KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF ENGINEERING

EFFECT OF MODIFIED ATMOSPHERE PACKAGING AND TEMPERATURE ON SHELF LIFE QUALITY OF MINIMALLY PROCESSED CARROT

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CERTIFICATION

I hereby declare that this submission is my own work and that to the best of my knowledge, it contains no material previously published by another person or material which has been accepted for the award of any other degree of the University, except where due acknowledge has been made in the text.

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TO MY DEAR SON, KWAKU AFRIYIE BINEY

ABSTRACT

Minimally processed fruits and vegetables are increasingly demanded by local consumers and for export purposes. However, the marketing potential of these produce is limited because of physiological ageing, biochemical changes and microbial spoilage that lead to a short life.
The quality and shelf life of minimally processed products can be extended by modified atmosphere packaging and cold storage by minimizing stress reactions.

A factorial experiment of 3 polymeric packaging films and 3 storage temperatures was conducted. The three films were Low Density Polyethylene (LDPE, control), High Density Polyethylene (HDPE) and co-extruded Polyvinylidene chloride and polyethylene (PVDC/PE) with thicknesses 9.9µm, 11.9µm and 78.2µm respectively. The O_2 permeability of a 25mm LDPE film and HDPE were 7900 and 2900 cm^3 $(m^2.24h.0.1MPa)^{-1}$ respectively and a 78.2 µm PVDC/PE film was 513.12 cm^3 $(m^2.24h.0.1MPa)^{-1}$. The water vapour transmission rates were 48 and 22 g $(m^2.24h)^{-1}$ at 40°C and 90% RH and 6.55g $(m^2.24h)^{-1}$ at 37.5°C and 38% RH respectively.

The storage temperatures were 3°C, 10°C and 20-25°C (ambient temperature, control).

Carrots were minimally processed into slices, sanitized and dipped in chitosan edible coating, then packed in the polymeric packaging films and stored for 10 days. Two packs were analyzed for each treatment combination on d2, d4, d6, d8 and d10 and d0 was taken as reference point.

During storage, the head space gas in the HDPE and LDPE packages showed an increase from 13% to between 17.5-20% oxygen and very little change of 0.9-1.2% from 1% carbon dioxide.

The PVDC/PE packages showed a rapid decrease from 13% to between 0 and 4% on the second day with a sharp rise on day 10.

A lower white blush formation was recorded on the surfaces of the carrot slices in the PVDC/PE package than carrot slices in HDPE and LDPE packages. Temperature showed a significant difference in the whitish index for all three packages with lower results observed at 3 and 10°C. The interaction effect between the packaging and temperature did not show a better control of the white blush formation and was probably due to differences in relative humidity gradient within the packages.

Vitamin C content for the minimally processed carrots did not present differences amongst all treatments throughout the storage period.

Average pH values ranged between 6.35-6.45 whilst contents of titratable acidity and total soluble solids were not affected. Incidence of decay was highest in samples packed in HDPE and LDPE at 20-25°C with 92 and 76% incidence respectively on day 6. The total microbial count was higher in packages stored at ambient conditions 20-25°C than in those stored at 10 and 3°C. The lowest count was observed in PVDC/PE up to day 10.

Carrot samples in PVDC/PE packages recorded the lowest weight loss of 3.6% on day 10 at 20°C-25°C and samples in LDPE packages showed the highest weight loss of 14.4% at 20°C on day 10.

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1. INTRODUCTION

Consumer demand for conveniently prepared fruits and vegetables with superior sensory and nutritional quality compared to processed products has led to the development of minimal processing technology (King and Bolin, 1989). Such trends together with busy lifestyles, increased purchasing power and health consciousness of the consumers have increased the demand for minimally processed fruits and vegetables (MPFV) (Sloan, 1995).

According to Wiley (1994), minimal processing generally refers to unit operations such as washing, sorting, peeling, coring, slicing; or any procedure short of traditional preservation (heat sterilization and freezing) that add value. For safety reasons and greatest retention of sensory and nutritional quality, these products are distributed and marketed in the cold chain.

As a result of the unit operations, MPFV have a limited shelf life as compared to the raw material (Ahvenainen, 1996). Minimally processed produce deteriorate because of physiological ageing, biochemical changes and microbiological spoilage, thereby resulting in degradation of colour, texture and flavor (King and Bolin, 1989). Methods such as refrigeration, use of chemical additives and irradiation have shown potential to extend the shelf life of MPFV (Huxsoll et al, 1989), but new approaches include creating a modified atmosphere using polymeric packaging films of selective permeability or edible coating or films (Ahvenainen, 1996). Seljasen et al. (2004) suggested that package type and storage temperature had the greatest influence on the sensory quality of carrots.

Polymeric packaging films have been shown to modify the atmosphere of the pack by controlling the diffusion of gases and water vapor. Thus it was found to extend the shelf life of MPFV (Carlin et al., 1990). Modified atmosphere packaging involves the use of polymeric film with a specific permeability to O_2, CO_2 and water vapor. Low O_2 concentration and high CO_2 concentration are widely assumed to maintain quality and extend shelf life of fruits and vegetables (Iqbal et al., 2009). Depletion of O_2 and elevation of CO_2 within the package can be achieved passively by respiration of the produce or actively by flushing a desired mixture of gases into the package (Zagory and Kader, 1988; Ballantyne et al., 1988). In the first instance the level of gas composition achieved depends on the permeability of the packaging film and the rate of respiration of the produce. Lowering the storage temperature together with modified atmosphere packaging of fruits and vegetables may reduce both physiological and biochemical activities.

Minimally processed products have a physiology that differs from intact product since they require different and more controlled handling. Minimal processing results in tissue and cell integrity disruption, with a concomitant increase in the respiration rate, enzymatic and microbial activity, which cause a negative impact on quality, affecting color, flavor, and texture (Olivas, et al., 2007). However modified atmosphere packaging (MAP) of fresh produce relies on modification of the atmosphere inside the package, achieved by the natural interplay between two processes, the respiration of the product and the transfer of gases through the packaging, that leads to an atmosphere richer in CO_2 and poorer in O_2. This atmosphere can potentially reduce respiration rate, ethylene sensitivity and production, decay and physiological changes, such as oxidation (Kader et al., 1989). Active or passive modified atmosphere packaging can be employed. Active modification occurs by changing initial air at packaging by a desired mixture of gases, and this is employed with high respiring produce such as carrot, while passive modification occurs when the product is packaged using air as initial package atmosphere composition (Farber et al., 2003). The use of a low O_2 concentration (1-5ml/100ml) and a high CO_2 concentration (5-10ml/100ml) (balance N_2) in combination with storage at refrigeration temperatures, is proposed by many researchers as optimal storage conditions for fresh fruits and vegetables to maintain the sensory as well as microbial quality (Jacxsens et al., 1999).

The choice of an MAP involves careful selection of the film, package type and size for each specific product (Farber et al., 2003). Permeability to O_2, CO_2 and water vapour transmission are the most important factors to be considered when selecting a film for MAP. These permeabilities are key factors in determining package atmosphere composition and humidity inside the packages and therefore might influence product deteriorating rate.

Minimally processed carrots constitute one of the major minimally processed vegetables. Their shelf-life and acceptance is limited because of the whiteness developed on the surface during storage. Whiteness may be the result of a partial dehydration of the surface deriving from physical and/or physiological damage (Durango et al., 2006). The main problems that limit their shelf-life are white blush discoloration caused by tissue dehydration, and microbial spoilage (Emmambux and Minnaar, 2003). Therefore, a treatment that could inactivate their natural micro-flora keeping the tissue hydrated seems appropriate to prolong their shelf-life.

Coatings of edible material applied as a thin layer can offer a possibility to extend the shelf life of minimally processed carrots by providing a semi permeable barrier for gases and water vapor, and therefore, reducing respiration and water loss (Perez-Gago et al., 2006; Olivas et al., 2007).

Minimally processed carrots consumed as ready-to-eat snacks or salad vegetables have become increasingly popular (Amanatidou et al., 2000; Barry-Ryan et al., 2000). However, sales are limited because of rapid deterioration during storage. The main problems that limit the shelf life of minimally processed carrots to 4 or 5 days are high respiration rate, development of off flavors, acidification, loss of firmness, discolouration, and microbial spoilage (Amanatidou et al., 2000; Barry-Ryan et al., 2000; Barry-Ryan and O'Beirne, 2000).

Minimally processed carrots are a growing segment in the food retail industry and their highly perishable nature necessitates a good preservation strategy.

The main objective of this work therefore was to investigate the effect of modified atmosphere packaging and storage temperature on the shelf life quality of minimally processed carrots.

The specific objectives were:

- To investigate the effect of HDPE, LDPE and PVDC/PE modified atmosphere packaging polymeric films on shelf life quality of minimally processed carrot stored for 10days.

- To investigate the effect of storage temperatures 3°C, 10°C and 20-25°C on shelf life quality of minimally processed carrot stored for 10days.
- To investigate the combined effect of HDPE, LDPE and PVDC/PE modified atmosphere packaging films and storage temperatures, 3°C, 10°C and 20-25°C of shelf life quality of minimally processed carrots stored for 10days.

2. LITERATURE REVIEW

This chapter reviews literature which explains the shelf life quality problems associated with minimally processed fruits and vegetables (MPFV) and suggestions to control these problems. The importance of polymeric packaging films and temperature will be emphasized.

2.1 Quality of minimally processed fruits and vegetables

Minimally processed fruits and vegetables undergo processes to increase their functionality without greatly altering their fresh like properties. Minimal or light processing, however changes the composition of these products and they become more perishable (Shewfelt, 1987). The perishability as determined by the shelf life is a combination of the quality changes (physiological, biochemical, microbiological and sensory) and the factors that influence these changes. The quality of minimally processed fruits and vegetables generally refers to the sensory quality as determined by texture, flavor and appearance; microbiological quality in terms of spoilage and safety; and to a certain extent the nutritional quality (Emmambux and Minaar, 2003). These quality changes and the factors causing the changes will be discussed as well as their ways of prevention.

2.1.1 Quality changes of minimally processed fruits and vegetables

During minimal processing, fruits and vegetables are peeled and sliced and this wounding changes the biological composition from a relatively stable fresh product to a more susceptible form which enhances quality deterioration. This wounding causes stress, resulting in metabolic activation to cause physiological ageing, biochemical changes and microbiological spoilage. These metabolic processes in turn result in loss of firmness, discolouration and off flavor development that are unacceptable to consumers (Varoquaux and Wiley, 1994).

The deteriorative quality changes that occur in MPFV can be classified as physiological biochemical and microbiological.

2.1.1.1 Physiological and biochemical changes of minimally processed fruits and vegetable

At the harvest stage, plant tissues start to undergo senescence and there is a shift to total degradative reactions becoming greater than biosynthetic reactions. In addition, partial processing that leads to wounding will induce or enhance these deteriorative changes (Watada, et al, 1990). These changes include a localized increase in respiration at the site of injury, stress ethylene production, and cellular disruption leading to a mixing of enzymes and substrates (Rolle and Chism, 1987). Consequently, there will be colour, texture and flavor changes that are indicative of quality deterioration.

Metabolic changes

An increase in respiration rate and ethylene production from intact to cut fruits and vegetables is quite considerable (Watada et al., 1990). Moisture loss increases when fresh fruits and vegetables are cut because of transpiration. This is because there is no skin barrier to control transpiration (Wills et al., 1998). Moisture loss and dry matter loss as a result of transpiration and excessive respiration respectively, can lead to decrease in weight and desiccation. Wilting is very common in leafy produce and shriveling is more common in bulky tissues. These defects can be easily managed by storing under high relative humidity (85-95%) and low temperatures (Wills et al., 1998).

