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Effect of several packaging films on biochemical characteristics and microbiological counts of shredded cabbage at different storage conditions

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Abstract

Shredded cabbage is becoming one of the most important minimally processed (MP)* vegetables mainly for salad and coleslaw production. This has led to a need to develop technology for extending the shelf life. This study was conducted to determine the effects of using different types of polymeric films (Polypropylene (PP), Low Density Polyethylene (LDPE), High Density Polyethylene (HDPE) and PVC cling wrap (served as Control)) of varying permeability to gases and water vapour and also with application of vacuum packaging on the biochemical characteristics and microbiological counts of the MP shredded cabbage during storage at 5±1°C with 90-95% RH. Biochemical characteristics were determined by quantitative measurements of carbon dioxide (CO_2) and ethylene (C_2H_4) production, polyphenol oxidase (PPO) activity, degree of browning and chlorophyll degradation. Meanwhile microbiological study involved mesophilic and psychrotrophic counts of bacteria, molds and yeasts. Data collected were analyzed using ANOVA and Duncan's multiple range test (DMRT) at 5% significant level. Among all the different packaging films used, PP showed the best packaging film in maintaining the storage quality of the shredded cabbage which has high CO_2 production due to its greater barrier property to CO_2 and lower ethylene production in the package atmosphere. PP also gave lower PPO activity, degree of browning and chlorophyll degradation compared to the other types of packaging films. Meanwhile for the microbiological study, the microbial flora of the shredded cabbage was predominantly bacterial, with smaller numbers of yeasts and only an occasional mold for both mesophilic and psychrotrophic microorganisms found in the packages. It was observed that different packaging system does not necessarily affect the microflora of the shredded cabbage in the same manner as the shelf life.

Key words: Minimally processed, cabbage (*Brassica oleracea* L. var. Capitata), packaging films, biochemical characteristics, microbiological counts.

Introduction

Modified atmosphere packaging involves the use of plastic films with known permeability to gases, for the packaging of products. The presence of the semi-permeable film causes the formation of an atmosphere inside the package resulting from the interaction of the respiration rate of the commodity and the gas permeability of the packaging film [1-3]. As a consequence of the respiration of the produce, there is an increase in CO_2 and a decrease in O_2. There is also gas interchange through the film with the external atmosphere depending on the speed of diffusion through the package [2].

Different atmospheres are achieved according to the film permeability and the respiratory intensity of the produce, and these affect the physiological and biochemical activities of the produce such as enzymatic browning, chlorophyll degradation, etc. [1, 4, 5]. Accumulation of carbon dioxide and depletion of oxygen were determined as a percentage of total headspace gas to find the effects of various holding conditions on the rate of respiration.

MAP used for shredded cabbage will partially inhibit the increase of PPO activity [6] in the shredded cabbage which is associated with browning. This implies that the packaging films used may form a protective barrier and reduce the supply of oxygen to the shredded cabbage.

Another area of assumed causality and conventional wisdom is the belief that modified atmospheres suppress microbial growth and in that way extend shelf life. There is ample evidence that elevated CO_2 extends the lag phase of bacterial growth and can slow the propagation of bacteria [7]. The potential for microbial contamination of fruits and vegetables is high because of the wide variety of conditions to which the produce is exposed during growth, harvest, distribution and processing. Their behaviour depends on the nature of the produce and the modified atmosphere (MA) created in the package [8].

Generally minimally processed vegetable supports rapid growth of microorganisms and counts of 10^7 to 10^8 CFU/g are frequently obtained [8]. The microflora of processed fruits and vegetables differs depending on their pH. Vegetables are usually contaminated by less-acid tolerant organisms, while fruits are contaminated by more acid-tolerant bacteria and fungi [9].

For a given product, mesophilic bacteria counts at the beginning of storage may be a more useful indicator of storage stability. Mesophilic bacteria counts on plate count are highly variable and range from 10^3 to 10^9 CFU/g. Product quality is often acceptable despite such high counts [8]. Some procedures may, nevertheless, increase the number of mesophilic bacteria. For example, shredding and slicing were found to increase counts from 10^3 to 10^4-10^6 CFU/g for a range of vegetables [10].

*List of abbreviations: CW - Cling wrap (Control), HDPE - High density polyethylene, IR - Respiratory intensity, LDPE - Low density polyethylene, MA - Modified atmosphere, MP - Minimally processed, PP - Polypropylene, PPO - Polyphenol oxidase, PVC - Polyvinyl chloride, RH - Relative humidity, SSC - Soluble solids concentration, TCD - Thermal conductivity detector, VCW - Vacuum cling wrap (Control), VHDPE - Vacuum high density polyethylene, VLDPE - Vacuum low density polyethylene, VPP - Vacuum polypropylene

The objectives of this study were to determine the effect of using different packaging films on the biochemical characteristics such as carbon dioxide and ethylene production, PPO activity, degree of browning, chlorophyll content and also on the microbiological count of shredded cabbage stored at chilled temperature (5±1°C) with high RH (90-95%) and also on the effect of the application of vacuum packaging.

Materials and Methods

Sample preparation: Freshly picked round white cabbage (*Brassica oleracea* L. Capitata) of hybrid KKY-Cross was supplied by MARDITECH Plantation in Cameron Highland, Malaysia. Four types of packaging materials were used to pack the shredded cabbage and their properties are as stated in Table 1.

Cabbage heads were washed under chlorinated water and then shredded after removing outer leaves and core area. Shredding/ slicing was carried out using National Food Processor model MK5070M to give a fine and uniformly cut shreds of 3-5 mm thick. The shredded cabbage was then washed for 30 s in distilled water and toss-dried in shaking baskets. The final product was packed (400 g/bag) in different packaging films (PP, LDPE, HDPE) and polystyrene tray with PVC cling wrap (served as control). Each bag has the same surface area of 2 x (270 mm x 200 mm). Polystyrene tray with PVC cling wrap was used as control due to the fact that it has been a normal commercial practice for packing fresh produce and minimally processed products especially on the supermarkets' shelves.

For non-vacuum condition, the bags of samples were heat-sealed whereas for vacuum packaging, the bags were heat-sealed under vacuum level at 63.5 cmHg using vacuum packing machine model VacMaster svp-40. All the packed samples were stored at 5±1°C with 90-95% relative humidity. Three packs of samples from each treatment were removed from the controlled temperature storage at weekly intervals to be evaluated (destructive samplings).

