.1.7 Antioxidant (AOX) activity

In the current study on twenty two apple cultivars, we have found that red cultivar, 'Silver Spur' had the greatest amounts of antioxidant (AOX) activity (13.20 µmole Trolox equivalent g^{-1}) whereas the non-red cultivar 'Granny Smith' had the lowest amount (2.64 µmole Trolox equivalent g^{-1}). Variation in the values might be as a result of difference in genotype since all cultivars were grown in the same location using similar horticultural practices. Previously, a range of 7.7–22.6 µmole Trolox equivalent g^{-1} antioxidant activity has been reported by Joshi *et al.*(2007) in apples. In 2015, Wang *et al.* reported that the white-fleshed genotypes had a lower antioxidant activity in comparison to red-fleshed genotypes that is due to the synthesis of pigments which are also a contributor towards the total antioxidant activity.

5.1.8 Total carotenoid content

In the 22 apple cultivars studied by us, the total carotenoid content varied significantly. The total carotenoid content varied from 29.03 mg kg^{-1} (Early Red-I) to 147.06 mg kg^{-1} (Red Chief). This variation might be due to a high level of genetic variability among the cultivars and the environmental factors since all cultivars otherwise received similar horticultural practices for cultivation at the Seobagh station. Previously also Delgado-Pelayo *et al.* (2014) and Péroumal *et al.* (2017) have reported a variation in the total carotenoid among different apple accessions.

5.1.9 Sugar profiling

The level of sugars in fruits is known to vary depending on the genetic variations, maturity of the tested fruit, environmental factors and horticultural practices followed in the plantations (Wu *et al.,* 2007; Hudina and Stampar, 2006). Sugar profiling of fruits is essential as it determines the sensory and biochemical attributes of fruits. A wide difference in terms of individual sugars was recorded among the apple cultivars in our study. The principal monosaccharide was observed to be fructose that ranged between 10.85 to 67.55 g L^{-1}. Owing to the higher fructose content, the intake of apple fruits is recommended by diabetic patients as it keeps the level of blood sugar stable (Hecke *et al.*, 2006). Sucrose was also observed to be present in small amount with an average of 20.40 g L^{-1}. The presence of different sugars in the apple cultivars may be due to the variation in synthesis and accumulation patterns during growth as a function of genotype. Presence of fructose, glucose, sucrose alongwith sorbitol in apple fruits has been reported previously by Zhang *et al.* (2010)**.** Wide variations in composition of sugar compounds in eleven Kei-apple fruit accessions have also been reported by Mpaia *et al.* (2018).

5.1.10 Organic acid profiling

Analysed apple fruits showed great variation in organic acids. Among the 22 apple fruits studied, Stark Spur Golden (9.50 g L^{-1}) showed the highest malic acid content followed by Winter Banana (8.25 g L^{-1}). It is believed that taste and the consumption pattern of fruits is influenced by the presence of organic acids in the apple fruits. Fruits possessing appreciable sweetness with low acidity are considered good for fresh consumption. All the non-red cultivars had a higher level of malic acid in comparison to the red coloured counterparts. Malic acid was the most predominant organic acid followed by succinic acid and traces of citric and acetic acid, among the studied apple cultivars. Surprisingly, out of the 22 apple cultivars studied only 3 cultivars showed the presence of citric acid, the maximum being in cultivar 'Red Delicious' (11 g L^{-1}). This shows the variation in synthesis of individual organic acids amongst the cultivars studied. Presence of malic, succinic and citric acid in apples has also reported by Wu *et al.* (2007). The quantitative variation of organic acids in different cultivars might be due to the genetic makeup and the accumulation pattern in the different cultivars.

5.1.11 Mineral analysis

Mineral composition of apple fruits is an indicator of the nutritional quality. According to the obtained results, the concentration for the macro and micro elements varied in the following order: K > Ca > Na > Mg and Fe > Zn > Mn > Cu, respectively. The cultivar 'Scarlet Gala' had a higher (1142 mg $100g^{-1}$) concentration of potassium while 'Winter Banana' recorded the least values (550 mg $100g^{-1}$). The results are comparable with the study of Horsley *et al.* (2014) and Joshi *et al.* (2007). The concentrations of sodium, calcium and magnesium recorded were comparable with the results reported by Fazli and Fazli (2014), Joshi *et al.* (2007) and Ornelas-Paz *et al.* (2018) in apple fruits. Micro elements are important for the body as they support various functions in the human body. The average concentrations of micro elements in the apple cultivars were: Fe (2.04 µg g^{-1}), Zn (0.64 µg g^{-1}), Mn (0.16 µg g^{-1}) and Cu

(0.12 µg g^{-1}). The results of Manzoor *et al.* (2012) and Ornelas-Paz *et al.* (2018) for micro elements were comparable with our findings. Factors such as cultivars, soil quality, production site, cultural practices and environmental conditions are attributed for the variation in mineral elements. Since the cultivars were grown at the same site with similar soil quality and cultural practices, genotypic variation and environmental conditions played a significant role for the variation in mineral composition.

5.1.12 Profiling of phenolic compounds

Phenolics are an important group of antioxidants that are found in horticultural plants. The phenolics compounds have a major role in enzymatic browning in apple. It has been emphasized that the health benefits of phenolics consumption is owing to the antioxidant, anti-inflammatory, anti-viral and anticancerogenic properties that helps in preventing heart disease and lower cholesterol levels (Alberti *et al.,* 2017; Borges *et al.,* 2010; Catel-Ferreira *et al.,* 2015; Zhang and Tsao, 2016; Craig and Beck, 1999). Therefore, there has been a growing interest for using apples in functional food products, such as functional beverages and healthy snack products. Chlorogenic acid was found to be the major phenolic compound in the 22 apple cultivars studied. The maximum concentration of chlorogenic acid was found in non-red cultivar 'Winter Banana' and minimum in 'Granny Smith' cultivar. Carbone *et al.* (2011) have also reported chlorogenic acid to be the major phenolic compound in various apple cultivars. Phloridzin dehydrate was the second major phenolic compound after chlorogenic acid in the apple cultivars.

5.1.13 Fatty acids profiling

Besides the structural roles, fatty acids and lipids often serve as precursors of important regulatory and aroma volatile substances. Free fatty acids liberated by lipase activity and further metabolized by β-oxidative enzymes and/or lipoxygenase, are the main precursors of ester, alcohol and aldehyde volatiles produced during development and maturation of apples (Fellman *et al.,* 2000). In our study, a total of seven different fatty acids were detected in the apple cultivars in varied quantities. The most abundant were levulinic acid and valeric acid followed by linoleic and palmitic acids. Variation in the fatty acid profile of different apple cultivars might be as a result of genetic makeup and the synthesis of fatty acids during growth and development (Wu *et al.,* 2007).

5.2 Evaluation of Nutritional Composition of Different Apple Cultivars Grown at Different Altitudes

5.2.1 Fruit dimensions and weight

The dimensional attributes of apple cultivars can be used to characterize the variety according to shape. An influence of cultivar and altitude was observed for the fruit dimensions and fruit weight. During the course of the investigation, fruits from lower elevation showed lower fruit weight. Solarte *et al.* (2014) reported that the fruit weight of different guava cultivars was influenced by altitude this might be due to the variations between day and night temperatures.

5.2.2 Fruit firmness

Firmness, juiciness and absence of mealiness are the most liked textural traits of apple fruit by the consumers (Brookfield *et al.,* 2011). Cultivars, season and the harvest time directly affect the fruit firmness (Beaudry, 1992). We observed that fruits grown at higher elevation yielded higher fruit firmness as compared to those grown at lower elevation. The maximum firmness was exhibited by the 'Golden Delicious' cultivar, while the lowest firmness was observed for 'Royal Delicious' cultivar grown at 1000-1400 msl.