The effects of minimal processing on the rate of respiration, transpiration and wound ethylene production differ between type of commodity, physiological ripening stage of climacteric produce and extent of tissue damage (Rosen and Kader, 1989; Barry-Ryan and O'Beirne, 1998).

Colour changes

The most important colour changes occurring in MPFV as a result of wounding are enzymatic browning, destruction of chlorophyll and formation of white translucent tissue on the surface of carrots (Couture et al., 1993). Membrane integrity disruption causes degradative enzymes and substrate to mix causing colour changes (Rolle and Chism, 1987).

The surface white discolouration, also known as white blush is a major defect in minimally processed carrots. The formation of white blush is attributed to surface dehydration (Tatsumi et al., 1991; Cisneros-Zevllos et al., 1995) and lignin formation (Bolin and Huxsoll, 1991; Howard and Grifin, 1993). According to Tatsumi et al (1991), fresh cut carrots examined by scanning electron microscopy revealed that surface cells were either damaged or layers were separated from tissue because of the processing knife. These cells and tissues dehydrated quickly and formed a white tissue.

Cisneros-Zevallos et al. (1995) showed that the mechanism for the white discolouration is both physical and physiological response to wounding. They used Whiteness Index (WI) as an objective measurement to show the extent of the defect. WI was also related to visual grading as shown in Table 1. They found that higher relative humidity conditions were associated with lower white blush incidence. In addition, wetting the white surface significantly decreased the WI, indicating a reversible change. Thus they concluded that this is a physical response reflected in a colour change which is reversible. However, an irreversible change was also noted. Dewetted peeled carrots that were exposed to 75 and 98% relative humidity and afterwards dipped in water did not regain their original colour. This irreversible change was explained by a physiological response because of lignin formation (Bolin and Huxsoll, 1991).

Table1. A visual description of the whiteness index (WI) for peeled carrots.

Description	WI[1] (average values with standard deviation)
Non white	32.6±2.4
Slightly white	38.4±1.3
Moderate white	43.0±1.8
Severe white	47.2±1.7
Extreme white	50.9±3.1

(Cisneros-Zevallos et al., 1995)

[1] $WI = 100-[(100-L)^2+a^2+b^2]^{1/2}$, L, a and b values were obtained from a colour meter

White tissue from the carrot surface was found to give positive lignin test as compared to the non white part, showing the involvement of lignin formation (Bolin and Huxsoll, 1991). Further research by Howard and Griffin (1993) confirmed the close relationship between development of white discolouration and lignin formation.

Textural changes

Textural changes in minimally processed carrots showed an increase in firmness in the first few days of storage followed by a decrease afterwards, but the changes were not consistent (Izumi and Watada, 1994). Other minimally processed products such as Kiwi fruit and banana showed significant decreases in firmness with storage time (Watada et al., 1990). The loss was suggested to be a consequence of enzymatic hydrolysis of the cell wall components that provide cell wall integrity. Moreover it was emphasized that ethylene played a role in enhancing loss of firmness.

2.1.1.2 Microbiological changes

The type of produce, processing and storage conditions will generally affect both the spoilage and safety of the MPFV (Brackett, 1987 and Zagory, 1999).

Microbiological spoilage

Fresh green salads and vegetables that are generally low acid foods are mainly spoiled by a variety of bacteria as compared to high acid foods such as apples that are mainly spoiled by yeasts and molds; and lactic acid bacteria (Brackett, 1994). Many authors suggested that there is no simple correlation between microbial load and chemical markers such as pH, lactic acid, acetic acid, carbon dioxide levels and sensory quality (Guerzoni et al., 1996; Zagory, 1999). In fact spoilage depends on several processing factors and quality changes. Minimally processed carrots were found to have a typical lactic acid fermentation type of spoilage by the lactic acid bacteria (Carlin et al., 1989). Principal component analysis showed that the head space composition of high carbon dioxide (over 30%) and low oxygen (below 1.5%), ethanol and lactic acid bacteria were the most discriminating variables between spoiled and well preserved packs regardless of storage time. The identified lactic acid bacteria were *Leuconostoc mesenteroides* (Babic et al., 1992).

Microbiological safety

Fruits and vegetables have a very low risk of causing food borne illnesses as compared to other carriers such as meat (Bryan, 1988). Brackett (1994) mentioned several reasons why minimally processed fruits and vegetables are relatively safer as compared to other foods and these include:

- Refrigeration conditions of fresh produce are generally unfavourable for the growth of most pathogens except the psychrotrophs.
- High acid food also eliminate the possible growth of most pathogens, and
- Normal spoilage microorganism generally have an antagonistic effect on the growth of most pathogens

On the other hand, Francis et al. (1999) suggested that mild preservation systems such as refrigeration and modified atmosphere packaging could not ensure the safety of a product. Psychrotrophic pathogens such as *Listeria monocytogenes* and *Aeromonas hydrophilia* and the pathogen *Clostridium botulinum* are capable of growing to infectious levels under these mild preservation regimes. In assessing the microbiological quality of ready to use vegetables for health care food services, it was found that only the level of *Listeria monocytogenes* increased among the pathogens. This increase is associated with temperature abuse from 4°C to 10°C (Odumeru et al., 1997). However *Listeria monocytogenes* was not found to be a threat in minimally processed carrots because of an inhibitory effect. They suggested that temperature is a critical factor and it should be kept below 5°C, but this is difficult to achieve during cold chain distribution. They also recommended that quality assurance programs to check for *Listeria* *monocytogenes* randomly, is critical to ensure safety of minimally processed fruits and vegetables.

2.1.2 Factors affecting the quality of minimally processed fruits and vegetables

These factors can be categorized into pre-processing, processing and post-processing factors.

2.1.2.1 Pre-processing factors

The pre-processing factors include growing conditions and cultural factors, cultivar, maturity at harvest, harvesting and handling methods, inspection standards and duration of storage (Shewfelt, 1987). As such these pre-processing factors will affect the raw material quality.

2.1.2.2 Processing factors

The processing factors can influence the stresses that will lead to physiological ageing and microbiological deterioration. Barry-Ryan and O'Beirne (1998) clearly showed that the sharpness of the slicing blade affected the quality and storage life of modified atmosphere packaged sliced carrots as shown in Table 2. Slicing was found to cause physical damage, physiological stress and enhanced microbial growth. These damages and stresses were enhanced by blunt machine blade compared with a sharp machine blade and by the sharp machine compared with a razor blade.

Table 2. Quality of carrots as affected by slicing methods.

Measured changes	Time	Slicing method		
		Razor	Sharp machine	Blunt machine
Total aerobic count	d1	5.77	5.80	6.27
Log10/g	d6	7.02	7.95	8.16
Lactic acid bacteria	d1	4.70	4.93	5.66
Log10/g	d6	7.20	7.84	7.94

Source: (Barry-Ryan and 'OBeirne, 1998)

Ahvenainen (1996) identified the following key requirements during processing to improve quality of MPFV.

- Strict hygiene, good manufacturing practices and hazard analysis, critical control point
- Low temperature processing
- Careful cleaning and/or washing before and after peeling
- Good quality of water (sensory, microbiological and pH) for washing
- Use of mild additives
- Gentle spin drying following washing
- Gentle peeling, slicing and shredding
- Correct packaging material and methods

2.1.2.3 Post processing factors

The post processing factors as stated by Watada (1997) and Watada et al. (1996) include temperature and storage, relative humidity, modified atmospheres and post-processing microbial contamination. These post-processing factors together with some processing factors are generally manipulated to improve the quality and shelf life of MPFV and are discussed in the section below.

2.2 Ways to improve quality of minimally processed fruits and vegetables

Minimally processed fruits and vegetables are very susceptible to deteriorative changes that limit their shelf life. Therefore ways to prevent or delay these changes will extend the shelf life. The most common practice to enhance the shelf life is the use of low temperature during processing and distribution under the cold chain (Wiley,1994). Low temperature preservation can only prevent the deteriorative changes to a certain extent and frequently the cold chain is abused. So it is important to consider other hurdles that will enhance the quality without changing the fresh like properties. Hurdles that are of interest are the modification of the atmospheres surrounding the MPFV with partially permeable polymeric packaging films and edible coatings (Emmambaux and Minaar, 2003).

2.2.1 Modifying atmospheres by polymeric packaging film

The basic principle underlying storage under modified and controlled atmospheres is the modification of the gas composition to minimize the respiratory rate and other biochemical processes. The decrease in O_2 concentration as well as the increase in CO_2 concentration lead to a decrease, up to a certain limit, in the respiration rate of fruits and vegetables (Saltveit, 2003).

Modified atmosphere packaging can be of two types, passive or active (Zagory and Kader, 1988). Passive MAP involves the packaging of produce in semi permeable (gas and water vapour) bags, sealing the package and allowing the fresh produce to decrease the level of oxygen and increase the carbon dioxide concentration in the pack by respiration until equilibrium is reached. This equilibrium should provide the desired effects without creating any detrimental or anaerobic conditions. Active MAP also involves the use of a gas permeable package, but before sealing, the gas in the package is evacuated and replaced by a pre-selected mixture of oxygen and carbon dioxide and nitrogen gases. This is followed by rapid sealing of the package. Active MAP also includes the utilization of adsorbers and /or absorbers to generally scavenge oxygen, carbon dioxide or ethylene (Kader et al, 1989).

There are several types of polymeric packaging films that can create a modified atmosphere passively and this influences the metabolic and microbiological status of MPFV. Thus polymeric packaging films have the potential to enhance the quality of MPFV.

2.2.1.1 Polymeric packaging films

The utilization of polymeric films or plastic materials (such as rigid trays) of appropriate permeability is very desirable. It creates an internal atmosphere of oxygen and carbon dioxide that results in substantial reduction of the respiration rate without inducing significant produce anaerobiosis (Schlimme and Rooney, 1994). Polymeric films as packaging have numerous functions that include control of gas transfer, control of moisture transfer, protection from external physical or mechanical damage and biological contamination.

The design of an MAP involves careful selection of the film, package type and size for each specific product (Farber et al. 2003). Permeability to O_2 and CO_2 and water vapour transmission are the most important factors to be considered when selecting a film for MAP. These permeabilities are key factors in determining package atmosphere composition and humidity inside the packages and therefore might influence product deteriorating rate.

Polymeric packaging films are generally thermoplastic polymers such as polypropylene, low density and high density polyethylene, polybutylene, polystyrene, polyvinyledene chloride ethylene and vinylacetate polymers.

2.2.1.2 Metabolic effects of modified atmosphere

The effect of reduced oxygen and elevated carbon dioxide on reducing respiration rate has been assumed to be the primary reason for the beneficial effects on fruits and vegetables. However modified atmospheres can also provide the following benefits (Kader, 1986; Powrie and Skura, 1991)

- Lowering ethylene production
- Inhibition of the initiation of ripening
- Decrease rate of ripening and senescence
- Reduction in tissue water loss and maintenance of cell integrity
- Minimization of nutrient loss and decomposition
- Reduction of specific physiological disorder such as chilling injury

Most research has been carried out on fresh fruits and vegetables rather than on minimally processed fruits and vegetables and conclusions were mainly drawn under a controlled atmosphere rather than a modified one. Watada and Qi (1998) experimented on the effects of low oxygen and high carbon dioxide on the quality of fresh cut products. They found that low oxygen was beneficial in maintaining the quality of the cut produce. The physiological response of low oxygen on carrots' respiratory pattern is known. When carrots were stored in an atmosphere of 0.5% oxygen, it was found to sustain itself for a short period of time without any anaerobic respiration. (Kato-Noguchi and Watada, 1996a). Under similar conditions, where carrot tissues were stored under a continuous flow of 0.5% oxygen, ethanolic fermentation was favoured by an increase in the activity of alcohol dehydrogenase and ethanol concentration (Kato-Nogochi, 1998).