Determination of ethylene and CO_2 production in the package atmosphere: Three representative samples of 400 g packages of shredded cabbage for each different packaging films and conditions were used for the determination of ethylene and carbon dioxide production. Assessments were conducted at weekly intervals during storage. The same samples were used for continuous assessment at day 0 and week 1, 2, 3 and 4. A piece of 2 cm x 2 cm double-sided tape was pasted on the package and 1 mL of gas sample was withdrawn from the inside of the package through this double-sided tape by using an injector syringe and the packages were resealed after each injection. The gas sample was injected into the 5890 Hewlett Packard Gas Chromatograph fitted with Thermal Conductivity Detector (TCD) and a stainless steel column (1.524 m in length and 3.175 mm OD, packed with 80-100 mesh 'Haye Sep D') for measurement. Helium served as the carrier gas at flow rate of 30 mL/min. Standard gases used for calibration were 99.9% CO_2 and gas mixture of ethylene in He at 100 ppm ± 5% (by volume).

$$CO_2/C_2H_4 \text{ production} = \frac{\text{Volume of space surrounding shredded cabbage } x \text{ Amount of } C_2H_4 \text{ or } CO_2\ x\ 100}{\text{Shredded cabbage weight } x \text{ Incubation time}}$$

Five injections were obtained from 3 packages for each treatment. The test was only done for the non-vacuumed packing condition since the vacuum-packed samples were observed to be shrink-wrapped after vacuum was applied. This made it difficult to extract out any gases from the packages.

Determination of PPO activity

Preparation of shredded cabbage for PPO extract: This method is a slight modification of the method of Arogba [11]. Forty grams of shredded cabbage was homogenized with 60 mL pre-chilled (0°C) 0.01 M phosphate buffer (pH 6.0) using a blender and made up to 100 mL with buffer in a measuring cylinder. The mixture was immediately filtered using suction through coarse filter (Whatman no. 4) paper into an Erlenmeyer flask (250 mL) placed on ice. No brown colour was observed in the crude extract before used.

Assay for PPO activity: PPO activity was determined using a colorimetric method [12] based on the initial rate of increase in absorbance at 420 nm. 10 mL of 0.01 M phosphate buffer (pH 6.0), 1 mL of 0.5 M catechol and 0.5 mL of the crude enzyme extract were pipetted in this sequence into a test tube, mixed and absorbance recorded at 30 s intervals. One unit of PPO activity causes a change in absorbance of 0.001/min at 420 nm.

Determination of degree of browning: According to method by Coseteng and Lee [13], approximately after an hour from the time of homogenizing, 15 mL 95% ethanol was added to 10 mL of the supernatant from the above centrifugation (from the pH and SSC), shaken and was centrifuged again at 800 x g for 15 min. The degree of browning of the supernatant was determined by measuring absorbance at 440 nm using a spectrophotometer. All analyses were carried out in triplicates.

Determination of chlorophyll content: A small amount of $CaCO_3$ was added to 1 g of sample and blended with 80% acetone (pre-chilled at 4°C) for 3 minutes. The mixture was filtered and washed with 80% acetone until colourless. The extract was transferred to a 100 ml volumetric flask and made up to volume with 80% acetone. Absorbance reading was recorded at 645 nm and 663 nm using spectrophotometer. 80% acetone was used as blank before absorbance reading of sample was taken [14].

$$Ch_t = (20.2\ x\ \text{OD at 645 nm} + 8.02\ x\ \text{OD at 663 nm})\ x\ \frac{\text{volume made up}}{100}\ x\ \text{weight of sample}$$

Ch_t = Total chlorophyll

Microbiological study: Samples were prepared for microbial analyses by stomaching 25 g of shredded cabbage in 225 mL sterile water for 1.5 min. A series of dilutions was made in 0.1% peptone water as needed (up to 10^{-6}), and 0.1 mL surface plated on agar. Viable microbial counts were obtained with plate count agar (PCA) and dichloran Rose Bengal chloramphenicol (DRBC) agar. PCA medium allows

Table 1. Properties of the packaging films used in the study.

Film Properties	Packaging Film: Polypropylene (PP)	Low density polyethylene (LDPE)	High density polyethylene (HDPE)	Polyvinyl chloride (PVC) Cling Wrap
Thickness (mm)	0.03	0.03	0.03	0.014
Bag dimension (cm^2)	20 x 31	20 x 31	20 x 31	*
Water vapour transmission rate (g/m^2/day)	5.89	4.26	5.12	338.42
Oxygen transmission rate (cc/m^2/day)	>2,000	>2,000	>2,000	>10,000
Carbon dioxide transmission rate (cc/m^2/day)	11,931	28,418	18,116	>100,000

*Polystyrene tray with dimension of 19.0 cm x 27.0 cm x 2.5 cm (depth) was used together with PVC cling wrap (as a base).

growth of most bacteria, while DRBC will allow growth of yeasts and molds. PCA plates were counted after 24 hours and DRBC can only be counted after 48 to 72 hours of upright incubation at 25-30°C (mesophilic condition). Meanwhile, PCA and DRBC plates were also incubated in the cool room at 5±1°C for 3-4 days (psychrotrophic condition). Microbial counts are expressed as $\log_{10}$ count per g sample [15].

Statistical analysis: The data collected were analyzed using the analysis of variance (ANOVA), significant differences ($p<0.05$) between treatments were determined using Duncan's multiple range test (DMRT). The statistical programme used was Statistical Analysis System [16].

Results and Discussion

CO_2 and C_2H_4 production in the package atmosphere: From the overall mean values (Table 2), no specific trend was observed for CO_2 production in the package atmosphere during storage. However, CO_2 production in the PP packages showed a significantly ($p<0.05$) higher rate of CO_2 due to the greater barrier property to CO_2, followed by HDPE and LDPE, whereas CO_2 in control packages showed significantly ($p<0.05$) lower concentration due to the high film permeability to CO_2. The production of CO_2 in the package atmosphere for PP, LDPE and HDPE showed a significant ($p<0.05$) increase during the first week of storage and the concentration then started to level off thereafter (Fig. 1a). In control packages the production of CO_2 showed a significant ($p<0.05$) decrease during storage.