5.2.3 Peel colour analysis

Instrumental colour analysis of apple fruits yielded that cultivars 'Golden Delicious' and 'Red Gold' grown at 1500-1800 msl altitude showed the maximum L* values. This may be due to genetic variability among cultivar to synthesize pigments and also the effect of growing elevation. González *et al.* (2011) have reported that the increase of L* values may be associated with the differential susceptibility to degradation of chlorophylls among cultivars. Cultivars 'Royal Delicious' and 'Red Gold' displayed higher a* values with dark red coloured fruits due to genetic variability and effect of elevation. The major environmental factor that influences the colour is temperature that increases the carotenoids in the skin of apple (González *et al.,* 2011). Similarly, Gautier *et al.* (2008) and Medlicott *et al.* (1986) reported that the increase in the carotenoids content was associated with the increase of temperature.

5.2.4 Soluble solid content and titratable acidity

Table 4.8 showed differences in soluble solids and titratable acidity among the apple cultivars with respect to the orchard site. We observed that the orchard elevation influenced the soluble solids content. Guerrero-Chavez *et al.* (2015) have reported that the soluble solid content in strawberries influenced by altitude. Titratable acidity (TA) was also significantly ($p < 0.05$) affected by the growing elevation of the three apple cultivars.

5.2.5 Ascorbic acid content

Cultivar such as ‘Golden Delicious’ and ‘Royal Delicious’ showed high contents of ascorbic acid grown at higher elevation (1500-1800 msl) (Table 4.8.), Considering cultivar and elevation influence over ascorbic acid content in different apple cultivars, the highest value was obtained in fruit of the cultivar ‘Golden Delicious’ (28.80 mg 100 g^{-1}) growing at 1500-1800 msl. The cultivar ‘Red Gold’ showed the lowest ascorbic acid concentration in both the elevations. Our findings also corroborate with the findings by Mphahlelea *et al.* (2014) and Crespo *et al.* (2010) who reported a significant effect of altitude on ascorbic acid concentration. According to Lee and Kader (2000), ascorbic acid in fruits is synthesised from sugars during photosynthesis under higher light intensity. This indicates that high ascorbic acid concentration recorded in apple fruits cultivated at higher elevation (1500-1800 msl) might be due to higher light intensity during the growing season as compared to apples grown at lower (1000-1400 msl) elevation . The fruit cultivation under cool temperatures during day and night influences the ascorbic acid content in fruit (Mercado-Silva *et al.,* 1998; Nauer *et al.,* 1974) probably due to a lower mean temperature which stimulates the synthesis of organic acids (Lee and Kader, 2000). It also lowers the utilization of organic acids due to reduction in respiration rate (Bron *et al.,* 2005) and enhances the translocation (Rathore, 1976).

5.2.6 Total phenolic content

Concentration of total phenols in apple fruit varied with cultivar and with maximum phenolics recorded in fruits grown at 1500-1800 msl as compared to those grown at 1000-1400 msl. The accumulation of total phenolic concentration in fruit harvested at higher altitude may be in response to high light intensity. Germ *et al.* (2009) have reported that the intense solar radiation at higher elevation has often been implicated to having an impact on secondary metabolite profiles. Earlier, Mphahlele *et al.* (2014) recorded higher total phenolic content in pomegranate fruits harvested from plantations situated at higher (898 msl) elevations. On the other hand, it could be suggested that fruit maturity among two locations varied due to climate effect thus resulting in variation in the phenolic content of the cultivars. Differences in the phenolic content of pomegranate juice have been reported to vary significantly due to geographical variation as well as exposure to extreme temperatures and maturity stage (Kondakova *et al.,* 2009). Recently, D'cunzo *et al.* (2017) reported that the gowning sites influence the total phenolic content of endives and chicory stems.

5.2.7 Total antioxidant activity

A significant ($p < 0.05$) variation in total antioxidant activity among the apple cultivars studied was observed in this study which was also affected by orchard elevation. Varied degree of total antioxidants are synthesized by individual cultivars as a response to genetic makeup and climatic response. Also, as stated earlier also, greater the elevation, greater is the intensity of radiation that ultimately results in higher production of secondary metabolites. Higher antioxidant activity has been associated to higher total phenolic compound present in fruit (Tzulker *et al.,* 2007). Our findings are in agreement with Hakkinen and Torronen (2000) and Mphahlele *et al.* (2014) who also reported higher antioxidant activity in strawberries and pomegranate, respectively grown at higher elevations.

5.2.8 Total carotenoid content

After interpretation of results, it was found that the apples grown at higher elevation retained higher total carotenoids compared with those grown at lower altitude. The total carotenoid content varied between 46.44 mg kg^{-1} and 104.50 mg kg^{-1}. Such huge difference might be due to genetic variability among the cultivars studied. Similarly, Ouni *et al.* (2012) investigated that the oils obtained from olives cultivated at higher altitudes showed higher carotenoid content compared with those from lower altitudes.

5.2.9 Sugar composition

Ratio of different sugars affects the taste balance of fruits. Of the studied cultivars, the significant differences ($p < 0.05$) among the cultivars and elevations in sugar compounds were found (Table 4.10). It was recorded that apple fruits grown at a lower elevation were found to contain low levels of glucose, fructose and sucrose in comparison to the apples grown at a higher elevation. The higher elevation site is characterized by lower a temperature that was found to favour an increase in accumulation of fructose, glucose as well as sucrose. Schwartz *et al.* (2009) and Fischer *et al.* (2007) have also reported a similar phenomena for strawberries and gooseberries, respectively. Solarte *et al.* (2014) have also reported high altitudes to improve fructose and glucose accumulation in certain guava genotypes. In contrast, Correia *et al.* (2016) have reported no significant differences in glucose, galactose and total free sugars contents among cultivars of blueberries grown at differing altitudes. Contrasting results were obtained in sucrose content at lower altitudes possibly due to genetic variability among the cultivars they studied. Crespo *et al.* (2010) have also reported that the compositional variations in fruits in response to orchard sites were cultivar specific.

5.2.10 Organic acid composition

Composition of organic acids in fruits is important, not only because of their correlation with fruit flavour and taste but also because they are an important substrate for respiration (Hulme *et al.,* 1963). In case of apples, malic acid is the predominant substrate for respiration during postharvest handling (Ackermann *et al.,* 1992). HPLC analysis of the apple cultivars we studied also showed predominance of malic acid followed by succinic and citric acids (Table 4.9). Cultivar 'Golden Delicious' and 'Royal Delicious' recorded the highest concentration of malic and citric acids while succinic acid content was highest in the 'Red Gold' cultivar. Fruits grown at 1800 msl synthesized maximum organic acids. Earlier, Crespo *et al.* (2010) reported that the genotypes significantly affect the organic acid content of four strawberry cultivars grown at two production sites (1060 and 480 m) due to genetic variability.

5.2.11 Mineral content

Our results of mineral content in the apple fruits are corroborate with the findings of Horsley *et al.* (2014), Todea *et al.* (2014) and Manzoor *et al.* (2012). The mineral contents in fruits are dependent on the cultivar, fertilization, soil and climatic conditions (Nour *et al.,* 2011). Similarly, Gonçalves *et al.* (2011) reported that the differences in the mineral contents are related to the intrinsic characteristics of each cultivar.

5.2.12 Phenolic acid composition

The quantity and quality of phenolic compounds in fruits are not only important to plant ecology but also impart nutraceutical properties to the fruit. Concentration of these compounds are influenced by several factors, including organs and tissues, variety, plant species, physiological and phenological stages, soil and climate conditions and several biotic and abiotic stresses (Michalek *et al.,* 1999; Scalbert and Williamson, 2000). In our study, fruits harvested from higher altitude (1800 msl) demonstrated higher content of phenolics than those from lower elevation. This might be as a result of cold stress induced accumulation of phenolic compounds as also reported previously by Thomai *et al,* (1998). In general, the total content of phenolics analysed were comparable to those reported by Wu *et al.* (2007) for apple. Previously, Hakkinen and Torronen (2000) and Guerrero-Chavez *et al.* (2015) have also reported influence of growing location on the total phenolic content in strawberry.