For minimally processed carrots stored under controlled atmosphere, an equilibrium of about 5% oxygen was found to be critical as respiration rate was highly dependent on the oxygen concentration as shown by applying the Michaelis-Menten model (Sode and Kuhn, 1998). However in a passive modified atmosphere Carlin et al. (1989) found a critical value of 1.5% for oxygen. No dependency of carbon dioxide was found by Sode and Kuhn (1998), but a carbon dioxide level greater than 30% was not recommended by Carlin et al. (1989) as it favoured lactic acid bacteria.

2.2.1.3 Microbiological effects of modified atmospheres

The microbiological effects of modified atmospheres can be of two dimensions: spoilage and pathogenic. Generally, it is agreed that elevated carbon dioxide might exert a biostatic effect on bacteria and it can extend the lag phase of bacterial growth (Farber, 1991). However, the effects were found to be quite variable and it depends on the initial numbers and type of the microorganism; ability of food to support the microbial growth (acidity, nutritional composition and presence of antimicrobial factors); water activity, temperature and the concentration of gases.

Under modified atmosphere packaging of MPFV stored at refrigeration conditions, food borne pathogens that are anaerobic or microaerophilic can be of concern (Farber, 1991). *Listeria monocytogenes* and *Clostridium botulinum* were the main pathogens under study. A study conducted by Amanatidou et al. (1999) showed that *Enterbacter agglomerans*, *Salmonella typhimurium*, *Salmonella enteritidis* and *Escherichia coli* were significantly decreased under modified atmospheres in an agar surface model, but *Listeria moncytogenes* were found to increase.

Similarly, *Listeria innocua* (indicator microorganism for *Listeria monocytogenes*) were found to increase on minimally processed cabbage (Omary et al., 1993). However, on minimally processed carrots *Listeria innocua* were found to have one log decrease when stored under modified atmosphere (Finn and Upton, 1997). This decrease might be due to inhibitory activity of carrots towards *Listeria spp*. (Nguyen-the and Lund, 1991).

2.2.1.4 Benefits of using polymeric packaging films

Polymeric films are commercially used to create passive modified atmospheres and to extend the shelf life of MPFV. Carrots stored in polymeric packaging films were found to maintain a good quality. The permeability of these films was critical in extension of shelf life. A high permeability polymeric packaging was recommended for minimally processed carrots because of high respirations (Carlin et al., 1990b). This suggested that permeability of polymeric packaging films should relate to the respiratory activities of the produce. Modelling approaches could be used to ensure this dynamic system between the respiring produce and the film permeability to achieve an internal gas concentration at a steady state (Solomos, 1994).

Other potential benefits of using polymeric packaging films on fresh and minimally processed fruits and vegetables are:

- Prevention of russet spotting and pink rib of Salinas lettuce packed in polypropylene packaging film (Artes and Martinez, 1996).
- Better retention of sugar, organic acids and soluble proteins in asparagus spears packed in a commercial polymeric film (Baxter and Waters, 1991).
- Chlorophyll preservation, petiole firmness and decreased vitamin C loss in parsley packed in a ceramic film (Park et al., 1999).
- Decreased anthocyanin degradation in shredded onions packed in perforated polypropylene films (Ferreres et al., 1996).

2.2.2 Effect of temperature

A major factor that must be considered in the use of MAP is the effect of temperature fluctuation (Exama et al., 1993). The influence of temperature change on the function of MAP has been one of the primary obstacles to successful package development (Prince, 1989).

Metabolic processes are sensitive to temperature. High temperatures increase respiration rate (Exama et al., 1993). The intensity of respiration in fruits responds within certain ranges to temperature in a manner characteristic of chemical reactions. Von't Hoff introduced the concept of the respiratory quotient, Q_{10} (Potey, 1988). Biochemical reactions generally increase 2-3 fold for every 10°C rise in temperature (Zagory and Kader, 1988).

Temperature also affects the permeability of the polymeric films (Liming, 1992; Exama et al., 1993). The effect of temperature on the permeability of films differs from film to film.

For instance, polyethylene (PE) is much more responsive to change in temperature than polystyrene (PS) (Liming, 1992). The permeability of packaging films vary log-linearly with the reciprocal of the absolute temperature (Prince, 1989). In general, the permeability increases with the increase of the temperature. Since temperature changes a commodity's metabolic processes and the properties of the film, therefore, as a consequence, it will also affect the actual equilibrium atmospheric levels in a package, and time required to obtain equilibrium.

Packages that were transferred from refrigeration to room temperature were generally unsuccessful because increasing temperature could lead to depletion of O_2 and the risk of fermentative reaction within the packages (Liming, 1992). While the packages may have obtained beneficial atmospheres at low temperatures, anaerobiosis and /or toxic levels of CO_2 quickly developed on transfer to warm temperatures, resulting in off-odors and flavors, but little has been published on the extent of this problem or possible solutions (Cameron et al., 1994).
It appears then, that the choice of film not only determines permeability at a given temperature, but also adaptability to temperature change (Prince, 1989). This implies that a film that is appropriate for MAP at one temperature may not be appropriate at other temperatures (Zagory and Kader, 1988). Exama et al., 1993, reported that MAP systems designed to produce optimal O_2 at a particular temperature could have complications from transient temperature increases during storage and /or transportation. More permeable gas pathways and temperature compensation to equalize Q_{10} values are needed for MAP systems to function effectively (Exama et al., 1993).

2.2.3 Other treatments

During minimal processing of fruits and vegetables, sanitizers such as sodium hypochlorite and hydrogen peroxide are often used to reduce initial microbial load. Microbial load was found to reduce by 1-2 log cycles compared with water when chicory was disinfected with 10% hydrogen peroxide for 2 minutes (Bennik et al., 1996). Similarly, application of active chlorine at 250ppm produced a one log decrease in washed salads (Adams et al., 1989).

Irradiation has the potential to improve the quality of minimally processed carrots. Irradiated samples of carrots that were minimally processed had a higher quality. Better carotene retention, better orange colour retention, inhibited aerobic mesophilic and lactic acid microflora compared with non irradiated ones (Chervin and Boisseau, 1994). After 9 days of storage, irradiated minimally processed carrots had a microbial load of 3.1 log CFU/g in contrast to 4.9 log CFU/g for the non irradiated shredded carrots (Hagenmaier and Baker, 1998).

Steam treatment has been found to control whitish discolouration. When carrot sticks were steam treated on both sides for 45 seconds at 99°C, control of the white discolouration was achieved and appeared to be reduced due to the retardation of phenlypropanoid metabolism for lignin formation (Howard et al., 1994). In addition, acidic or basic solution has shown potential to control surface white discolouration of minimally processed carrots (Bolin and Huxsoll, 1991b).

2.3 Summary

- MPFV are very susceptible to deteriorative changes and pre-processing, processing and post-processing factors influence these changes.
- Moisture loss, increased respiration rate, wound ethylene production, colour changes, loss in firmness and off flavor development are the most important changes that occur in MPFV.
- The main microbial spoilage concerns are the pseudomonads and lactic acid bacteria; and for microbiological safety, *Listeria monocytogenes* is of concern in low acid foods.
- Modified atmosphere using polymeric packaging films of specific permeabilities can be very desirable as it reduces metabolic activities to an optimum level without resulting in anaerobiosis.
- White blush is a visual defect in minimally processed carrots and microbial spoilage of this produce is mainly through fermentation by the lactic acid bacteria
- Modified atmosphere packaging generally decreases the microbial load, but can alter the microbial flora to anaerobiosis or microaerophilic bacteria such as lactic acid bacteria.
- A high permeability polymeric packaging film is generally recommended for MPFV because of high respiration rates.

3. MATERIALS AND METHODS

3.1 Minimal processing of carrots

The experiment was conducted at the Central Laboratory of the School of Biosystems Engineering and Food Science, Zhejiang University, China from September 28 to October 7, 2012.

3.1.1 Raw material processing

The Carrots (*Daucus carota*) were purchased from Zhejiang University wholesale fruits and vegetable market. According to the supplier, the carrots were from the same harvest batch and were of the cultivar 'Nantes'. They were sorted according to their size, colour and general physical appearance. They were then thoroughly washed under running water, peeled and both the lower and upper ends cut and discarded. Carrot slices (20-40 mm diameter and 5 mm thick) were cut crosswise with a stainless steel knife, rinsed with water at room temperature (~23°C) (Izumi et al., 1996) and then air dried. The carrots slices were sanitized by immersion into a 50mgL^{-1} sodium hypochlorite solution for 20 minutes as described by Barbosa et al. (2011). The samples of sliced carrots were submerged in chitosan treatment at 0.02g mL^{-1} to reduce deteriorative processes, maintain the quality and increase the shelf life for 3 min and subsequently air dried at ambient temperature for 3h (Durango et. al., 2006).

Figure 1: Photograph of unprocessed carrots from market

Figure 2: Photograph showing slicing of carrots.

Figure 3: Photograph showing rinsing of carrots after slicing.

Figure 4: Photograph showing air drying of carrots slices after rinsing and sanitizing.

Figure 5: Photograph showing carrot slices in packages of LDPE film and tray, PVDC/PE and HDPE.

3.1.2 Packaging and Storage

The samples were packed into three types of packages, HDPE (commercial food bag), Polystyrene tray with LDPE wrapping film (control) and co-extruded PVDC and PE (consisting of three layers, two layers of polyethylene and polyvinylidene chloride in the middle). The thickness, O_2 gas permeabilities and water vapour transmission rates of the packaging films were tested for using a film thickness tester, O_2 gas permeability tester (Perme, VAC-VBS, Beijing, China) and water vapour transmission tester (Perme, W3/ 030, Beijing, China) respectively. The thickness for HDPE film was 11.9µm, LDPE film was 9.9µm and PVDC/PE film was 78.2µm. The O_2 permeabilities of HDPE and LDPE films could not be read because the film was too thin, PVDC/PE film however had an O_2 permeability of 513.12 cm^3 $(m^2.24h.0.1MPa)^{-1}$ at 21.8°C and 38% RH and water vapour transmission rate of 6.55g $(m^2.24h)^{-1}$ at 37.5°C and 38% RH. According to Elsayed (2003), HDPE and LDPE films with 25mm thickness had an O_2 permeability of 2900 and 7900 cm^3 $(m^2.24h.0.1MPa)^{-1}$ and water vapour transmission rates of 22 and 48g $(m^2.24h)^{-1}$ at 40°C and 90% RH respectively. The polyvinylidene chloride has low permeability to water and gases, and thus the PE and PVDC is co-extruded for its low permeability to vapour and mechanical resistance (Varoquaux and Nguyen 1994). The packages were then stored under three different temperatures; 3°C, 10°C and ambient (20°C-25°C, control).

A total of 9 experimental units were used in two replications. The packages were heat-sealed immediately after filling and the microenvironment atmosphere actively modified with a gas mixture of 13% O_2, 1% CO_2 and 86% N_2 as initial gas atmosphere. (Gómez-López et al., 2007). Three packages consisting of each film type were stored at 3°C, 10°C and 18-20°C with a relative humidity ranging from 80-90% (Workneh et al., 2001) for the 10-day storage period. Three storage temperatures were used because fresh-cut carrots are commonly held at 5°C or higher (Hardenburg et al., 1986) and the temperature during handling, holding, transit and marketing usually ranges from 5°C to 15°C and at times may approach 20°C. (Watada et al., 1996).