In the presence of O_2 there was an increase in the respiration of the injured tissues, resulted in the increased accumulation of the CO_2 in the package atmosphere, and after one week of storage the concentration of CO_2 in PP, LDPE and HDPE packages seemed to reach equilibrium when their values level off. According to Pretel et al. [17], the modified atmosphere inside the bags (O_2 and CO_2) and the time necessary to reach equilibrium depend on the respiratory intensity (IR) of the produce and the permeability of the film.

When commodities are cut or otherwise damaged mechanically, they start respiring more rapidly, and ethylene production is stimulated [18]. Some variation in ethylene production rate was noted in the different packages. The general trend was a sharp rise due to wounding, followed by a decline. The marked ethylene variability was due to the fact that gas concentrations within each package evolved differently [19].

The ethylene concentration decreased during storage, and normally this occurred after one week of storage (Fig. 1b), this decrease being more pronounced in the films of least permeability due to a combination of its diffusion through the film and the inhibition of its synthesis, as the CO_2 concentration increased and O_2 concentration decreased [17].

In this study PP showed significantly ($p<0.05$) lower concentration of ethylene than LDPE and HDPE after the first week of storage before their concentration started to level off after two weeks of storage. In contrast to the other films used, control (CW) showed a rapid increase after the first week until the second week of storage and its concentration started to level off thereafter at a significantly ($p<0.05$) higher concentration compared to the other types of films. It seems that ethylene concentration in the package atmosphere was inversely proportional with the concentration of CO_2 during storage (Figs 1a-b). This might be due to the fact that the packaging films are more permeable to CO_2 as compared to ethylene gas especially in the control film.

There was a significantly ($p<0.05$) higher concentration of CO_2 and lower concentration of ethylene in the package headspace for control samples with vacuum compared to the non-vacuum-packed samples (Table 2). This may be due to the initial low O_2 concentration in the package headspace at the early days of storage as a result of the vacuum-package. Since control film was a very permeable film to gases, therefore the concentration of CO_2 later on became more pronounced with a slightly lower concentration of ethylene.

PPO activity: PPO, like other oxidases, is an enzyme involved in several metabolic changes and reactions of plant tissue responsible for tissue browning. In this study, PPO activity of MP shredded cabbage was determined by measuring changes in absorbance of 0.001/min at 420 nm with spectrophotometer. The enzyme activity was tested towards catechol which is one of the substrates for PPO extracted from shredded cabbage.

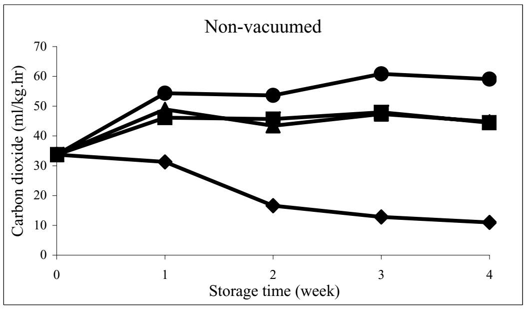
PPO activity was affected significantly by storage. During low temperature storage, PPO activity in the shredded cabbage packed in the film bags were significantly ($p<0.05$) lower than before they were packed and stored. However, the activity will increase significantly ($p<0.05$) until it reached maximum and will then decrease gradually when there were some factors limiting its activity (Figs 2a-b). The overall mean value of PPO activity in PP showed significantly ($p<0.05$) lower activity compared to other types of packaging films (Table 3). Meanwhile in vacuum condition, control (VCW) showed the highest PPO activity followed by VPP, VLDPE and VHDPE.

Generally, vacuum-packed samples showed a significantly ($p<0.05$) lower PPO activity compared to the non-vacuum-packed samples except for PP vs. VPP, and the differences were significant ($p<0.05$) in LDPE vs. VLDPE, where LDPE gave higher PPO activity as compared to VLDPE. From the results obtained, it was observed that PPO activity was more pronounced in the samples packed in the film with higher permeability to gases (in this case CW), especially O_2, which is a second substrate in this catecholase activity [20].

Table 2. Overall mean values for CO_2 and C_2H_4 produced of minimally processed shredded cabbage packed in different packaging films vacuum and non-vacuum conditions during storage.

Main effects	Gas production (ml/kg h)	
	CO_2	C_2H_4
Non-vacuumed treatments		
PP	52.33^a	9.35^c
LDPE	43.58^b	12.51^b
HDPE	43.64^b	13.02^b
Control (CW)	21.09^c	23.59^a
Vacuumed Treatment		
Control (VCW)	24.72^a	18.45^a
Test of Significance		
Control (CW vs. VCW)	*	*
Storage time (week) (Non-vacuumed)		
0	33.73 ± 1.23^c	14.19 ± 1.08^{ab}
1	45.19 ± 0.88^a	12.95 ± 0.96^b
2	39.83 ± 1.19^b	16.70 ± 1.36^a
3	42.24 ± 0.92^{ab}	13.95 ± 1.28^{ab}
4	39.82 ± 1.19^b	15.29 ± 0.52^{ab}

Note : Values are means of 3 replicates (5 representative samplings/replicate).
Means (n=15) ± standard deviation
Means with the same superscript letter within each column are not significantly different at 5% level ($p<0.05$)
ns , * = Nonsignificant or significant at $p \leq 0.05$



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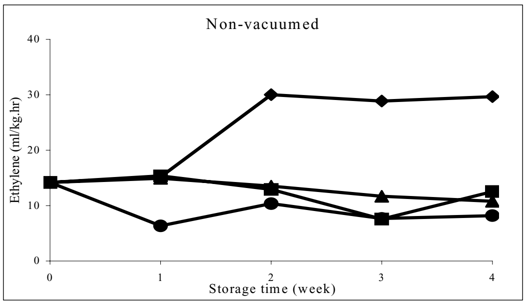

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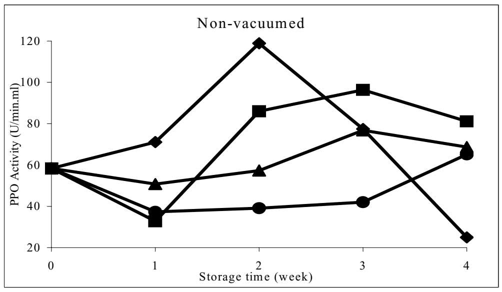
Figure 1. Effect of different packaging films on the (a) carbon dioxide and (b) ethylene production in the package atmosphere of minimally processed shredded cabbage during low temperature storage.