5.2.13 Fatty acid composition

Fatty acids form the outer coating of the apple fruit that gives it a glossy look. Seven fatty acids, namely, valeric, levulinic, palmitic, stearic, oleic, linoleic and 13-docosenoic acid were analyzed by FAMEs. Among these, linoleic, oleic and palmitic acids were found to be the major ones. The content of fatty acids we obtained in the twenty three cultivars of apple in this study is similar to that reported in a previous study of Wu *et al.* (2007). Ouni *et al.* (2012) studied the composition of Chétoui olive oils of various altitude and found significant variations in the levels of the main fatty acids.

5.3 Synergistic Effect of Edible Coatings and Anti-browning Agents on Quality of Minimally Processed Apple Slices

5.3.1 Fruit firmness

The loss of fruit firmness in the fresh-cut fruits was the most notable change. We observed that fruit firmness continuously decreased during storage in all treated as well as untreated samples except those that were treated by calcium chloride. This is the consequence of degradation of the cell wall that results from cutting operations during minimal processing which accelerates metabolic activity and moisture loss in products (Olivas *et al.,* 2007; Qi *et al.,* 2011; Rojas-Graü *et al.,* 2008). The infiltration of calcium into the apple tissues might have resulted in better firmness of the apple slices owing to the reaction of calcium with pectic acid present in the cell wall to form calcium pectate that imparts strength to the cell wall (Olivas *et al.,* 2007). Besides this, the significant reduction of fruit firmness of the untreated apple slices and those not treated with $CaCl_2$ over the 7 days of storage could be due to the pectic acid hydrolysis (Ponting *et al.,* 1972). CMC and AVG coatings effectively retarded softening of the apple slices. CMC (1 %) + HR (0.01 %) + AA (0.5 %) + $CaCl_2$ (0.2 %), AVG (15 %) + HR (0.01 %) + AA (0.5 %) + $CaCl_2$ (0.2 %) and CMC (1 %) + AA (1 %) + $CaCl_2$ (1 %) treated apples had the highest firmness, since $CaCl_2$, a known firming agent helped apple slices maintain firmness as described above. Previous studies also report the increase in firmness of apple slices by calcium chloride treatment by strengthening the middle lamella and cell wall (Qi *et al.,* 2011). Apple slices coated with CMC and AVG in combination with anti-browning agents were found better to retain the fruits firmness. Our results are in accordance with Guerreiro *et al.* (2017) who reported the positive effect of coatings mixed with anti-browning agent in maintenance of fresh-cut apple firmness. Similaly, Saba and Sogvar (2016) have reported that the apple slices treated with CMC in combination with $CaCl_2$ and ascorbic coating has better retention of firmness.

5.3.2 Browning index (BI)

Fig. 4.3 shows the effect of applying coatings with anti-browning agents on the browning index of minimally processed apple slices during cold storage (5 ± 2 °C) revealing that the treated samples had a statistically significant effect on reducing the BI in comparison to control samples. Browning index was used to determine the changes associated with the development of browning which is one of the major biochemical indicators of browning in minimally processed fruits (Pathare *et al.,* 2013; Chiumarelli and Hubinger 2012). We observed that apple slices coated with CMC and AVG in combination with anti-browning agents has significantly lower BI. Our results are in line with previous researcher (Qi *et al.,* 2011) who reported the synergistic effect of edible coatings and anti-browning agents to maintain lower BI, better cut surface color and quality of apple slices. Saba and Sogvar (2016) reported that the browning index was strongly suppressed in samples coated with carboxymethyl cellulose alone or in combination with ascorbic acid. Recently, Guerreiro *et al.* (2017) observed that the edible coatings in combination with anti-browning agents (ascorbic acid, citric acid and sodium chlorite) effectively reduced browning in fresh-cut apples.

5.3.3 Whiteness Index (WI)

The results of this experiment show that surface coating and browning inhibitor treated samples had the higher values of WI than uncoated samples during the 7 days of storage, indicating the positive effect of CMC and AVG coatings towards control of enzymatic browning. Chen *et al.* (2016) observed that the UV-C and citric acid + UV treated samples have the lowest BI and a* value and highest whiteness index and L* value throughout the storage period of 15 days compared with citric acid (CA) alone and control samples. It has been reported that the decrease of L* and whiteness index associated with the loss of water.

5.3.4 Headspace gas composition

Headspace gas composition in packed apple slices involved a continuous decrease in oxygen and increase in carbon dioxide as storage period progressed. Slices applied with edible coatings with anti-browning agents exhibited lower O_2 consumption and CO_2 production while the untreated apple slices showed highest O_2 consumption and CO_2 production during storage. As far as the ΔCO_2 and ΔO_2 is concerned, it was observed that apple slices dipped in CMC containing 4-HR+AA+$CaCl_2$ showed the lowest CO_2 production and O_2 consumption during storage whereas treatment with only cysteine resulted in highest CO_2 production and O_2 consumption as a result of greater metabolic changes. Similar trend of reduction of ΔO_2 and increase of ΔCO_2 levels in apple slices has been reported previously also (Rocculi *et al.,* 2004) might be due to higher metabolic changes.

5.3.5 Weight loss

The loss in weight is associated with the moisture exuded from the minimally processed apple slices during storage at low temperature (Mantilla *et al.,* 2013). In general, the PLW continually increased during storage of the minimally processed produce as also observed previously by Antunes *et al.* (2010). A browning inhibitor dip in conjunction with the edible coatings resulted in lowest weight loss till 7 days at 5 ± 2°C. Our observations are in agreement with reports of Guerreiro *et al.* (2017) and Liu *et al.* (2016) who reported that the incorporation of anti-browning agents into edible coatings maintained minimum weight loss of apple slices. Coatings are considered to decrease weight loss due to their effects as semipermeable barrier against water loss.

5.3.6 Browning potential

At the termination of experiment, lowest browning potential was observed in apple slices treated with edible coatings (CMC and aloe vera) in combination with anti-browning agents (4-HR, AA, $CaCl_2$) and the highest for water dipped apple slices. Maximum degree of inhibition was found for combined treatment with browning inhibitors and edible coatings. These findings were in agreement with Chiabrando and Giacalone (2011) where they studied the effect of ascorbic acid and calcium chloride, citric acid and calcium chloride and 1-MCP for fresh-cut apples because anti-browning agents inhibit the enzymatic browning.

5.3.7 Soluble solids content (SSC) and titratable acidity (TA)

The soluble solids content showed only a minimal variation in all the treated minimally processed apple slices. Generally, the SSC increased initially followed by a decline. In untreated samples, the peak value was obtained on second day of storage but in case of treated samples there was a delayed peak (15.7 °B). The increasing trend of soluble solid content during storage, indicates the ripening process. Titratable acidity declined as storage period progressed regardless of the treatment used but remained higher in apple slices treated with anti-browning agents and edible coatings especially of those treated with carboxymethyl cellulose. At the end of experiment, for all the treated apple slices, soluble solids and titratable acidity content was higher than that of the control apple slices. Both CMC and aloe vera coating in combination with anti-browning agents reduced the loss of soluble solids content and TA when compared with control. The most effective treatment was T_9 followed by T_5 and T_{10} for all edible coatings and anti-browning combinations. Organic acids get utilized during ripening process thereby increasing the soluble solids contents initially and thereafter showing a decreasing trend (Olivas *et al.,* 2007). Saba and Sogvar (2016) reported that the apple slices coated with CMC coating in combination with $CaCl_2$ and ascorbic acid maintains better soluble solids and titratable acidity during low temperature storage.