Samples were analyzed on day 0 (after peeling and cutting) and used as reference and 2, 4, 6, 8 and 10 days during the storage period to evaluate the changes with time. Samples were analyzed in terms of the physiological and biochemical quality as measured by the headspace gas composition, weight, pH, soluble solids, and titratable acidity, microbiological quality as measured by the total microbial count and Sensory quality as measured by colour, texture, and decay.

3.2 Experimental layout

The experimental layout was a completely randomized factorial design with two replications. The treatments were HDPE (High density polyethylene), LDPE (low density polyethylene), (polyvinylidenechloride/polyethylene) PVDC/PE packages and 3°C, 10°C and 20-25°C storage temperatures. To address the research objectives, a factorial experiment of 3 packaging films x 3 storage temperatures x 5 sampling days were investigated.

Data was subjected to a 3-way analysis of variance by the Statistical Package for the Social Sciences (SPSS). The effect of modified atmosphere packaging, temperature and sampling time and the interactions on the quality parameters was investigated.

3.3 Shelf-life quality analysis

For the shelf-life study, replicate packages for each treatment combination were taken at 2, 4, 6, 8 and 10 days to measure headspace gas concentration, decay, weight, soluble solids, color, texture, vitamin c content, titratable acidity, and pH .

3.3.1 Headspace oxygen and carbon dioxide analysis

The headspace concentrations of oxygen (%) and carbon dioxide (%) in the packs were determined with a gas analyser (PBI Dansensor, Ringstead, Denmark.). The apparatus was first calibrated with air (20% O_2 and 0.3% CO_2). Then the needle was inserted in the packaging material to sample the head space oxygen and carbon dioxide.

Figure 6: Photograph showing Gas analyzer sampling headspace gas of a PVDC/PE carrot package

3.3.2 Colour assessment for whiteness index

The colour measurements of the surface of the carrot slices were determined by a color difference meter (WSC-S, Hinotek Tech., Ningbo, Zhejiang, China). The apparatus was first calibrated to a standard white tile to the corresponding L*, a* and b* values. The colour was analyzed using the CIE: $L^*a^*b^*$ uniform colour space (Lab), where L^* indicates luminosity, a^* corresponds to a coloration on an axis from green (−) to red (+), and b^* to a coloration on an axis from blue (−) to yellow (+). The discoloration of the surface was calculated through the whiteness index (WI) calculated by the equation $WI = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$ (Bolin and Huxsoll, 1991). The carrot slices were gently removed from the package by holding the sides to prevent any possible hydration as this could affect the WI (Cisneros-Zevallos et al., 1995). Colour measurements were done on 9 slices of carrot per package.

Figure 7: Photograph showing color difference meter

3.3.3 Texture analysis

The firmness of the carrot slices was measured with a texture analyzer (TA-XT Plus, Stable Micro systems, Surrey, UK). The equipment was first calibrated using standard weight and force.

The carrot slices were then analyzed using a test speed of 1 mm/s and penetration distance of 3mm and the firmness was expressed as maximum cutting force (N). The data was presented as mean of 20 independent measurements.

Figure 8: Photograph showing texture analyzer sampling a sliced carrot.

3.3.4 Vitamin C

Vitamin C was determined by using the procedure as outlined by AOAC International Methods of Analysis, Method 967.21. 10g of the sample was accurately weighed and grinded using mortar and pestle with an addition of 20 ml 2% oxalate solution. The mixture was further grinded and strained through muslin and the extract made up to 100 ml with 2% oxalate solution. 10 ml each of the sample extract was pipetted into three of 100 ml Erlenmeyer flask. The samples were then titrated separately with the indophenol dye solution until a light rose pink persisted for 5s. The amount of dye used in the titration was determined and used in the calculation of vitamin C content. In the calculation of the Vitamin C content of the carrots, standard vitamin C was titrated against the indophenol dye solution to determine the volume to compare with the Vitamin C content in the stored carrots as in the equation below:

$$\text{Vitamin C} = \frac{V \times (V_1 - V_0) \times \ell}{V_S \times m} \times 100 \text{ (mg/100g)}$$

Where V- Total volume of sample (ml)

V_1-Volume of titrant (ml)

V_0- Standard VC content (ml)

Vs- Volume of sample (ml)

m-weight of sample (g)

ℓ-amount of titrant used in standardization (mg/ml)

3.3.5 Titratable acidity

A 25g sample of carrot was accurately weighed and homogenized with a mixer (Midea food blender, MJ- 250BP02A, Foshan, Guangdong, China) with 250mL of distilled water. After dilution, the mixture was filtered and 25mL of this solution was titrated with 0.1 $molL^{-1}$ of NaOH, with phenolphthalein dye as an indicator. The end point was reached when the sample colour changed into a slight pink and persisted after 30sec. The change in volume of the titrant was read and recorded and used in the acidity calculations expressed as % malic.

$$\% \text{ titratable acidity} = \frac{\text{ml NaOH x N NaOH x M malic acid x 100}}{\text{Volume of sample ml x 1000}}$$

M –molecular weight of malic acid
N-normality of NaOH

3.3.6 pH measurement

A sample of 10 g of carrots was homogenized by using a mixer (Midea food blender, MJ-250BP02A, Foshan, Guangdong, China) with 50 ml of distilled water. The pH was measured by using a portable pH meter (Sartorious, PB 10, Beijing, China).

3.3.7 Total Soluble solids

The carrot was homogenized with a food processor (Midea food blender, MJ- 250BP02A, Foshan, Guangdong, China) to extract the juice. Soluble solid content was determined by using a hand-held refractometer, (ATAGO N-50E, Japan) at 20°C (Rocha et al., 2007) and the results expressed as °Brix.

3.3.8 Decay

The incidence of decay was expressed as a percentage of the total number of pieces in each package (Izumi et al., 1996).

3.3.9 Weight loss

Carrot packages were weighed (Sartorious BS 224S, Beijing China) before and after storage, and the respective weights recorded. The results were presented as a percent loss of the initial weight.

$$WL (\%) = \frac{\text{Initial weight - final weight}}{\text{Initial weight}} \times 100$$

3.3.10 Microbiology

The nutrient agar media was prepared by suspending 14g nutrient Agar in 500ml distilled water in a beaker and boiled to dissolve completely. The media was sterilized by autoclaving (YXQ-LS-18SI, Boxun Industry, Shanghai, China) at 121°C for 20 min.

For total bacteria count, a 10g sample of carrots was taken from packages under aseptic conditions and homogenized with 90 ml of sterile saline solution (0.9% w/v) using a mixer (MJ-250BP02A,Midea food processor, China) . The plates were inoculated with 1ml of dilutions 10^{-1}, 10^{-2}, $10^{3}$and agitated with circular movements. After discarding the excess, the plates were inverted and incubated (Jinlong, Devices, Zhejiang, China) at 37°C for 24 -36h for a later reading of the results (AOAC 970301) (Pilon et al, 2006). The results were presented as log CFU (colony forming units) g^{-1}.

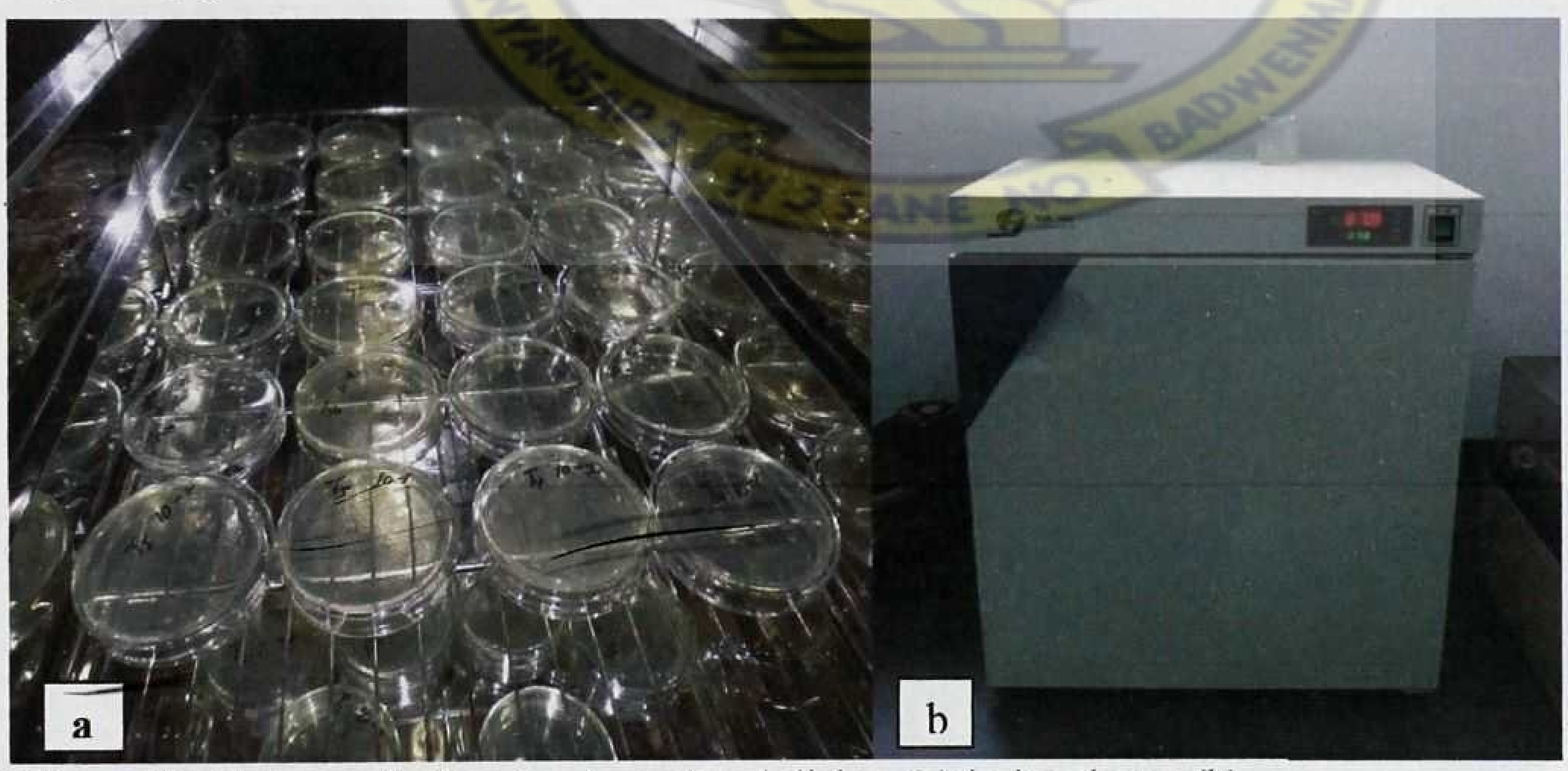

Figure 9: Photograph showing inoculated dishes (a) in incubator (b).

4. RESULTS

4.1 Headspace oxygen and carbon dioxide composition

A rapid increase in the headspace oxygen concentration was observed for all HDPE and LDPE carrot packages on the second day and remained throughout the storage period, with a parallel decrease in the carbon dioxide concentration. In PVDC/PE carrot packages however, headspace oxygen dropped to 0% at 20°C, 1% at 10°C and 4% at 3°C on the second day of experiment with a corresponding increase in carbon dioxide. On the fourth day, however, oxygen concentration dropped further to 0% at 3°C and 10°C and remained till day 10 when there was a sharp rise to 9% and 2.5% respectively, whilst concentrations at 20°C rose slightly each day until a sharp rise to 11.3% was observed on day 10.

The changes in oxygen and carbon dioxide concentration in PVDC/PE, HDPE and LDPE carrot packages are shown in Fig 10a and 10b respectively.