-●- PP/VPP; -■- LDPE/VLDPE; -▲- HDPE/VHDPE; -◆- CW/VCW



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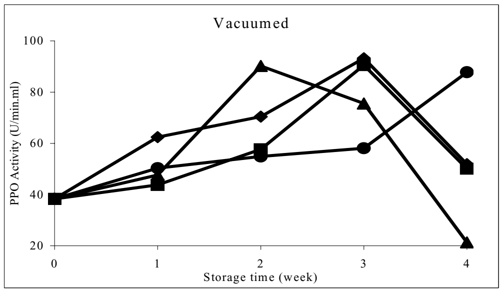
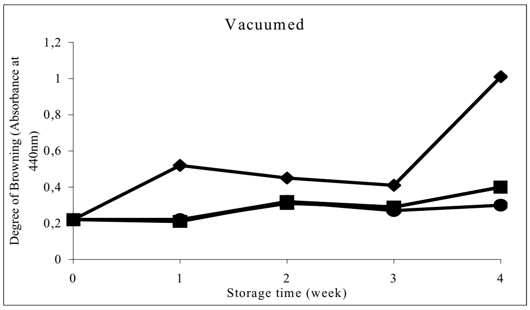


(b)

Figure 2. Effect of different packaging films on the polyphenol oxidase (PPO) activity of minimally processed shredded cabbage (a) non-vacuum packaging and (b) vacuum packaging during low temperature storage.

-●- PP/VPP; -■- LDPE/VLDPE; -▲- HDPE/VHDPE; -◆- CW/VCW



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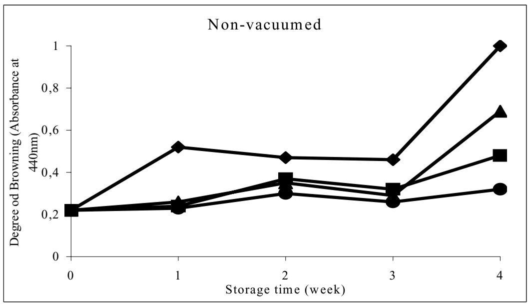
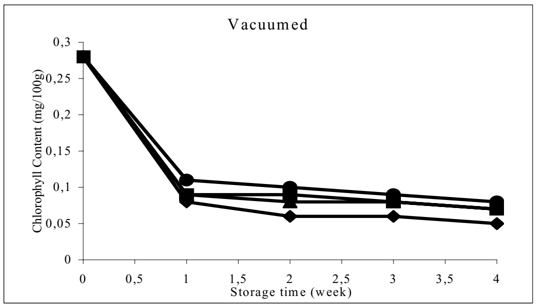


(b)

Figure 3. Effect of different packaging films on the degree of browning of minimally processed shredded cabbage (a) non-vacuum packaging and (b) vacuum packaging during low temperature storage.

-●- PP/VPP; -■- LDPE/VLDPE; -▲- HDPE/VHDPE; -◆- CW/VCW



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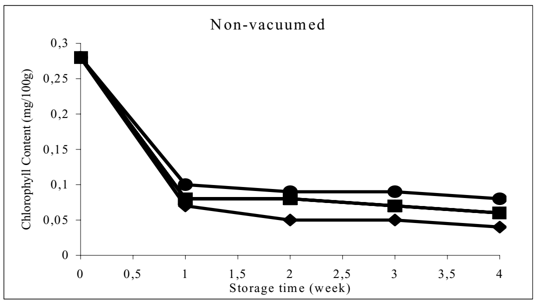

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Figure 4. Effect of different packaging films on the chlorophyll content of minimally processed shredded cabbage. (a) non-vacuum packaging and (b) vacuum packaging during low temperature storage.

-●- PP/VPP; -■- LDPE/VLDPE; -▲- HDPE/VHDPE; -◆- CW/VCW

Degree of browning: The degree of browning is related to the total phenolics or polyphenoloxidase (PPO) activity and is proportional to the absorbance value at 440 nm [13]. The *o*-quinones formed from phenolic compounds by phenolases (such as PPO) are the precursors of the brown colour [21].

The absorbance at 400 nm of the supernatant allowed estimation of the soluble pigments. Reflectance measurement (L* value) was carried out in order to evaluate the insoluble polymerized pigments bound to membranes. The latter compounds are not taken into account when only absorbances between 400 and 440 nm are considered [13, 22] for the browning estimation. Moreover, the method applied to determine the degree of browning in this study avoided possible interferences among brown polymers and other natural pigments such as chlorophylls and carotenoids, which absorbed in the same wavelength range.

There were significant (p<0.05) increase in the absorbance values at 440 nm during storage and there were also significant (p<0.05) differences between different packaging films (Table 3, *Figs 3a-b*). Table 3 also indicates that PP showed the least degree of browning followed by LDPE and HDPE while control (CW) gave the highest value. Similar trend was also applied to samples packed in vacuum condition, where control (CW) showed a significant (p<0.05) difference in the degree of browning compared to the other 3 types of packaging films. The degree of browning of the 4-week stored samples was significantly (p<0.05) higher in all packaging conditions compared to the values at other time intervals.

Samples packed in vacuum condition were significantly (p<0.05) lower in degree of browning compared to those packed in non-vacuum condition, mainly in LDPE vs. VLDPE and HDPE vs. VHDPE. Generally, the degree of browning was greater in the samples packed in the packaging films with high permeability to gases, especially O_2 (in this case CW), since the general course of brown pigmentation is known to be involved in enzymatic oxidation.

Chlorophyll content: Changes in chlorophyll content correspond to changes in colour, which in turn are indicative of changes in quality. Chlorophyll content during storage of minimally processed shredded cabbage packed in different packaging films are shown in Table 3 and Fig. 4. Chlorophyll content decreased considerably during storage in all packaging films and no significant differences were observed among different films used, even though control (CW) showed slightly higher chlorophyll degradation as compared to the other films. A general rapid decrease in chlorophyll a and b, ranging from 64.3% in PP to 75% in control (CW) from its initial content, was noted in the shredded cabbage during the first week of storage in all the packaging films.

There were also no significant differences found in the chlorophyll degradation between vacuum and non-vacuum-packed samples for all the different types of packaging films used. In a study done in spinach, degradation of chlorophyll was hastened by exposure to ethylene which resulted in the increase of chlorophyllase activity [23, 24].