5.3.8 Ascorbic acid content

Both CMC and AVG coatings retarded the loss of ascorbic acid in apple slices when compared with control and treatment with anti-browning alone (Table 4.16). CMC coating in conjunction with anti-browning agents performed best. Ascorbic acid in fruits is lost due to the activities of phenol oxidase and ascorbic acid oxidase enzymes. Saba and Sogvar (2016) reported that the reduction in ascorbic acid in coated slices was slower than uncoated ones which might be due to the low oxygen permeability of carboxymetyhl cellulose (CMC) coating that resulted in decreased activity of the oxidizing enzymes hence preventing the loss of ascorbic acid. Similarly, Liu *et al.* (2016) have also reported retention of higher ascorbic acid in fresh-cut apples coated with chitosan containing ascorbic acid or calcium chloride. Among the anti-browning agents, HR (0.01 %) + AA (0.5 %) + $CaCl_2$ (0.2 %) resulted in highest retention of ascorbic acid in comparison to the control at end of storage. Among the coatings alone and coatings combination with anti-browning agents, CMC and CMC (1 %) + HR (0.01 %) + AA (0.5 %) + $CaCl_2$ (0.2 %) shows the least reduction in ascorbic acid.

5.3.9 Total phenols and antioxidant activity

Edible coatings and anti-browning agents were found to significantly affect the total phenols and antioxidant activity of the apple slices. Total phenol levels were significantly higher in ascorbic dips than in the other treatments; this was true for both edible coatings. The apple slices treated with edible coating alongwith anti-browning agents showed significantly retarded decline in the two attributes. Similarly, Saba and Sogvar (2016) also found that CMC in combination with anti-browning agents was more effective to maintain total phenol in apple slices during storage as compared to the uncoated ones. These results are in agreement with previous reports where anti-browning treatments reduced the loss of phenols in fresh-cut fruits (Gonzalez-Aguilar *et al.,* 2005). It has been suggested that ascorbic acid exerts a protective effect on phenolic compounds by self-oxidation (Mayer and Harel, 1979) and also by acting as an oxygen scavenger and avoiding polyphenol oxidase-catalyzed reactions. Therefore, it seems that the ascorbic acid treatment inhibited browning by increasing antioxidant capacity in apple slices. According to Robles-Sanchez *et al.* (2013) antioxidant activity in fresh-cut mangoes treated with the edible coating in combination of anti-browning agents was significantly higher than in coating alone and control fruits.

5.3.10 Polyphenol oxidase (PPO) and peroxidase (POD) activity

PPO and POD activities in all samples increased continuously along storage times. Since, oxidising enzymes such as PPO and POD get activated during minimal processing operations, they result in oxidation of phenolic substrates resulting in undesirable effects such as browning. The rise in respiration caused by the cutting operation is responsible for induction of these oxidizing enzymes. Application of CMC and AVG in conjunction with browning inhibitors on apple slices showed significant reduction in the activities of the oxidizing enzymes. Maximum inhibition of PPO and POD activity was observed after treatment of slices with CMC + HR (0.01 %) + AA (0.5 %) + $CaCl_2$ (0.2 %). At the end of storage period the PPO and POD activities in control sample were 48 and 77 U min^{-1} g^{-1}, respectively while in CMC (1 %) + HR (0.01 %) + AA (0.5 %) + $CaCl_2$ (0.2 %) treated apple slices the activity of PPO and POD were 36 and 61 units g^{-1} min^{-1}, respectively. Our data is in agreement with that of Saba and Sogvar (2016) who reported that carboxymethyl cellulose (CMC) coatings in combination with $CaCl_2$ and ascorbic acid decreased PPO and POD activities in fresh-cut apples. Jang and Moon (2011) also reported that ascorbic acid effectively reduced the peroxidase activity in the fresh cut apples as a result of lower oxidative stress on the surface of produce because of the antioxidant nature of ascorbic acid. Similar reduction in PPO and POD activities by application of coatings and anti-browning agents has been previously reported by Qi *et al.* (2011) and Gonzalez-Aguilar *et al.* (2005).

5.3.11 Microbiological analysis

Microbial spoilage of food is one of the main causes of fresh-cut fruit deterioration. In this regard, the microbial safety is one of the foremost factors to be considered for the preservation and to maintain the commercial marketability of fresh-cut produce (Graça *et al.,* 2015; Sanchís *et al.,* 2016). However, fruits such as apple are a good source of water and nutrients and are excellent substrates for microbial growth. At the termination of experiment it was observed that untreated apple slices had the highest microbial count whereas all other apple samples revealed lower counts. Treated apple slices presented a lower increase in microbial load that might be due to the addition of acidulants such as citric acid/ ascorbic acid and calcium chloride (components of the dip) as antioxidant/firming agents. They are reported to confer antimicrobial activity to coatings and help control microbial growth in fresh-cut apples (Sanchís *et al.,* 2016). Calcium ion, whose mode of action is mainly associated with maintaining cell wall structure and firmness has also been reported to retard microbial growth in fresh-cut produce (Cefola *et al.,* 2014). Regarding organic acid salts, optimal antimicrobial activity is at low pH values when the undissociated form is present. In our research work with CMC and AVG coatings, the addition of organic acids lowered the pH of the formulations which fall within the optimum range for the antimicrobial activity of these organic acid salts. Sanchís *et al.,* (2016) reported that the edible coating showed a synergic effect with the antioxidant solution and the counts of total microbial growth significantly reduced in treated samples during storage. As shown in Table 4.19, reducing the changes of oxygen and carbon dioxide by controlled atmosphere as provided by the coatings may not only inhibit respiratory processes but also suppress microbial count. In the present study, it was found that AVG and CMC with anti-browning agents showed more pronounced antimicrobial properties. Benítez *et al.* (2015) reported that the apple slices coated with aloe vera gel were effective in inhibition of microbial count.

5.3.12 Sensory quality

The results of this study show that the browning inhibitors in combination with edible coatings imply an increase in the sensory scores. The sensory scores allotted to different attributes exhibited that treated samples were well within the limit of marketability till 7 days of storage at 5 ± 2 °C, whereas the untreated apple samples lost marketability within a day of storage. At the end of this study, the apple slices coated with the CMC and AVG coatings containing anti-browning agents, were better than those which were treated either with anti-browning agents and coatings alone. Moreira *et al.* (2015) reported that combined application of coating and pulsed light treatment of fresh-cut apples maintained the sensory attribute scores above the rejection limits after prolonged storage. CMC coating maintained fruit flavour of the slices till last day of storage. Benítez *et al.* (2015) observed that the polysaccharides based coating enhanced the fruity flavour of the fresh-cut kiwifruit slices from the sixth day which was liked by the sensory panel. The lowest rated samples were those treated with cysteine, CMC and AVG alone due to loss of fruit flavor. Similarly, Moreira *et al.* (2015) found that the combination of coatings and pulsed light treatments led to the lowest scores for aroma. However, Benítez *et al.* (2015) reported that the kiwifruit slices treated with aloe vera coating was preferred by the sensory panel.

5.4 Effect of Modified Atmospheres on Quality of Minimally Processed Apple Slices

5.4.1 Fruit firmness

Softening in apple slices is a major factor affecting its acceptability, shelf life and quality. Decline of firmness is associated to the loss of moisture which leads to a decrease in crispness and turgor. Rate of softening is fast in minimally processed products due to the absence of an outer protective layer and exposure of internal tissue. These undesirable changes can be delayed by appropriate packaging of cut produce (Toivonen and Brummel, 2008). During the course of our investigation, fruit firmness of apple slices continuously declined over storage for both MAP as well as air packs slices. At the end of storage period there was a ~5 % reduction of fruit firmness for MAP samples stored in packs containing 5 % O_2, 5 % CO_2 (T_8) while the control samples (T_0) showed a significant 31 % reduction of firmness. This indicates that the modified atmospheres retarded the rate of fruit softening and maintained firmness of apple slices throughout storage. Similarly, Fagundes *et al.* (2015) in their study on the MAP of cherry tomatoes documented that the atmosphere composition of 5 % O_2 + 5 % CO_2 helped in better retention of firmness. Cortellino *et al.* (2015) have reported the mixtures 1 % O_2 + 99 % N_2 and 5 % O_2 + 5 % CO_2 + 90 % N_2 to preserve better firmness than normal atmosphere. Low temperature storage also restrict the softening of tissue which may be due to reduction in respiration and transpiration rates, inactivation of cell-wall hydrolytic enzymes as well as decreased ethylene production. The apple slices with the higher weight loss showed a maximum reduction in fruit firmness. Fruit softening occurs due to deterioration in the cell structure, cell wall composition, and intracellular materials (Seymour *et al.,* 1993). These biochemical processes involve the direct suppression of the activities of pectin methyl esterase and polygalacturonase enzymes leading to postharvest softening of fruit structure or blockage of the synthesis of ethylene, which controls the activities of these enzymes especially with MAP treatment (Akbudak *et al.,* 2012). Salunkhe *et al.* (1991) reported that the low respiration rate can restrict the activities of cell wall degrading enzymes and allow retention of firmness during storage. Similarly, Rocculi *et al.* (2004) found that fruit firmness preserved in all samples of ‘Golden Delicious’ apple slices packed in MAP.