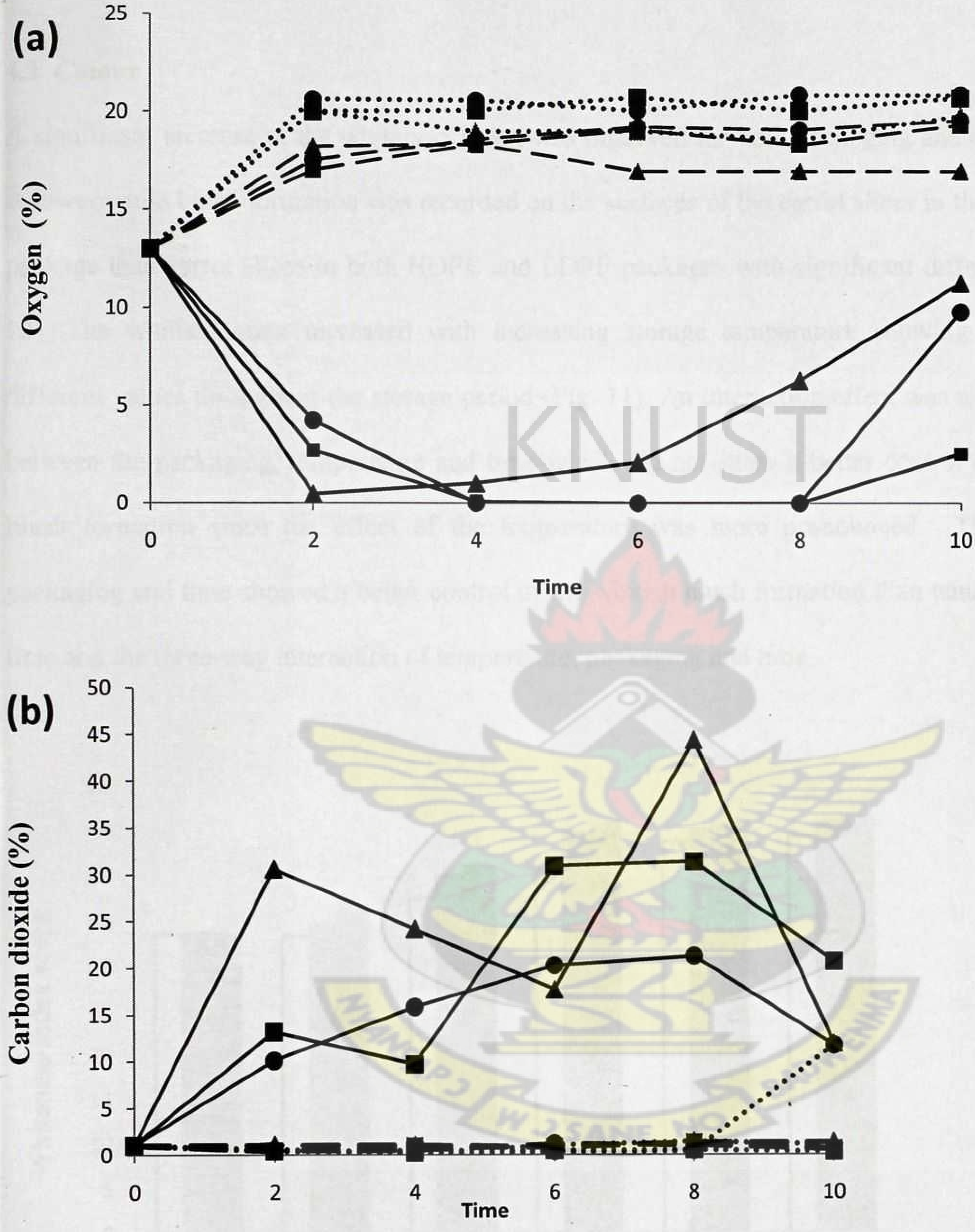

Figure 10: Changes in O_2 (a) and CO_2 (b) concentration in carrot packages PVDC/PE (—) HDPE (– –) and LDPE (······) at 3°C (●), 10°C (■), and 20°C (▲)

4.2 Colour

A significant increase of the whiteness index was observed for both packaging and temperature. A lower white blush formation was recorded on the surfaces of the carrot slices in the PVDC/PE package than carrot slices in both HDPE and LDPE packages with significant differences (Fig. 12). The whitish index increased with increasing storage temperature showing statistically different values throughout the storage period (Fig. 11). An interaction effect was also observed between the packaging, temperature and time which did not show a better control of the white blush formation since the effect of the temperature was more pronounced. The effect of packaging and time showed a better control of the whitish blush formation than temperature and time and the three-way interaction of temperature, packaging and time.

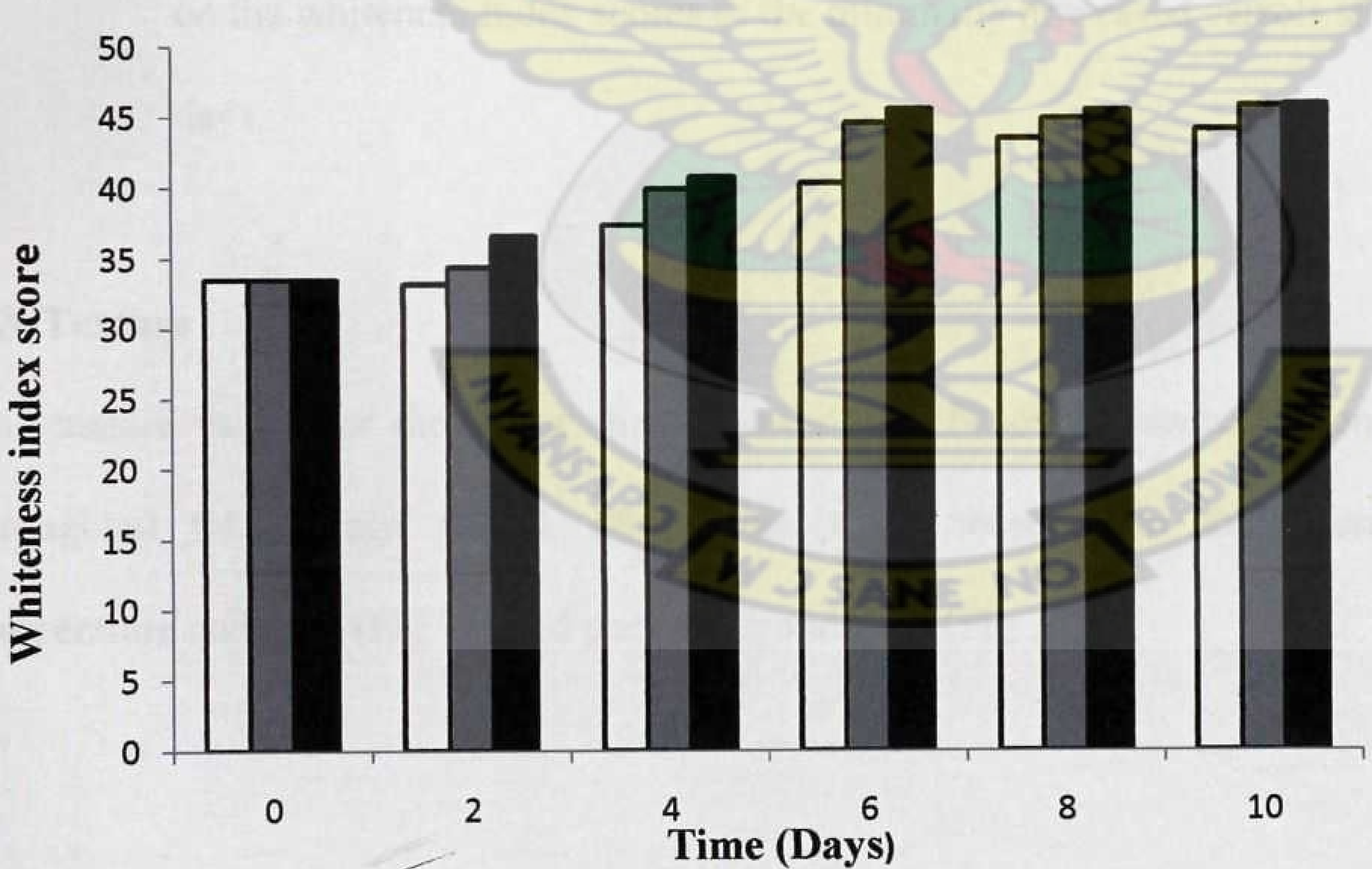

Figure 11: Interaction effect of storage temperatures, 3°C (□), 10°C (■) and 20°C (■) and time on the whiteness index scores of the minimally processed carrots during the 10-day storage period.

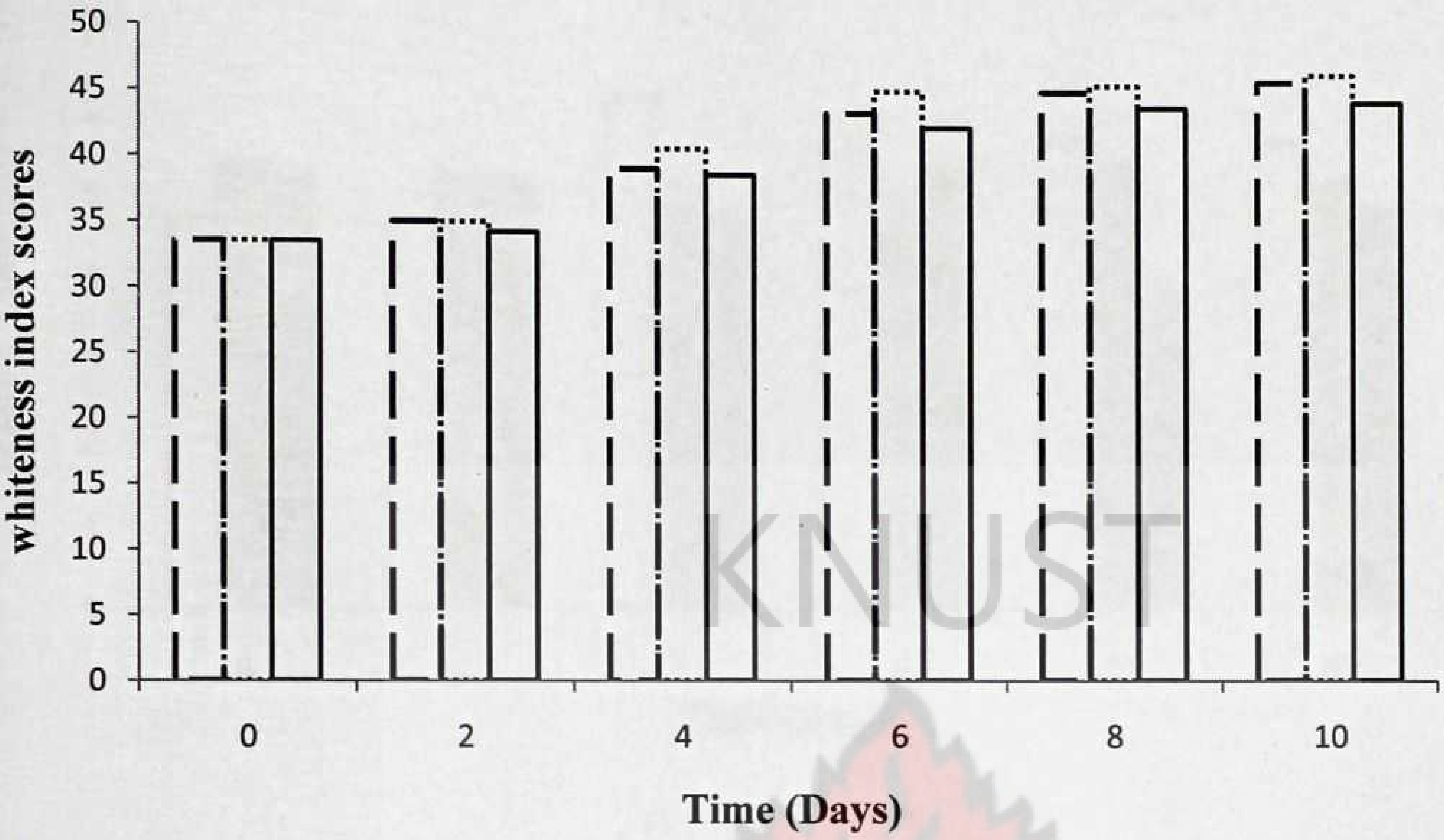

Figure 12: Interaction effect of packaging, PVDC/PE ( ), HDPE ( ) and LDPE ( ) and time on the whiteness index scores of the minimally processed carrots slices stored for 10 days.