An effect of slicing on the rate of respiration and ethylene production was reported in several fruits and vegetables. Therefore the dependence of chlorophyll degradation with damage area in shredded cabbage could be related to higher ethylene production induced by higher metabolic rates. After reaching a maximum decrease in the chlorophyll content, the degradation of chlorophyll was then reduced. This might be due to the high CO_2 and/or O_2 concentrations which reduce the breakdown of chlorophyll to pheophytin [25, 26].

Mesophilic microorganisms: Mesophilic bacterial counts could be used as an indicator of storage stability [27]. Generally population of aerobic mesophilic bacteria decreased significantly (p<0.05) after package and storage at low temperature. The population will then increase significantly as the storage progressed and the mesophilic bacteria populations were most pronounced at week 4 of storage in all packaging films (Table 4, Fig. 5). Samples packed in control (CW) film showed the highest number of mesophilic bacteria counts followed by LDPE, PP and HDPE. In vacuum condition, samples packed in VPP showed significantly (p<0.05) lower viable count of mesophilic bacteria compared to control and other packaging films.

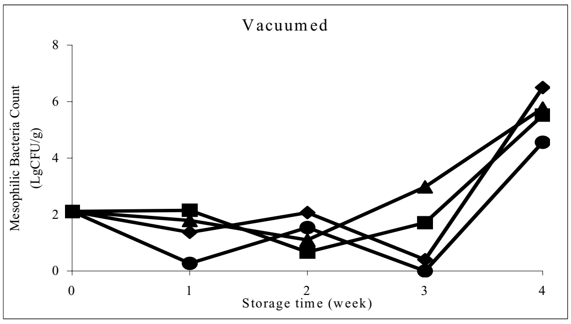
There was also no significant difference observed in the populations of bacteria between samples in vacuum and non-vacuum-packed for all types of packaging films. Basically, the viable population of mesophilic aerobes presented on day 0 (fresh) and after 3 weeks of storage was still below the threshold level of causing spoilage or hazards in both vacuum and non-vacuum conditions. Only after 4 weeks of storage in non-vacuum condition, the populations of mesophilic bacteria increased above the threshold level of 6.0 $\log_{10}$ according to Beauchat [28] especially in samples packed in LDPE and PP. However, for minimally processed vegetables, French legislation allows a maximum of 5 x 10^4 CFU/g at production stage and 5 x 10^7 CFU/g at consumption stage [29].

Mesophilic mold and yeast counts were found to be very much lesser than the mesophile bacterial counts (Table 4, Fig. 6). Generally the population of molds and yeasts increased during the first few weeks of storage and the count will then decrease or even no growth was observed after 4 weeks of storage in all packaging films. There was a significant (p<0.05) difference in mesophilic mold and yeast counts in the different packaging films used. Samples packed in LDPE showed significantly (p<0.05) higher mesophilic mold and yeast counts, followed by control (CW), HDPE and PP, whereas there were no significant difference found in the total mold and yeast counts in the different packaging films in vacuum condition.

Mold and yeast counts in PP and HDPE in vacuum condition (VPP and VHDPE respectively) were significantly (p<0.05) higher compared to the counts in their respective non-vacuum-packed samples, but in VLDPE significantly (p<0.05) higher count was found in its respective non-vacuum-packed sample. Meanwhile the mold and yeast counts in the samples packed in CW and VCW were not significantly different. Barriga et al. [30] reported that molds were present occasionally and yeasts were always present but at low levels (10^1 to 10^2 CFU/g). After an increase from 10^3 to 10^5 CFU/g, the yeast population returned to initial levels. Yeasts grow well at neutrality and under alkaline conditions but they do not compete well with bacteria at these pH values [31].

Psychrotrophic microorganisms: Low temperature storage may select for psychrotrophic microorganisms such as *Aeromonas hydrophila* and *Listeria monocytogenes*. *A. hydrophila* has a high CO_2 requirement [32] and it is more likely to grow under cold modified atmosphere storage. Population of psychrotrophic microorganisms exceeded those of mesophiles in all the packaging films used (Table 4). This was probably due to the storage at 5±1°C and 90-95% RH for MP shredded cabbage that was more conducive for growth of psychrotrophic than mesophiles.

Total bacterial populations increased significantly (p<0.05) during storage in all the packaging films (Fig. 7). This might be because microorganisms were likely to grow on MP produce due to the presence of cut surfaces and high moisture in the MAP storage [33]. Psychrotrophic bacterial counts in LDPE and HDPE films showed no significant difference with control (CW), whereas the count was significantly (p<0.05) lower in PP as compared to CW. Meanwhile in vacuum condition, the psychrotrophic bacterial count in VHDPE had no significant difference with control (VCW) but the counts in VLDPE and VPP were significantly (p<0.05) lower than in control (VCW). Overall, no significant difference was observed in the total psychrotrophic bacterial counts in all different types of packaging films both in vacuum and non-vacuum conditions.



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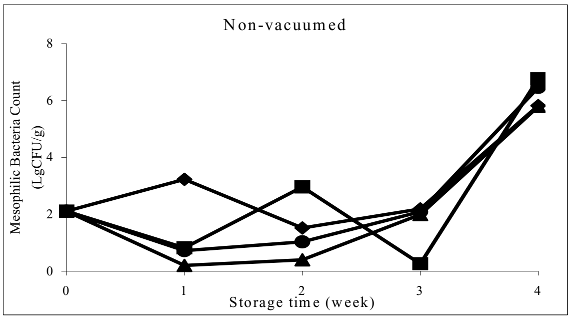
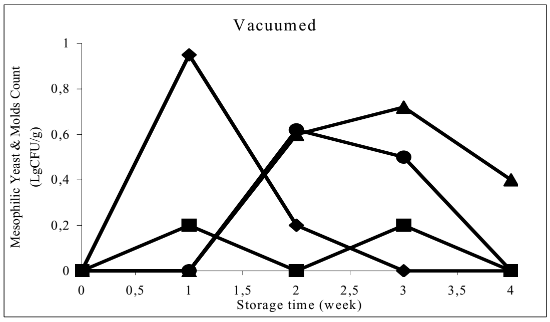


(b)

Figure 5. Effect of different packaging films on the mesophilic bacteria count of minimally processed shredded cabbage (a) non-vacuum packaging and (b) vacuum packaging during low temperature storage.
-●- PP/VPP; -■- LDPE/VLDPE; -▲- HDPE/VHDPE; -◆- CW/VCW