5.4.2 Browning index (BI)

Browning incidence and the loss of colour are common characteristics of minimally processed fruits due to the tissue damage provoked by peeling and slicing and release of enzymes which can induce browning reactions promoting loss of natural colour (Chiumarelli and Hubinger, 2012). The browning index which integrates the colour attributes of CIE Lab, is an indicator of the intensity of brown colour. BI of the air packs samples was significantly higher than that of the samples packed in modified atmospheres throughout the storage. Previously, Gonzalez-Aguilar *et al.* (2004) have reported that the combinations of anti-browning agents and modified atmosphere packaging (MAP) resulted in a reduction of browning. Enzymatic browning in terms of browning index (BI) values has been presented in Table 4.23 of the apple slices under different modified atmosphere conditions during storage. After 42 days of storage, control samples (air packs) recorded higher BI values (137.7) whilst MAP samples showed a restricted rise of BI under different modified atmosphere conditions. High humidity created in the in-package atmosphere alleviated tissue dryness and was an important factor in the ability of the anti-browning solutions to prevent browning.

5.4.3 Whiteness index (WI)

In the present work, the minimally processed apple slices stored under active modified atmosphere conditions gave a significantly higher WI than apple slices packed in normal atmospheric condition (Control). Whiteness index of samples indicates the suppression of browning and better acceptability of the product due to the fresh-like look. Although WI in all samples reduced during storage, higher CO_2 atmospheres showed a negative influence on the WI of the apple slices. Enhanced PPO activity under such conditions might have been the major cause for such an effect. Catalano *et al.* (2007) have earlier reported a similar phenomena wherein an increase in carbon dioxide increased the polyphenol oxidase activity of fresh-cut eggplants. Similarly, Gorny *et al.* (2002) have also observed that atmospheres with high carbon dioxide accelerated browning in fresh-cut pears as compared to the control (air). At the end of storage, although the WI values decreased in all the treatments but the slices stored under 5 % O_2 + 5 % CO_2 (T_8) conditions where those with the highest values. Jacxsens *et al.* (2001) reported that the high oxygen atmosphere packaging proved particularly effective at inhibiting enzymatic browning in sliced mushrooms as compared with low oxygen atmosphere packaging. Limbo and Piergiovanni (2006) also showed the positive effect of high oxygen partial pressures combined with dipping in acid solutions to control enzymatic browning of fresh-cut potato. Rocculi et al. (2004) measured the changes in whitening index of fresh-cut apples packed in polypropylene boxes and conditioned in Air, 90 % N_2 + 5 % CO_2 + 5 % O_2, 90 % N_2O + 5 % CO_2 + 5 % O_2, 65 % N_2O + 25 % Ar, + 5 % CO_2 + 5 % O_2. Apple slices packed with 65 % N_2O + 25 % Ar, + 5 % CO_2 + 5 % O_2 had increased WI and brightened after 8 days of storage WI whereas apples slices were packed in air and 90 % N_2 + 5 % CO_2 + 5 % had lower WI.

5.4.4 Headspace gas composition

The headspace gas comprises of O_2 and CO_2 that are produced during the respiration of the packed produce. Table 4.20 and 4.21 showed the in package (O_2 and CO_2) changes of minimally processed apple slices under different MAP conditions. Oxygen levels decreased continuously in these treatments, whereas CO_2 levels increased. A sharp drop in O_2 and an increase in the CO_2 concentrations were observed in the packs flushed with normal air composition. Several studies have described the beneficial effects of MAP on reducing the respiration rate of fresh-cut products (Gonzalez-Aguilar *et al.,* 2004).

5.4.5 Weight loss

Physiological loss in weight of minimally processed apple slices is an important attribute because it leads to the quality loss and decreases the shelf life. Minimally processing of fruits increases wound induced C_2H_4, water activity and surface area which may also increase water loss particularly at higher temperatures where a vapor pressure deficit is large (Sandhya, 2010). Modified atmospheres differentially affected weight loss of the apple slices during 42 days of storage at 5 ± 2 ºC. Least weight loss was incurred under gaseous composition of 5 % O_2 + 5 % CO_2followed by atmospheres having 5 % O_2 + 2.5 % CO_2. Lower weight loss found in the modified atmospheric packages was consistent with previous reports on packaged broccoli florets (Serrano *et al.,* 2006; Jia *et al.,* 2009; Fernández-León *et al.,* 2013). The reduction in weight loss for modified atmospheric packaged fresh-cut apples could be attributed to the limitation of water vapour diffusion which results in generation of a higher relative humidity inside the package (Serrano *et al.,* 2006). The slight increase in weight loss observed may be due the effect of respiration, wherein a carbon atom is lost from the fruit in each cycle (Tano *et al.,* 2008). Similar trend was also observed by Xing *et al.* (2011) and Tano *et al.* (2008) in the case of bell peppers and Rahman *et al.* (2012) for green chillies packed under different packaging materials. Overall, the weight loss was low that maybe attributed to the retardation of physiological processes such as respiration and transpiration at the low temperature of storage (Edusei *et al.,* 2012).

5.4.6 Browning potential

Browning potential of minimally processed apple slices packed in polypropylene trays is shown in Fig. 4.16. MAP differentially affected browning potential of apple slices during 42 days storage at 5 ± 2 ºC. After 42 days, apple slices packs under modified atmospheric packaging were found to have restricted increase in browning potential as compared to control samples (air). Control samples showed a rapid rise in browning potential from the 14th day onwards. Low O_2 atmosphere conditions also play an important role in terms of anti-browning function due to the anti-respiratory activity and lower availability of molecular oxygen required for the PPO mediated enzymatic browning.

5.4.7 Soluble solids content (SSC)

During storage the SSC increased initially in both control and MAP samples. The increase in SSC and sugars during storage may possibly be due to breakdown of complex organic metabolites into simple molecules. Further, it may be attributed to hydrolysis of starch into sugars. On complete hydrolysis of starch no further increase in sugars occurred and subsequently a decline in these parameters is evident as they along with other organic acids are primary substrate for respiration (Wills *et al.,* 1980). The increase in SSC of MAP samples was slower which could be attributed to lower respiratory activity and retardation in metabolic activity of the apple slices. Hence, the MAP could be more useful for maintaining the SSC of minimally processed apple slices. The above results are in good agreement with those of Saxena *et al.* (2008) for minimally processed jackfruit, Waghmare and Annapure (2013) for fresh cut papaya and Almenar *et al.* (2008) for blueberry.

5.4.8 Titratable acidity

Titratable acidity content of minimally processed apple slices stored in MAP are shown in Table 4.23. The titratable acidity for all the samples decreased during storage. A statistically significant difference was observed between MAP samples and showed a lower loss of TA compared to the air packs ($P < 0.05$). The combination of MAP and low temperature significantly reduced the reduction of TA in apple slices. Reductions observed in organic acid values in relation to ripening resulted from the utilization of acids in respiration and other physiological processes together with carbohydrates (Kader and Ben-Yehoshua, 2000). According to Sadler and Murphy (1998), the concentration of organic acids decreases during postharvest storage periods due to their use as a substrate in the respiration or their transformation into sugars.