4.3 Texture

The texture values for the carrot slices showed significant differences amongst all treatments throughout the storage period. Differences were observed in the interaction effects of temperature and time (Fig 13) and packaging and time (Fig 14).

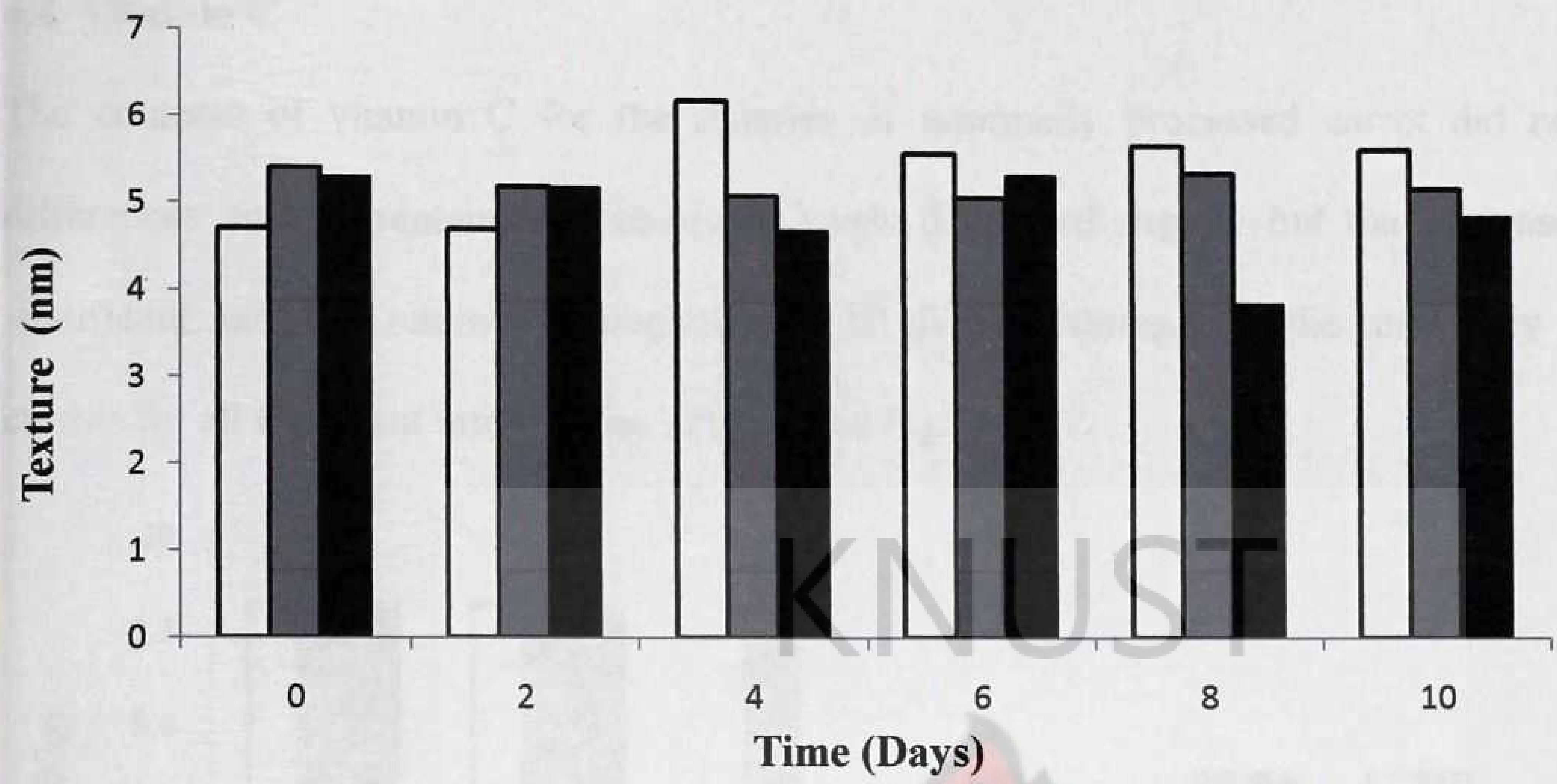

Figure 13: Interaction effect of storage temperature, 3°C (□), 10°C (■) and 20-25°C (■) and time on the texture values of minimally processed carrots stored for 10 days.

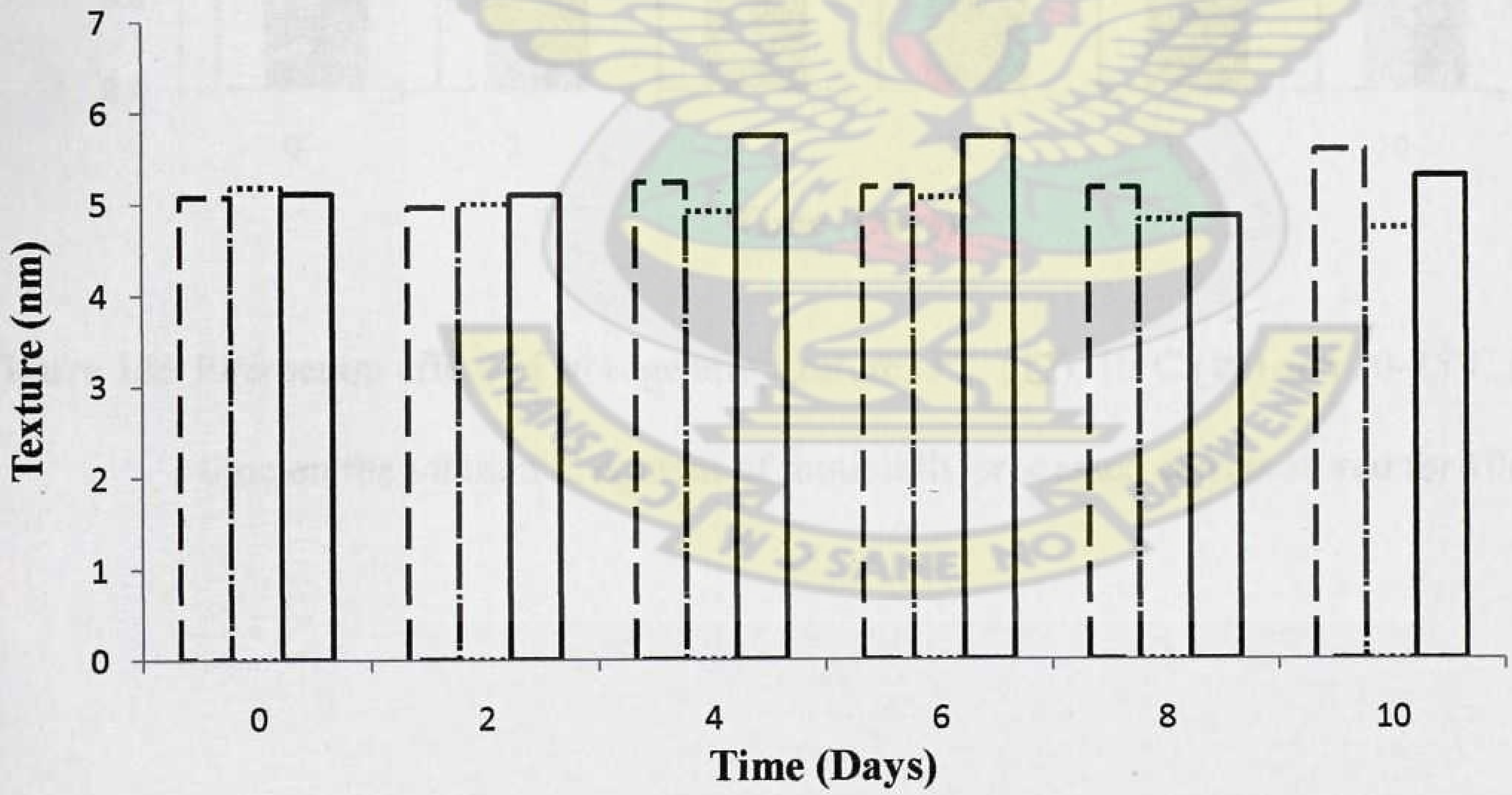

Figure 14: Interaction effect of packaging, HDPE (), LDPE () and PVDC/PE ( ) and time on the texture values of minimally processed carrots stored for 10days.

4.4 Vitamin C

The contents of vitamin C for the samples of minimally processed carrot did not present differences among treatments. Vitamin C levels decreased slightly but the decrease was not significant and was retained throughout the 10 days of storage for the minimally processed carrots for all treatment interactions. (Fig 15 and Fig 16).