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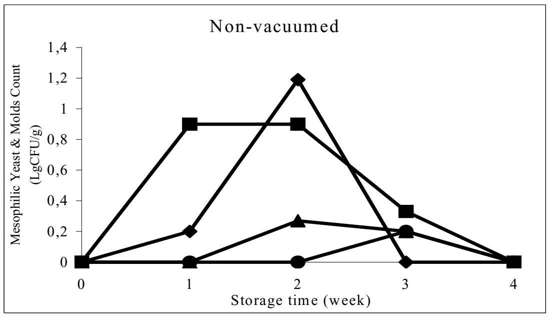
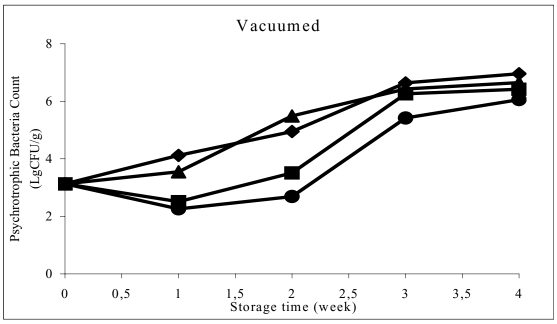
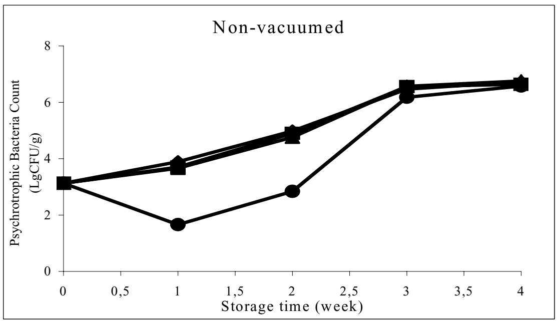


(b)

Figure 6. Effects of different packaging films on the mesophilic yeast and mold count of minimally processed shredded cabbage (a) non-vacuum packaging and (b) vacuum packaging during low temperature storage.
-●- PP/VPP; -■- LDPE/VLDPE; -▲- HDPE/VHDPE; -◆- CW/VCW

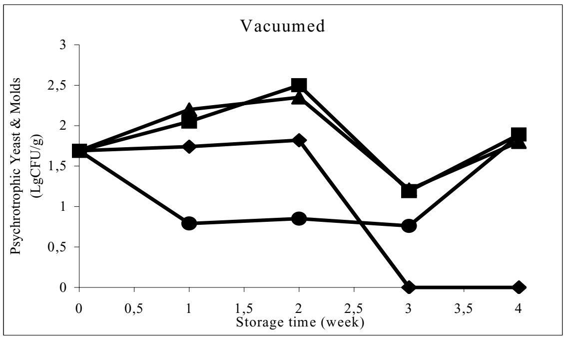


(a)

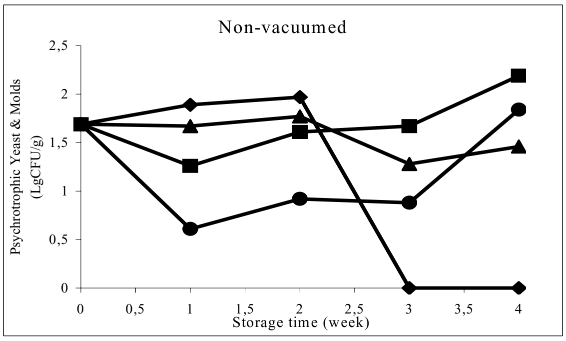


(b)

Figure 7. Effects of different packaging films on the psychrotrophic bacteria count of minimally processed shredded cabbage (a) non-vacuum packaging and (b) vacuum packaging during low temperature storage.
-●- PP/VPP; -■- LDPE/VLDPE; -▲- HDPE/VHDPE; -◆- CW/VCW



(a)



(b)

Figure 8. Effects of different packaging films on the psychrotrophic yeast and mold count of minimally processed shredded cabbage (a) non-vacuum packaging and (b) vacuum packaging during low temperature storage.
-●- PP/VPP; -■- LDPE/VLDPE; -▲- HDPE/VHDPE; -◆- CW/VCW

Different morphology of the bacteria colonies found on mesophilic and psychotrophic plate counts had turned out to be Gram-negative rods and cocci. King et al. [15] reported that 80-90% of mesophilic bacteria found in MP vegetables are Gram-negative rods such as *Pseudomonas* spp., *Enterobacter* spp. or *Erwina* spp.

Mold and yeast counts in psychrotrophic condition were also found to be higher than in the mesophilic counts (Table 4) in all the different types of packaging films used. No significant difference in the total counts of psychrotrophic molds and yeasts was observed among different packaging films used in both vacuum and non-vacuum conditions. There were also no

Table 3. Overall mean values for PPO activity, degree of browning and chlorophyll content of minimally processed shredded cabbage packed in different packaging films with vacuum and non-vacuum conditions during storage.

Main effects	Biochemical analysis		
	PPO activity ($Umin^{-1}ml^{-1}$)	Degree of browning	Chlorophyll content (mg/100g)
Non-vacuumed treatments			
PP	48.38^{b}	0.27^{d}	0.13^{a}
LDPE	70.91^{a}	0.33^{c}	0.12^{a}
HDPE	62.38^{a}	0.36^{b}	0.11^{a}
Control (CW)	70.16^{a}	0.53^{a}	0.10^{a}
Vacuumed treatments			
VPP	57.89^{ab}	0.26^{b}	0.13^{a}
VLDPE	56.09^{ab}	0.29^{b}	0.12^{a}
VHDPE	54.62^{b}	0.29^{b}	0.12^{a}
Control (VCW)	63.27^{a}	0.52^{a}	0.10^{a}
Test of Significance			
PP vs. VPP	*	ns	ns
LDPE vs. VLDPE	*	*	ns
HDPE vs. VHDPE	ns	ns	ns
Control (CW vs. VCW)	ns	ns	Ns
Storage time (week) (Non-vacuumed)			
0	58.33±1.52^{bc}	0.22±0.01^{d}	0.28±0.03^{a}
1	48.00±2.69^{c}	0.31±0.01^{c}	0.08±0.00^{b}
2	75.33±5.46^{a}	0.38±0.01^{b}	0.08±0.00^{b}
3	73.14±5.24^{a}	0.33±0.00^{c}	0.07±0.00^{b}
4	59.97±3.92^{b}	0.62±0.01^{a}	0.06±0.00^{b}
Storage time (week) (Vacuumed)			
0	38.33±1.00^{d}	0.22±0.01^{d}	0.28±0.03^{a}
1	51.06±2.03^{c}	0.29±0.01^{c}	0.09±0.00^{b}
2	68.31±3.17^{b}	0.35±0.01^{b}	0.08±0.00^{b}
3	79.36±3.85^{a}	0.31±0.01^{bc}	0.08±0.00^{b}
4	52.78±4.09^{c}	0.52±0.01^{a}	0.07±0.00^{b}