5.4.9 Ascorbic acid content

Ascorbic acid has an important role as a phytochemical due to its functionality as an antioxidant. The physiological stress imposed upon minimal processed products results in a significant reduction in ascorbic acid content. Initial ascorbic acid content of apple samples was recorded as 26.0 mg 100 g^{-1} which decreased during storage. In the present study the apple slices kept under modified atmospheric conditions showed a significantly ($p < 0.05$) higher retention of ascorbic acid as against in the case of air packs samples. Reports exist about higher retention of ascorbic acid in fresh-cut commodities subjected to MAP (Odriozola-Serrano *et al.,* 2008). Lower respiratory activity could be attributed to higher retention of ascorbic acid content due to restriction in enzymatic oxidation of ascorbic acid into dehydroascorbic acid through oxygen in the modified atmospheric packaged samples during storage. The control samples (air packs) showed a rapid loss in ascorbic acid from the 7th days onwards. The maximum restriction in loss was found to be in case of 5 % O_2 + 5 % CO_2 (T_8) modified atmospheric packaging. These results agree with experiments on shelf-life of fresh-cut peppers under passive MAP done by Senesi *et al.* (2000) and Gonzalez-Aguilar *et al.* (2004). The combined effect of low storage temperatures and MAP was beneficial in retaining the initial ascorbic acid (Manolopoulou *et al.,* 2010)

5.4.10 Total phenolic content

In the present study, it was observed that the total phenolic content decreased during storage of minimally processed apple slices in air packs as well as in MAP. Apple slices under MAP showed a significantly ($p < 0.05$) lower loss of total phenols as compared to air packed apple slices which recorded a higher degree of degradation after just 7 days of storage at 5 ± 2 °C. Gas flushing with 5 % O_2 + 5 % CO_2 (T_8) showed maximum retention of total phenolics followed by 5 % O_2 + 2.5 % CO_2 (T_7). The overall effect was based on synergism leading to extension in shelf life as well as a decrease in the respiration rate and oxidative stress (Saxena *et al.,* 2008). The low temperature and the modified atmospheric packaging in synergism caused a higher retention of total phenol due to decrease in the biological activity. Our results are in agreement with Saxena *et al.* (2009) and Cocci *et al.* (2006) who reported a restricted degradation in total phenolics due to the reducing action of ascorbic acid added in the dip treatment given to minimally processed apple slices stored under modified atmospheric packaging.

5.4.11 Total antioxidant activity

Amongst the modified atmospheres applied, atmospheric composition of 5 % O_2 + 5 % CO_2 showed significantly ($p < 0.05$) higher retention of total antioxidant activity as compared to other treatments. Physiological stress during processing of apples, results in decrease of radical scavenging activity, due to loss in phytochemical moieties responsible for antioxidant activity (Lana and Tijskens, 2006). Therefore, stabilisation of the phytochemical content responsible for antioxidant activity is essential to retain anti-oxidative functions in terms of radical scavenging activity. Restriction of respiratory activity by the modified atmospheres could be the causative agent for the higher retention of phytochemicals responsible for antioxidation in terms of radical scavenging activity. Additive-based minimal processing, devoid of thermal processing and coupled with modified atmosphere could be an ideal process for retention of phytochemicals in apple slices.

5.4.12 Polyphenol oxidase (PPO) activity

Polyphenol oxidases are a group of copper protein complex enzymes that catalyze the oxidation of phenolic compounds to produce brown pigments to damaged surfaces of fruits (Lamikanra, 2002). The apple slices packed in modified atmosphere packaging showed a significant lower browning level throughout the storage period. In our experiments, higher PPO activity was seen at the beginning of storage that sharply decreased after achieving a peak in air packed samples during the storage. Compared with the control samples, PPO activities of MAP samples changed more smoothly during storage. The modification of gaseous composition in the package atmosphere surrounding the apple slices which allows control of respiration rate, enzymatic activity and oxidation (Brecht, 1995). Rocha and Morais (2001) studied the effect of controlled atmosphere storage on polyphenol oxidase (PPO) activity and phenolic content of fresh cut cubed apples (Jonagoed). The atmosphere composition was 2 % O_2 + 4 % CO_2, 2 % O_2 + 8 % CO_2, 2 % O_2 + 12 % CO_2 and the cubes were stored for 7 days at 4 °C. After 7 days, the samples stored in controlled atmosphere with concentrations of CO_2 higher than 4 % had lower PPO activities than the air stored cubes.

5.4.13 Peroxidase (POD) activity

Peroxidase is found in almost all living organisms and the main function is to control the level of peroxides generated in oxygenation reactions to avoid excessive formation of radicals which are harmful to all living organisms. Peroxidase enzyme is heat stable and because of this reason is used in the processing of fruit and vegetables as a marker enzyme (Alzamora *et al.,* 2000). Peroxidase contributes to enzymatic browning because of its affinity to accept a wide range of hydrogen donors (Lamikanra, 2002). Initially, in our study, MAP showed a significantly ($p < 0.05$) lower POD activity due to lower metabolic activities as compared to air packs samples which recorded a higher degree of phenolics degradation after 21 days.

5.4.14 Microbial analysis

Microbial growth is influenced by the physiology of the minimal processed product and thus, must also be considered as a physiological processes leading to tissue senescence and deterioration can be minimized or modulated through the implementation of an integrated approach, involving appropriate genotypes selection, pre and post-processing treatments and application of an appropriated packaging that provides optimal atmospheres (Gonzalez-Aguilar *et al.,* 2004). The initial microbial count were 3.06 log CFU g^{-1} (average) and the loads increased in all samples during storage with significant differences among treatments. Increase in microbial count upon storage of fresh-cut papaya has also been reported by Gonzalez-Aguilar *et al.* (2009). After 42 days of storage at 5 ± 2 °C minimally processed apple slices packaged in different modified atmospheric conditions showed significant lower microbial counts (average 3.47 CFU g^{-1}) compared to air packs samples (6.10 CFU g^{-1}). The United States and most European countries have regulations relative to fresh-cut produce which limit the counts of aerobic microorganisms to 6 log (cfu g^{-1}) at expiration date (Martın-Belloso *et al.,* 2006). The effect of low O_2 and high CO_2 concentration at low storage temperature on microbial load of fruits and vegetables has been extensively reported. Babic and Watada (1996) reported that low O_2 atmosphere effectively controlled the growth of spoilage microorganisms on fresh-cut spinach leaves stored at 5 ºC for up to 7 days. Berrang *et al.* (1990) demonstrated that controlled atmosphere storage with low O_2 concentration significantly reduced the growth of microorganisms on broccoli stored at 4 ºC for 21 days. Tudela *et al.* (2013) reported that low O_2 and high CO_2 concentration resulted in lower microbial counts for baby spinach stored at 7 ºC for 12 days. The inhibitory effect of low O_2 and high CO_2 concentration could be attributed to both decrease in available O_2 for metabolic processes and acidification of the microbial environment due to high CO_2 concentration. The inhibitory effect of high O_2 atmospheres to microbial growth on minimally processed produce has also been reported in other studies on fresh-cut pineapple and berries (Zheng *et al.,* 2008; Zhang *et al.,* 2013). High O_2 atmospheres have been suggested to lead to intracellular generation of reactive oxygen species such as superoxide (O_2^-), hydroxyl (OH^-), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) which damage vital cellular components and reduce cell viability when oxidative stresses overwhelm cellular protection systems (Kader and Ben-Yehoshua, 2000).