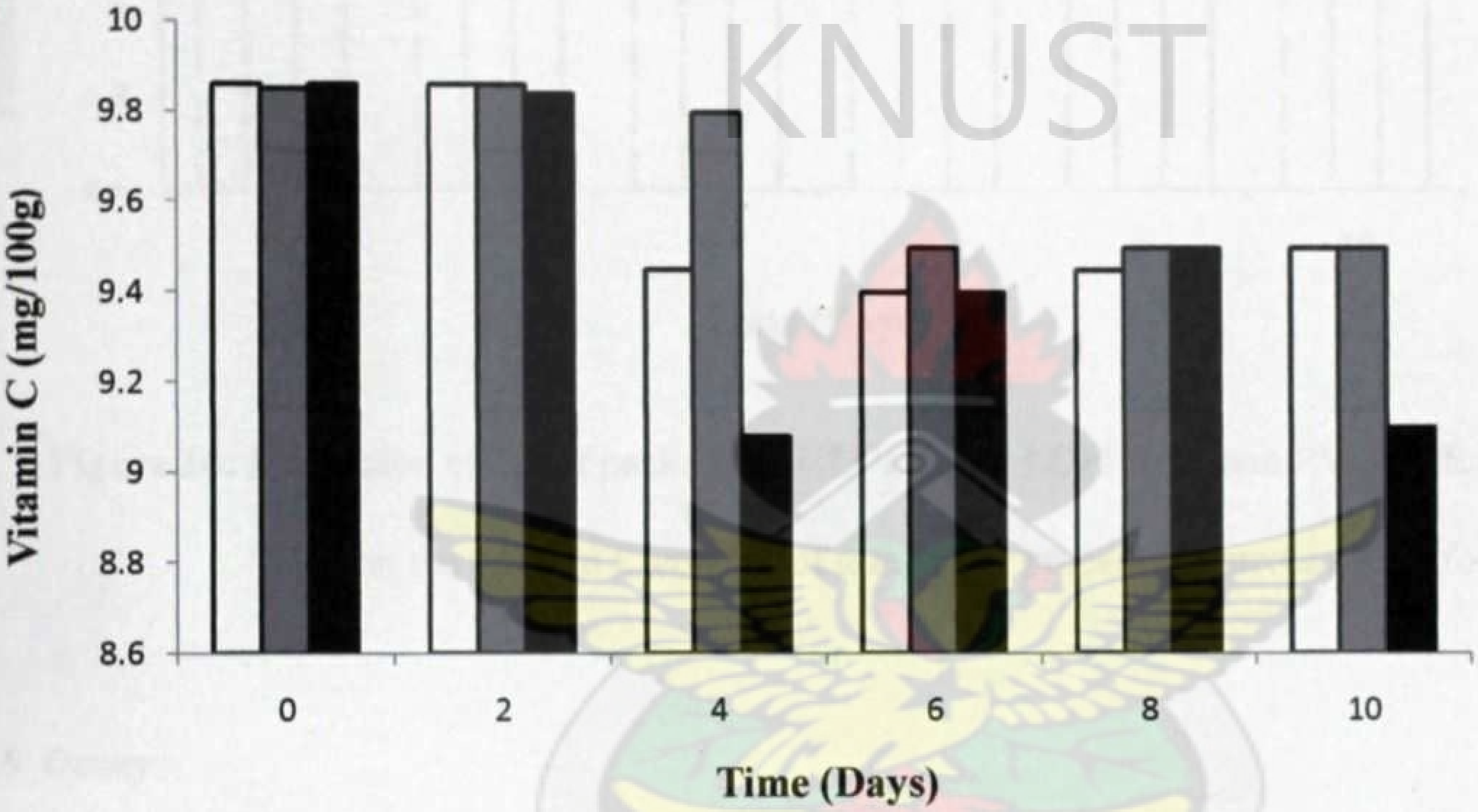

Figure 15: Interaction effect of storage temperature, 3°C (□), 10°C (■) and 20-25°C (■ and time on the vitamin C content of minimally processed carrots stored for 10days.

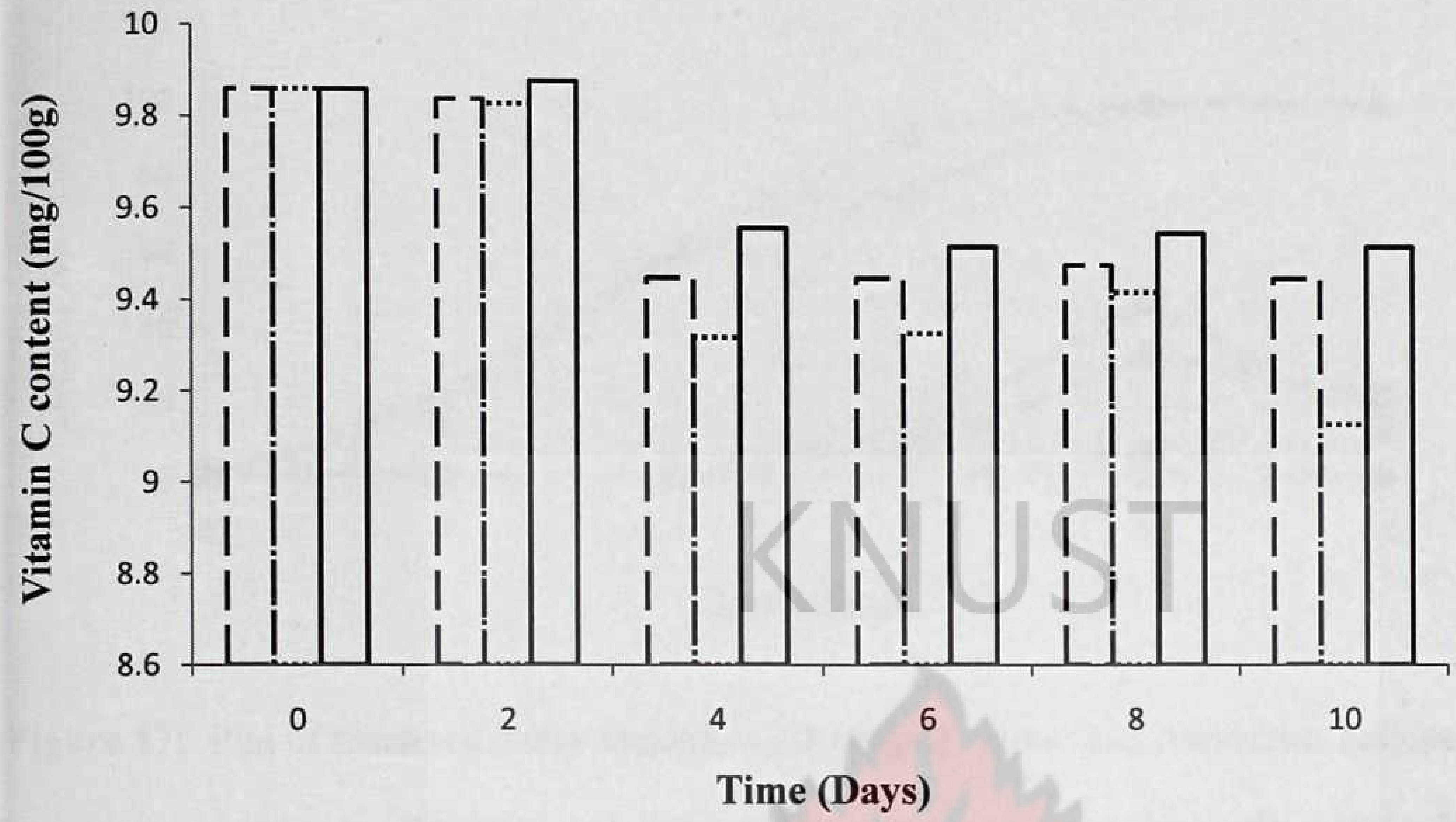

Figure 16: Interaction effect of packaging, HDPE ( ), LDPE ( ) and PVDC/PE ( ) and time on the vitamin C content of minimally processed carrots stored for 10days.

4.5 Decay

Black spots, black patches and exudates were the typical symptoms for spoiled carrots and were most severe in HDPE and LDPE packages at 20°C. On day 8 and 10, 100% rot incidence was recorded in both packages at 20°C. The black spots occurred once in the PVDC/PE carrot packages at 20°C on day 10 with 14.25% incidence. From Fig. 17 and Fig. 18, considering only the data from HDPE and LDPE packages, it can be seen that the rot incidence increased with increasing temperature. Similarly, a significant increase in rot incidence was noted from d6 to d10. The time and temperature interacted together to spoil the carrots at a faster rate.

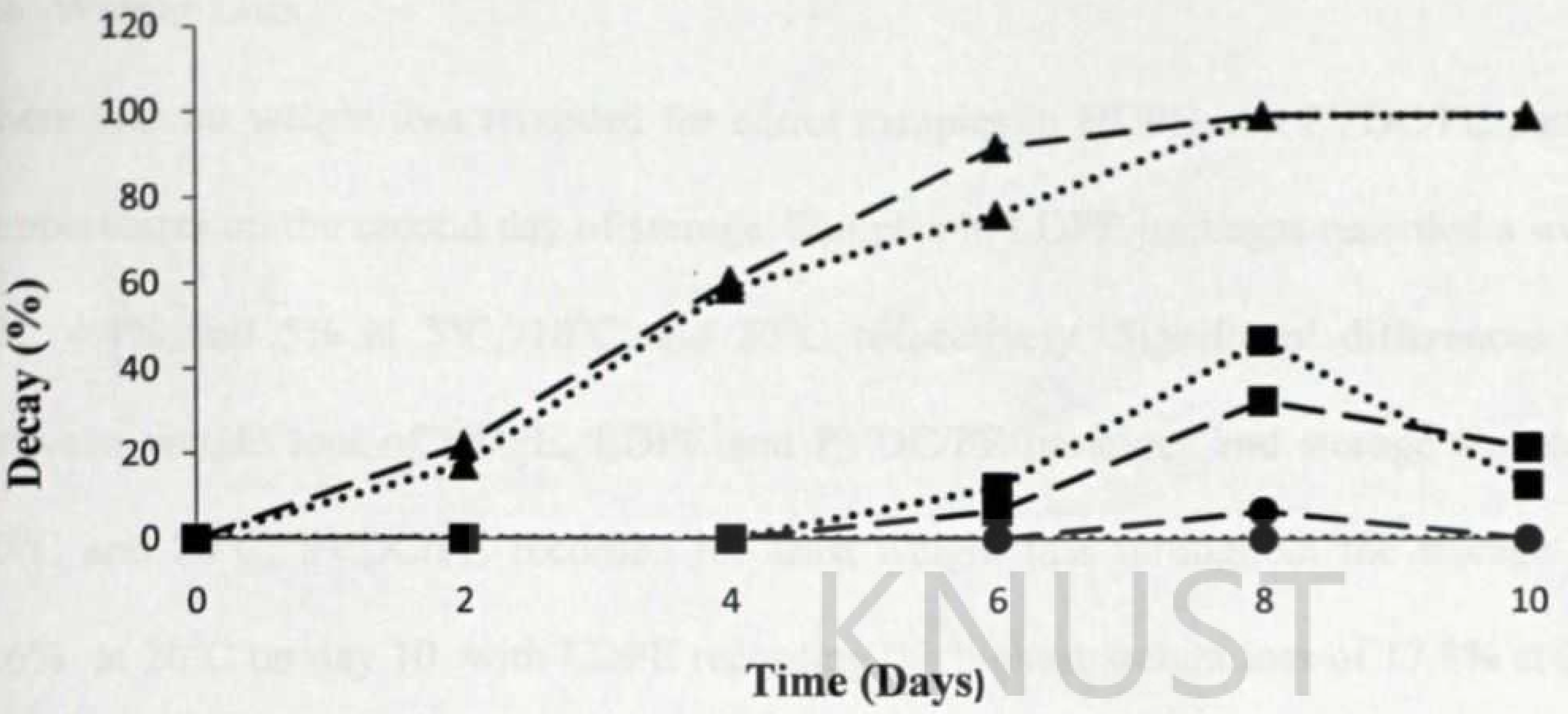

Figure 17: Plot of means of decay incidence showing the three-way interaction between packaging, HDPE (– –) and LDPE (.......), temperature, 3°C (●), 10°C (■) and 20°C (▲) and time.