Note : Values are means of 3 replicates (3 representative samples/replicate), Means (n=9) ± standard deviation
Means with the same superscript letter within each column are not significantly different at 5% level ($p<0.05$)
ns , * nonsignificant or significant at $p<0.05$

Table 4. Overall mean values for microbiology counts (mesophilic and psychrotrophic bacterial, yeast and mold) of minimally processed shredded cabbage packed in different packaging films with vacuum and non-vacuum conditions during storage.

Main effects	Microbial analysis (Log_{10}CFU/g)			
	Mesophilic bacterial Count	Psychrotrophic bacterial count	Mesophilic mold &yeast count	Psychrotrophic mold & yeast count
Non-vacuumed treatments				
PP	2.48^{ab}	4.08^{b}	0.04^{b}	1.19^{a}
LDPE	2.58^{ab}	4.99^{a}	0.43^{a}	1.68^{a}
HDPE	2.10^{b}	4.98^{a}	0.09^{b}	1.57^{a}
Vacuumed treatments				
VPP	1.70^{b}	3.91^{b}	0.22^{a}	1.19^{a}
VLDPE	2.43^{a}	4.37^{b}	0.08^{a}	1.86^{a}
VHDPE	2.75^{a}	5.05^{a}	0.34^{a}	1.85^{a}
Control (VCW)	2.49^{a}	5.16^{a}	0.23^{a}	1.05^{a}
Test of significance				
PP vs. VPP	ns	ns	*	ns
LDPE vs. VLDPE	ns	ns	*	ns
HDPE vs. VHDPE	ns	ns	*	ns
Control (CW vs. VCW)	ns	ns	ns	ns
Storage time (week) (Non-vacuumed)				
0	2.11±0.31^{b}	3.13±0.34^{c}	0.00±0.00^{b}	1.69±0.27^{a}
1	1.24±0.27^{c}	3.23±0.32^{c}	0.27±0.14^{ab}	1.36±0.31^{a}
2	1.48±0.29^{bc}	4.37±0.35^{b}	0.59±0.20^{a}	1.57±0.33^{a}
3	1.63±0.32^{bc}	6.45±0.12^{a}	0.18±0.11^{b}	0.96±0.28^{a}
4	6.21±0.19^{a}	6.68±0.12^{a}	0.00±0.00^{b}	1.37±0.36^{a}
Storage time (week) (Vacuumed)				
0	2.11±0.31^{b}	3.13±0.34^{c}	0.00±0.00^{a}	1.69±0.27^{ab}
1	1.39±0.30^{b}	3.11±0.31^{c}	0.29±0.14^{a}	1.69±0.33^{ab}
2	1.34±0.30^{b}	4.16±0.35^{b}	0.36±0.16^{a}	1.88±0.35^{a}
3	1.27±0.31^{b}	6.19±0.17^{a}	0.35±0.14^{a}	0.79±0.25^{b}
4	5.59±0.20^{a}	6.52±0.13^{a}	0.10±0.10^{a}	1.39±0.35^{ab}

Note: Values are means of 3 replicates (5 plates/replicate). Means (n=15) ± standard deviation
Means with the same superscript letter within each column are not significantly different at 5% level ($p<0.05$)
ns , * = nonsignificant or significant at $p<0.05$

significant differences found in the mold and yeast population between samples packed in vacuum and non-vacuum conditions in all the different packaging films (Fig. 8).

Yeast on MP vegetables are generally unaffected by modified atmosphere. Molds are aerobic microorganisms, therefore the modified atmosphere observed in minimally processing with high CO_2 and low O_2 concentration should inhibit their growth [34]. Many different yeast species of comparable numerical importance including *Candida* spp., *Rhodotorula* spp. and *Cryptococcus* spp have been identified in MP vegetables. Fungi have been isolated less frequently and belonged to the genera *Sclerotinia, Mucor, Aspergillus, Cladosporium* and *Rhizopus* [8]. Yeasts cause deterioration through fermentative production of CO_2, ethanol and off-flavours [35].

Overall, it can be considered that the most important factor that kept the microbial growth at a relatively low rate in this microbiological study was the constant temperature control at 5±1°C; 90-95% RH, during storage combined with the low initial load of microorganisms present in the prepared shredded cabbage. However, these conditions are rarely found commercially. The development of microorganisms and the decrease in quality score is not linear. In many instances, total bacterial counts at the end of the storage are unrelated to sample quality. Indeed, in shredded carrots, total counts of mesophilic flora were similar in spoiled samples and in samples with good appearance [8].

Conclusions

In conclusion, among all the different packaging films applied, PP showed to be the best packaging film in maintaining the storage quality of the MP shredded cabbage, which gave lower respiration rate in terms of CO_2 production and ethylene production in the package atmosphere. PP also gave lower PPO activity and degree of browning and chlorophyll degradation. The microbial flora of the shredded cabbage was predominantly bacterial, with smaller numbers of yeasts and only an occasional mold for both mesophilic and psychrotrophic microorganisms. It was observed that different packaging system does not necessarily affect the microflora of the shredded cabbage in the same manner as the shelf life.

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References

[1]Yahia, E.M. and Gonzalez-Aguilar, G. 1998. Lebensm.- Wiss. Technol. **31:**602-606.

[2]Kader, A.A. 1992. Postharvest technology of horticultural crops. 2nd ed., Univ. of California, Division of Agriculture and Natural Resources; Publication 3311.

[3]Labuza, T.P. and Breene, W.M. 1989. Application of active packaging for improvement of shelf life and nutritional quality of fresh and extended shelf-life foods. J. Food Processing and Preservation **13**:1-69.