5.4.15 Sensory characteristics

In comparison with air packed samples, apple slices packed in MAP showed significantly delayed decrease in sensory scores in terms of overall acceptability during storage ($P < 0.05$). Sensory attributes of minimally processed apple slices shows better preference by the panelists for the treated samples kept under the atmosphere of 5 % O_2 + 5 % CO_2 (T_8) due to better maintenance of colour, flavour and texture than others. On the other hand, the overall acceptability score decreased significantly in control (air packs) sample after 7 days. Colour, texture and flavor were considered as the most important attributes by the panelists in deciding the shelf-life of apple slices. The sensory scores in terms of overall acceptability varied significantly ($P < 0.05$) during storage in all the MAP samples compared with each other along with browning and loss in surface texture which agreed well with the changes in firmness and instrumental color values. Ayhan and Estürk (2009) reported a lower shelf-life for minimally processed pomegranate arils packaged in low oxygen atmospheres compared to those packaged in air, nitrogen and enriched oxygen. As shown in Figure 4.17, the MAP samples were considered as the most favourable for controlling the browning and extending the shelf life of apple slices. Results of Saxena *et al.* (2008) confirmed the contribution of MAP to maintain the overall acceptability of fresh cut apple wedges. For panellists, all MAP samples had slightly higher scores for firmness than air packs freshly prepared apple slices. These observations are supported by Soliva-Fortuny *et al.* (2002) results showing that MAP on the texture preservation of fresh-cut apples. Acidity scores also have changes, whereas sweetness scores decreased during the storage, suggesting a reduction in the amount of sugars. Soliva-Fortuny *et al.* (2002), was suggesting that high CO_2 concentrations were more detrimental to the sensory quality than storage under anoxic conditions. Considering that the exposure to O_2 and CO_2 levels outside the limits of tolerance led to anaerobic respiration with the production of undesirable metabolites and other physiological disorders, as found by Soliva-Fortuny *et al.* (2002), it was important to consider the flavour judgment.

6. SUMMARY AND CONCLUSIONS

In the present study, four experiments were conducted to develop shelf stable minimally processed apple slices by application of hurdle technology.

In the first experiment, wide quantitative variations were found in the nutritional profile among the cultivars with respect to all the nutritional attributes. In view of the results, the cultivar 'Oregon Spur II' was found to have maximum fruit size and weight while the least was observed for cultivar 'Starkrimson'. Cultivars of Gala series recorded a higher red colouration (means higher a* values) in comparison to others. Non-red apple cultivars such as 'Golden Delicious' and 'Granny Smith' exhibit higher b* values. The non-red cultivar 'Golden Delicious' shown a higher concentrations of soluble solid content followed by the red cultivar 'Royal Delicious'. The maximum titratable acidity was observed in non-red cultivars, 'Winter Banana' and 'Stark Spur Golden'. Maximum antioxidant activity recorded in 'Silver Spur' (13.20 µmole Trolox equivalent g^{-1}) and the minimum in 'Granny Smith' (2.64 µmole Trolox equivalent g^{-1}) cultivar. 'Red Chief' cultivar has the maximum total carotenoid content (147.06 mg kg^{-1}). The ascorbic acid content ranged between 19.38mg 100 g^{-1} ('Well Spur') and 32.08 mg 100 g^{-1} ('Starkrimson') while the antioxidant activity varied between 2.64 µmoles Trolox equivalent g^{-1} ('Granny Smith') and 13.20 µmoles Trolox equivalent g^{-1} ('Silver Spur').The highest total carotenoid was found in 'Red Chief' (147.06 mg kg^{-1}) while in 'Early Red-I' the total carotenoid was only 29.03mg kg^{-1}. Fructose (average 50.79 g L^{-1}) was the most abundant sugar. Among the organic acids, the malic acid (average 6.03 mg L^{-1}) was foremost individual organic acids. In respect of minerals, the potassium (average 795.14 mg 100 g^{-1}) and iron (average 2.04 µg g^{-1}) were the major macro and micro elements, respectively. Chlorogenic acid was the major phenolics constituent among phenolic compounds.

In the second experiment, three cultivars harvested from the two growing sites. After analyzing the results of this experiment it was concluded that there is wide variation in the fruit weight amongst different apple cultivars was larger (133.40 to 186 g) than firmness (10.32 to 11.87 N). The cultivar 'Golden Delicious' grown at 1800 msl had the highest ascorbic acid (28.80 mg 100 g^{-1}) and total antioxidant (9.98 µmol Trolox g^{-1}) content among the cultivars and elevations studied. The total phenol content was positively correlated with the elevation ranging from 82.5 mg 100 g^{-1}

(1000-1400 msl) to 231 mg 100 g^{-1} (1500-1800 msl). Fruits grown at higher elevation had greater concentrations of sugars (average 67.75g L^{-1}) and organic acids (average 16.31g L^{-1}). Such differences indicate that the elevation range affect the nutritional composition of fruit. A significant cultivar × elevation interaction was found for sugars (fructose, glucose and sucrose) and organic acid content (malic, citric and succinic), fatty acids, minerals and physical attributes. This study confirms the role of elevation as effective factor on the physical and nutritional quality in apple fruits.

In the third experiment, a edible coatings and anti-browning agents were used to develop healthy, ready to eat, convenience fresh cut apples slices after screening of the tested cultivars. As a general trend, carboxymethyl cellulose and aloe vera coatings alongwith anti-browning agents helped in preserving quality of apple slices during storage. Microbial load was significantly low for slices coated with carboxymethyl cellulose and aloe vera. Polyphenol oxidase and peroxidase enzyme activity was also low in the coated samples. Firmness of the uncoated apple slices declined more rapidly than the coated ones during storage. At the end of storage period, control slices showed the lowest firmness (1.87 N) while all other samples recorded higher firmness (> 2 N). Our findings exhibited that the carboxymethyl cellulose and aloe vera coating in combinations with anti-browning agents improved the quality of stored apple slices. Anti-browning dip treatment combined with edible coatings prevented loss of colour during the 7 days of storage. The treatment inhibited browning, retained colour and decreased CO_2 production and O_2 consumption, soluble solids content and titratable acidity of the apple slices. Browning incidence with carboxymethyl cellulose and aloe vera in combination with anti-browning agents was lower than when they were used alone. These findings suggest that the combined use of anti-browning agents and carboxymethyl cellulose (CMC) may be useful for preserving quality and reducing browning of minimally processed apple slices.

In the fourth experiment, the minimally processed apple slices packed under different conditions of modified atmospheres, recorded reduced changes in BI and WI, firmness and microbial growth and sensory characteristics of apple slices. Significant variations were observed among the MAP apple slices in all the attributes investigated. MAP, showed the best results among the treatment in terms of retaining sensory and quality characteristics and extending the shelf life of 42 d for apple slices. The air packed samples had the highest browning index (BI = 81.80) after 5 days of storage. At the end of 42 days, the MAP samples (5 % CO_2 + 5 % O_2) showed the lowest polyphenol oxidase activity and peroxidase activity. The high overall quality demonstrated that MAP treatment could provide a better inhibitory effect on the browning and extend the shelf life of fresh-cut apple slices.

Conclusions

From our experiments we concluded that shelf stable minimally processed apple slices can be developed by application of different hurdles (preservation techniques). On studying the twenty two apple cultivars, we observed that wide variability existed between the physical and biochemical attributes. The variation was significantly different for the apple cultivars grown in other countries of the world. The orchard elevation and cultivar also had a significant influence on the physical and nutritional attributes, with apples grown at higher elevation recording higher content of glucose, organic acids, minerals and phenolics than those grown at lower elevation. The combination of the edible coating with the browning inhibitors proved to be better to maintain the aesthetic, nutritional and microbial quality of apple slices till 7 days of storage under 5 ± 2 °C. Combination of CMC (1 %) coating with hexyl resorcinol (0.01 %) + ascorbic acid (0.5 %) + $CaCl_2$ (0.2 %) was found to be the best treatment for maintaining quality and reducing surface browning of fresh-cut slices. Under modified atmospheres, the gas composition of 5 % O_2 + 5 % CO_2 + 90 % N_2 proved to be the most effective for extending the shelf life of the apple slices till 42 days of storage. Fresh-cut apples should move under cold chain conditions during marketing in order to avoid microbiological spoilage in the final finished product. The preservation techniques proposed in the current work can be easily adopted at industrial scale. The outcome from this study indicates that the use of browning inhibitors in conjunction with MAP or edible coatings can be considered as a safe and effective treatment, resulting in reduced browning of the apple slices without significantly impairing the nutritional value.

Development of minimally processed apple slices by hurdle technology

ABSTRACT

To accomplish this investigation, three objectives were formulated. First objective dealt with the investigation of nutritional attributes of 22 apple cultivars and the effect of orchard elevation on the quality of three specific apple cultivars. The second and third objectives pertained to the development of minimally processed apple slices of cv. 'Royal Delicious' by hurdle technology (Anti-browning agents, edible coatings and MAP). A considerable variation in physical and biochemical characteristics among cultivars was observed. The total soluble solids ranged between 10 ºB (Red Delicious) and 16.10 ºB (Gale Gala) and the total antioxidant activity between 2.64 (Granny Smith) and 13.20 µmole Trolox g^{-1} (Silver Spur). Fructose and sucrose were the chief sugars in the evaluated cultivars. The content of fructose ranged between 10.95 g L^{-1} (Granny Smith) and 67.55 g L^{-1} (Scarlet Gala) and that of sucrose between 9.85 (Royal Delicious) and 48.90 g L^{-1} (Granny Smith). The average content of malic acid for the twenty two apple cultivars was 6.03 g L^{-1}. Potassium was the most accumulated elements in apple fruits followed by sodium, calcium and magnesium, regardless of the cultivars. Orchard elevations (1000-1400 and 1500-1800 msl) were found to affect the fruit quality of the three apple cultivars studied (Golden Delicious, Royal Delicious and Red Gold). The variation in the fruit weight amongst different apple cultivars was larger (133.40 to 186 g) than firmness (10.32 to 11.87 N). The cultivar 'Golden Delicious' grown at 1500-1800 msl had the highest ascorbic acid content (28.80 mg 100 g^{-1}) and total antioxidant activity (9.98 µmol Trolox g^{-1}) among the cultivars studied at the two orchard elevations. The total phenolic content was positively correlated with the elevation ranging from 82.5 mg 100 g^{-1} (1000-1400 msl) to 231 mg 100 g^{-1} (1500-1800 msl). Fruits grown at higher elevation had greater concentrations of sugars (average 67.75 g L^{-1}) and organic acids (average 16.31 g L^{-1}). The influence of edible coatings (CMC and AVG) and anti-browning agents (4-hexylresorcinol (4-HR), ascorbic acid and $CaCl_2$) as well as their combination on shelf life and quality of fresh-cut 'Royal Delicious' apple slices was studied. Edible coatings improved the shelf life of fresh-cut apple by reducing physicochemical, biochemical and microbial changes without significantly affecting sensory and nutritional attributes. Based on nutritional and sensory quality changes, treatment of fresh-cut apples by CMC (1 %) in conjunction with 4-HR (0.01 %) + AA (0.5 %) + $CaCl_2$ (0.2 %) was found to be the best method to preserve quality till 7 days of storage at low temperature. Minimally processed apple slices were packed under 1.5 to 5% O_2 and 2.5 to 7.5% CO_2 (modified atmosphere packaging) for up to 42 days at 5±2 ºC. Modified atmosphere packing of apple slices showed that 5 % O_2 + 5 % CO_2 and 90 % N_2 was the most effective modified atmospheric composition to enhance the shelf life and preserve the quality of the apple slices upto 42 days at 5±2°C.

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इस अध्ययन हेतु तीन उद्देश्य प्रस्तावित किए गए थे। पहले उद्देश्य में सेब की 22 किस्मों के विभिन्न गुणों का अध्ययन, दूसरे में विभिन्न बाग की उंचाईयों का सेब की तीन किस्मों के गुणों पर प्रभाव एवं तीसरे में हर्डल प्रौद्योगिकी द्वारा रॉयल डिलिसियस किस्म की फांकों का न्यूनतम प्रसंस्करण किया गया। वर्तमान अध्ययन से यह पाया गया कि सेब की 22 विभिन्न किस्मों के भौतिक एवं जैव रासायनिक गुणों में सार्थक भिन्नता पाई गई। इनमें कुल घुलनशील ठोस, 10 से 16.10^0 ब्रिक्स तथा कुल प्रतिऑक्सीकारक सक्रियता, 2.64 (ग्रैनी स्मिथ) से 13.20 माइक्रो मोल (सिल्वर स्पर) के बीच पाए गए। मूल्यांकित किस्मों में मुख्यतः फ्रक्टोस एवं सुक्रोज, शर्कराएं पाई गई। फ्रक्टोस की मात्रा, 10.95 (ग्रैनी स्मिथ) और 67.55 ग्राम/ली. (स्कार्लेट गाला) एवं सुक्रोज की मात्रा 9.85 (रॉयल डिलिसियस) और 48.90 ग्राम/ली. (ग्रेनी स्मिथ) पाई गई। सेब की 22 किस्मों में औसतन 6.03 ग्राम/ली. मैलिक अम्ल की मात्रा थी। किस्मों में भिन्नता के बावजूद सेब के फलों में सोडियम, कैल्सियम और मैग्निशियम की तरह पोटेशियम भी एक प्रमुख संचित पोषक तत्व पाया गया। समुद्र तल से भिन्न–भिन्न उंचाईयों (1000-1400 तथा 1500-1800 मी.) पर उगने वाली सेब की तीन किस्मों (गोल्डन डिलिसियस, रॉयल डिलिसियस तथा रेड गोल्ड) की गुणवत्ता में भिन्नता पाई गई। विभिन्न किस्मों में भार का अन्तर (133.40 से 186 ग्राम) एवं उनकी दृढ़ता (10.32 से 11.87 न्यूटन) में काफी अन्तर था। विभिन्न उंचाईयों पर उगाई जाने वाली उन सभी किस्मों में समुद्र तल से 1500-1800 मी की उंचाई पर उगने वाली किस्म, गोल्डन डिलिसियस में एस्कॉर्बिक अम्ल (28. 80 मि.ली./100 ग्राम) तथा कुल प्रतिऑक्सीकारक (9.98 माइक्रो मोल ट्रोलॉक्स/ग्राम) कम उंचाई (1000-1400 मी.) वाले सेबों की अपेक्षा अधिक पाए गए। वास्तविक रूप से कुल फिनॉल की मात्रा (231 मि.ग्रा./100 ग्राम) कम उंचाई वाले सेबों से अधिक उंचाई (समुद्र तल से 1500-1800 मी.) पर अधिक थी। अधिक उंचाई पर उगने वाले किस्मों के फलों में शर्करा (औसतन 67.75 ग्राम/ली.) और कार्बनिक अम्ल (औसतन 16.31 ग्रा. /ली.) की मात्रा भी अधिक पाई गई। रॉयल डिलिसियस सेब की निधानी आयु एवं फांकों की गुणवत्ता पर खाद्य लेपों के प्रभाव (सी.एम.सी. एवं ए.भी.जी.) एवं भूरेपन विरोधी घटक (4–एच.आर., एस्कार्बिक अम्ल एवं कैल्सियम क्लोराईड) संयोजन के अध्ययन से पता चलता है कि सामान्य एवं संवेदी गुणवत्ता के आधार पर, सेब की फांकों को कार्बोक्सी मिथाईल सेलुलोस (1 प्रतिशत) में रखना लाभदायक सिद्ध हुआ। निधानी आयु एवं न्यूनतम प्रसंसकृत सेब की फांकों को संशोधित वातावरण पैकेजिंग में (1.5 से 5 प्रतिशत ऑक्सीजन, 2.5 से 7.5 प्रतिशत कार्बन डाईऑक्साइड तथा संतुलित नाइट्रोजन में पैक कर) 5^0 सेल्सियस पर 42 दिनों तक रखा गया। प्रयोगों से पता चला कि सभी संशोधित वातावरण पैकेजिंग एवं सामान्य पैकेजिंग में महत्वपूर्ण अन्तर पाया गया। अंततः यह पाया गया कि 5 प्रतिशत कार्बन डाईऑक्साइड, 5 प्रतिशत ऑक्सीजन एवं 90

प्रतिशत नाइट्रोजन से उपचारित संशोधित वातावरण पैकेजिंग, सेब की फांकों की निधानी आयु को बढ़ाने तथा भंडारित करने के लिए सर्वाधिक प्रभावी सिद्ध हुआ।

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