[4]Riquelme, F., Pretel, M.T., Martinez, G., Serrano, M., Amoros, A. and Romojaro, F. 1994. Packaging of fruits and vegetables: Recent results in food packaging and preservation. Mathlouthi, M. (eds). Blackie. pp. 141-158.

[5]Folchi, A., Pratella, G.C., Tian, S.P. and Bertolini, P. 1995. Italian J. Food Sci. **3**:245-253.

[6]Jiang, Y. and Li, Y. 2001. Effects of chitosan coating on postharvest life and quality of longan fruit. J. Food Chemistry **73**:139-143.

[7]Zagory, D. 1999. Effects of post-processing handling and packaging on microbial populations. Postharvest Biol. Techn. **15**:313-321.

[8]Nguyen-the, C. and Carlin, F. 1994. The microbiology of minimally processed fresh fruits and vegetables. CRC Crit. Rev. Food Sci. & Nutr. **34**:371-401.

[9]Gunes, G., Splittstoesser, D.F. and Lee, C.Y. 1997. Microbial quality of fresh potatoes: effect of minimal processing. J. Food Protection **60**:863-866.

[10]Robertson, R. 1992. Modified atmosphere packaging of horticultural products – present and future. Food Technol. New Zealand. June 1992:32-34.

[11]Arogba, S.S. 2000. Mango (*Mangifera indica*) kernel: chromatographic analysis of the tannin, and stability study of the associated polyphenoloxidase activity. J. Food Comp. & Anal. **13**:149-156.

[12]Lee, C.Y., Kagan, V., Jaworski, A.W. and Brown, S.K. 1990. Enzymatic browning in relation to phenolic compounds and polyphenol oxidase activity among various peach cultivars. J. Agric. Food Chem. **38**:99-191.

[13]Coseteng, M.Y. and Lee, C.Y. 1987. Changes in apple polyphenoloxidase and polyphenol concentrations in relation to degree of browning. J. Food Sci. **52**:985-989.

[14]Amar Singh, 1977. Practical plant physiology. 1st ed. Kalyani Publishers, New Delhi. pp. 197-198.

[15]King, A.D. Jr., Magnuson, J.A., Torok, T. and Goodman, N. 1991. Microbial flora and storage quality of partially processed lettuce. J. Food Sci. **56**:459-461.

[16]SAS Institute Inc. 1989. SAS/STAT User's guide, SAS Institute. Ver. 6.12. 4th ed., Vol. 2 Inc., Cary, N.C.

[17]Pretel, M.T., Souty, M. and Romojaro, F. 2000. Use of passive and active modified atmosphere packaging to prolong the postharvest life of three varieties of apricot (*Prunus armeniaca*, L.). Eur. Food Res. Technol. **211**:191-198.

[18]Kader, A.A., Zagory, D. and Kerbel, E.L. 1989. Modified atmosphere packaging of fruits and vegetables. Crit. Rev. Food Sci. Nutr. **28**:1-30.

[19]Piga, A., D'Aquino, S.D., Agabbio, M., Emonti, G. and Farris, G.A. 2000. Influence of storage temperature on shelf-life of minimally processed cactus pear fruits. Lebensm.-Wiss. u.-Technol. **33**:15-20.

[20]Martinez, M.V. and Whitaker, J.R. 1995. The biochemistry and control of enzymatic browning. Trends in Food Sci. & Techn. **6**:195-200.

[21]Lee, C.Y. and Smith, N.L. 1979. Blanching effect on polyphenol oxidase activity in table beets. J. Food Sci. **44**:82-86.

[22]Goupy, P., Fleuriet, A., Amiot, M.J. and Macheix, J.J. 1991. Enzymatic browning oleuropein content and diphenol oxidase activity in olive cultivars (*Oleo europaea* L.). J. Agric. Food Chem. **39**:92.

[23]Amir-Shapira, D., Goldschmidt, E.E. and Altman, A. 1987. Chlorophyll catabolism in senescing plant tissue: *in vivo* breakdown intermediates suggest different degradative pathways for citrus fruits and parsley leaves. Proc. Natl. Acad. Sci. USA **84**:1901-1905.

[24]Shimokawa, K., Shimada, S. and Yaeop, S. 1978. Ethylene-enhanced chlorophyll activity during degreening of Citrus var. Shiu Marc. Scientia Hortic. **8**:129.

[25]Weichman, J. 1986. The effect of controlled atmosphere storage on the sensory and nutritional quality of fruits and vegetables. Hort. Rev. **8**:101-127.

[26]Zagory, D. and Kader, A.A. 1989. Quality maintenance in fresh fruits and vegetables by controlled atmosphere. In Jen, J. (ed.). Quality factors of fruits and vegetables. American Chemical Society, USA, pp. 175-188.

[27]Brocklehurst, T.F., Zaman-Wong, C.M. and Lund, B. M. 1987. A note on the microbiology of retail packs of salad vegetables. J. Applied Bacteriology **63**:409-411.

[28]Beauchat, L.R. 1996. Pathogenic microorganisms associated with fresh produce. J. Food Protection **59**:204-216.

[29]Anonymous 1988. New film doubles shelf life of fresh produce. Food Production/Mgmt. pp. 16.

[30]Barriga, M.I., Trachy, G., Willemot, C. and Simard, R.E. 1991. Microbial changes in shredded iceberg lettuce stored under controlled atmosphere. J. Food Sci. **56**:1586-1588.

[31]Brackett, R.E. 1987. Microbiological consequences of minimally processed fruits and vegetables. J. Food Qual. **10**:195-206.

[32]Sinell, H.J. 1980. Packaging. In Silliker, J.H., Elliot, R.P., Baird-Parker, A.C., Bryan, F.C., Christian, J.H.B., Clark, D.S., Olson, J.C.J. and Roberts, T.A. (eds). Microbial ecology of foods. Academic Press, New York. pp. 193-204.

[33]Osornio, M.M.L. and Chaves, A.R. 1998. Quality changes in stored raw grated beetroots as affected by temperature and packaging film. J. Food Sci. **63**:327-330.

[34]Janardhana, G.R., Raveesha, K.A. and Shetty, H.S. 1998. Modified atmosphere storage to prevent mould-induced nutritional loss in maize. J. Sci. Food Agric. **76**:573-578.

[35]Kays, S.J. 1991. Postharvest physiology of perishable plant products. 1st ed. US. AVI Press, pp. 78-195.