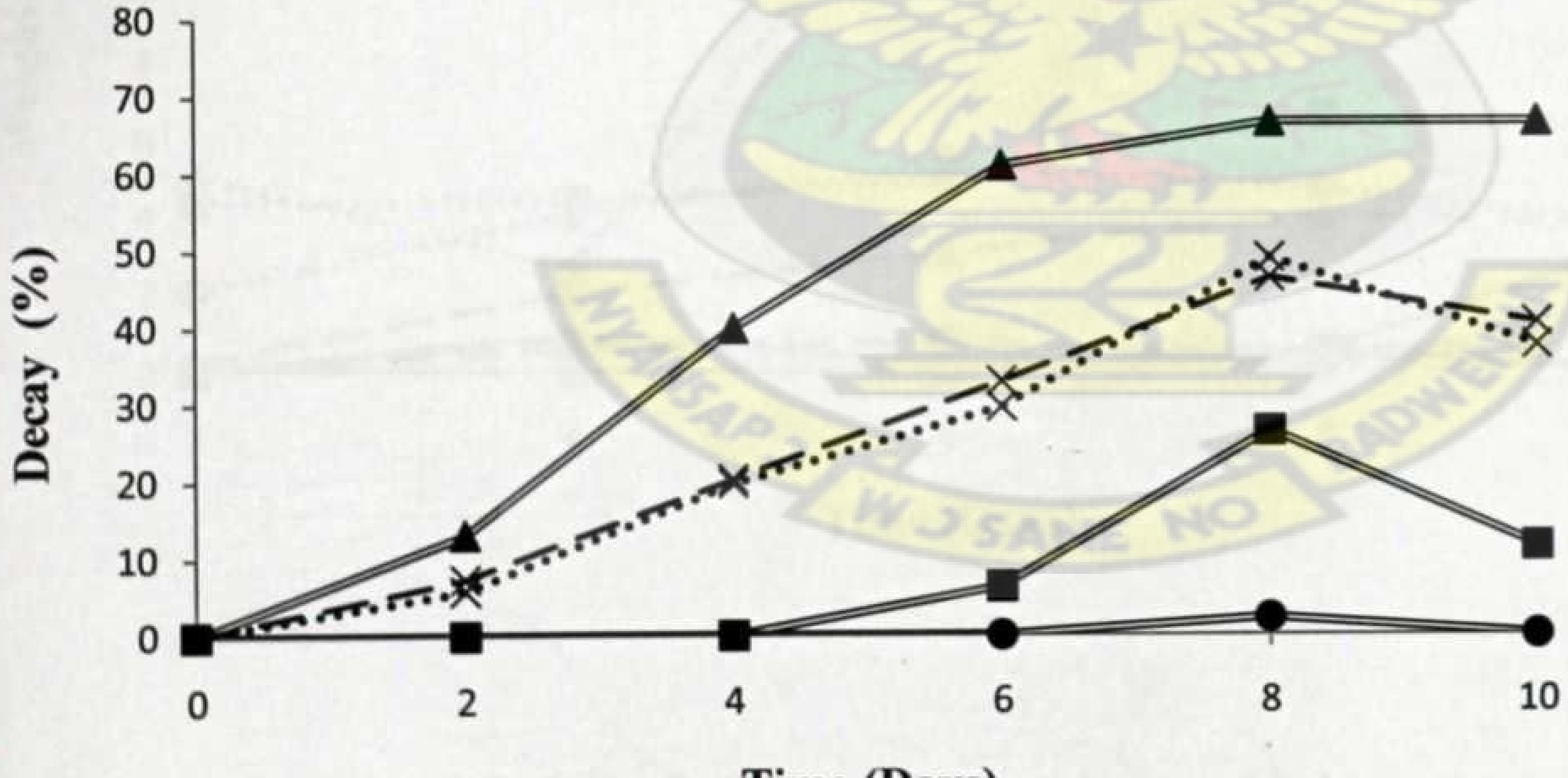

Figure 18: Plot of means of the two- way interaction between Packaging, HDPE (– –) LDPE (.......) and time, and temperature 3°C (●), 10°C (■), and 20°C (▲) and time on decay incidence on minimally processed carrot stored for 10 days.

4.6 Weight Loss

There was no weight loss recorded for carrot samples in HDPE and PVDC/PE packages at all temperatures on the second day of storage. Samples in LDPE packages recorded a weight loss of 2%, 4.4% and 5% at 3°C, 10°C and 20°C respectively. Significant differences were found between weight loss of HDPE, LDPE and PVDC/PE packages and storage temperatures 3°C, 10°C and 20°C. PVDC/PE recorded the least weight loss throughout the storage period with 3.6% at 20°C on day 10 with LDPE recording the highest weight loss of 17.8% at 20°C on day 8 (Fig 19).

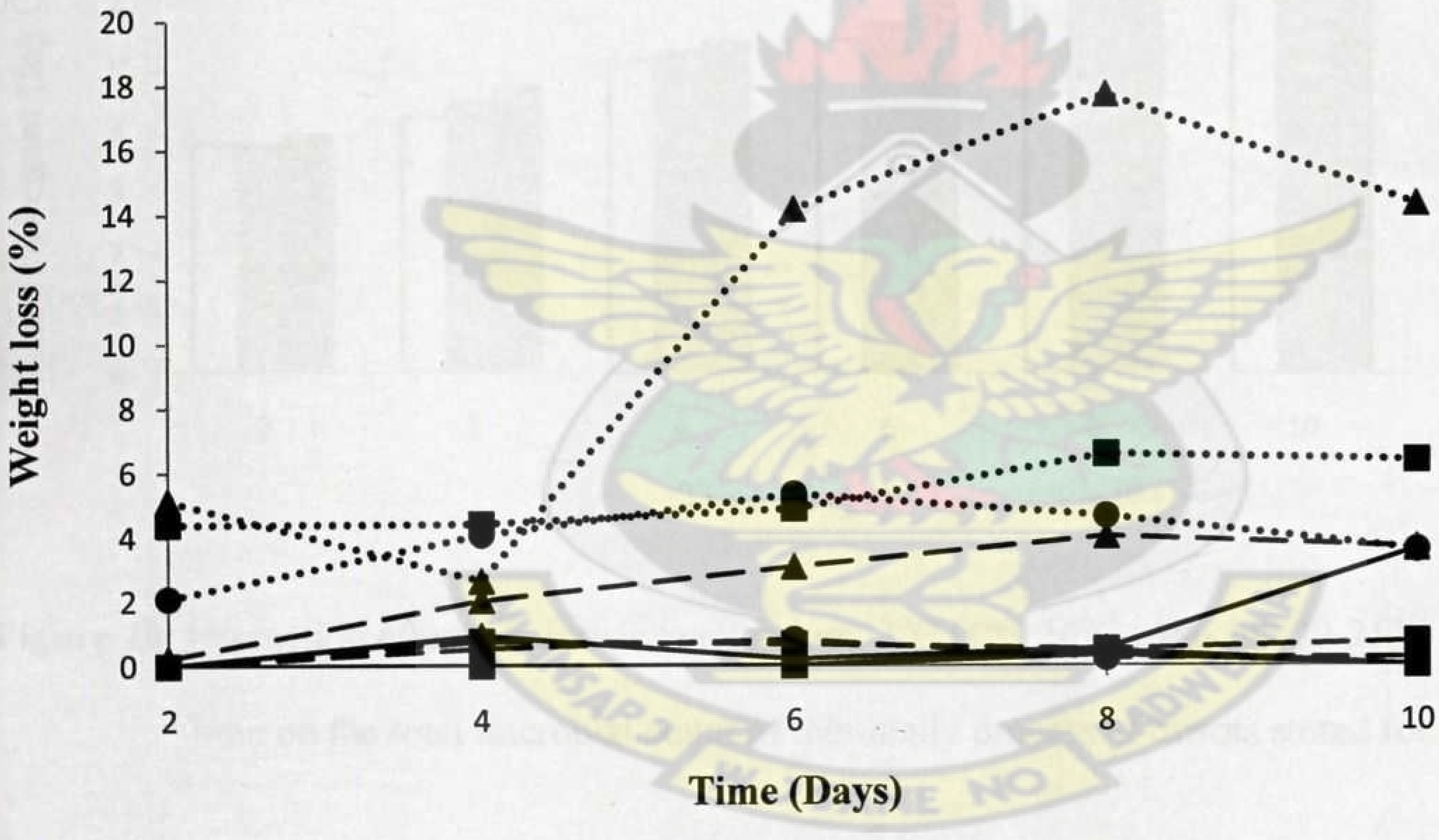

Figure 19: Weight loss of minimally processed carrot during a 10-day storage period showing a three-way interaction between temperature 3°C (●), 10°C (■) and 20°C (▲), time and packaging, HDPE (– –) LDPE (……) and PVDC/PE (—).

4.7 Microbial count

The occurrence of microbial growth was significantly affected by the packaging and temperature as well as their two-way interactions with time from the second day of storage(Fig. 20 and Fig. 21). The total counts of micro-organisms at 37°C never exceeded the maximum acceptable limit of log CFU g^{-1}=8 for all treatments (Jacxsens et al., 2002).

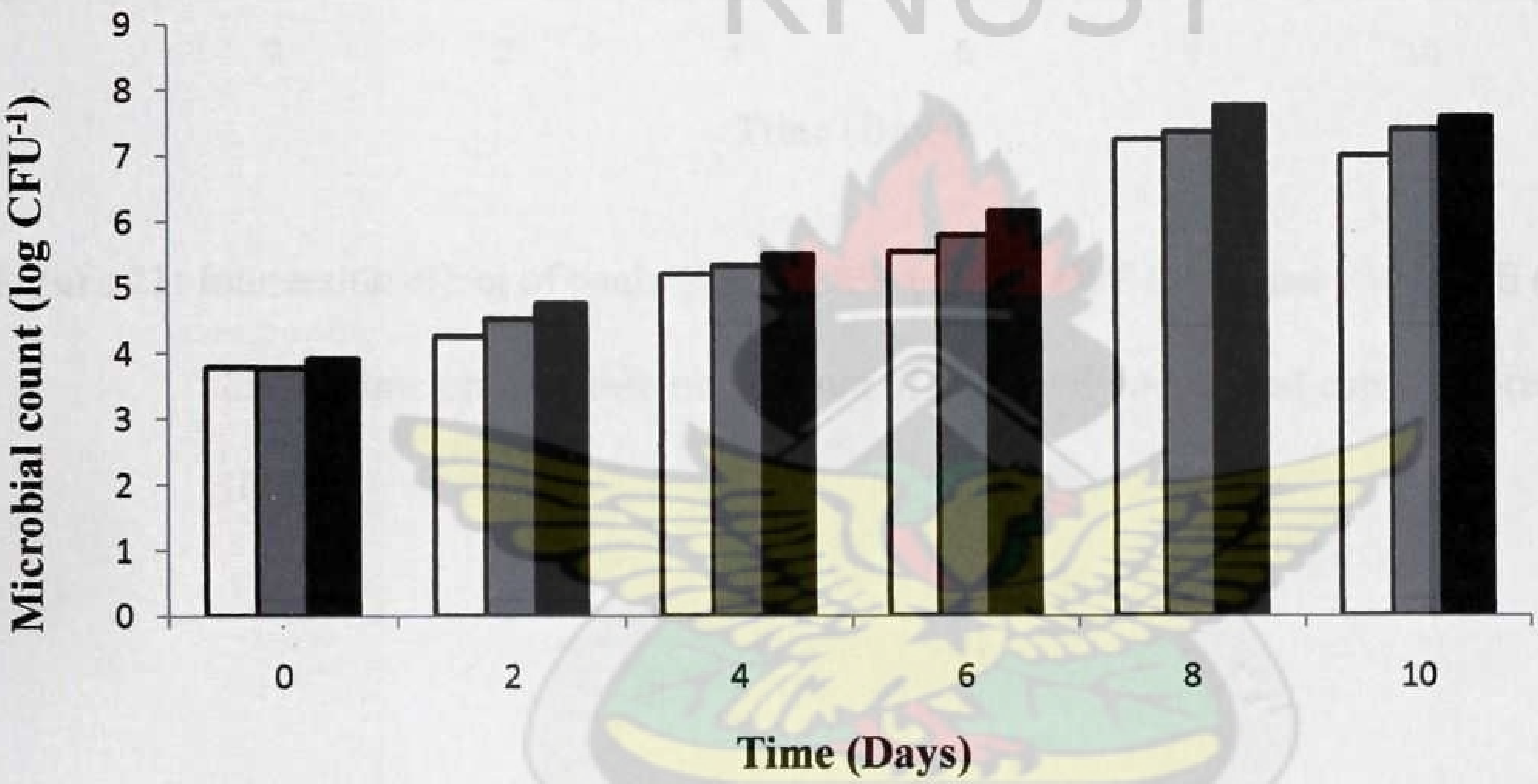

Figure 20: Interaction effect of storage temperature, 3°C (□), 10°C (▩) and 20-25°C (■ and time on the total microbial count of minimally processed carrots stored for 10 days.

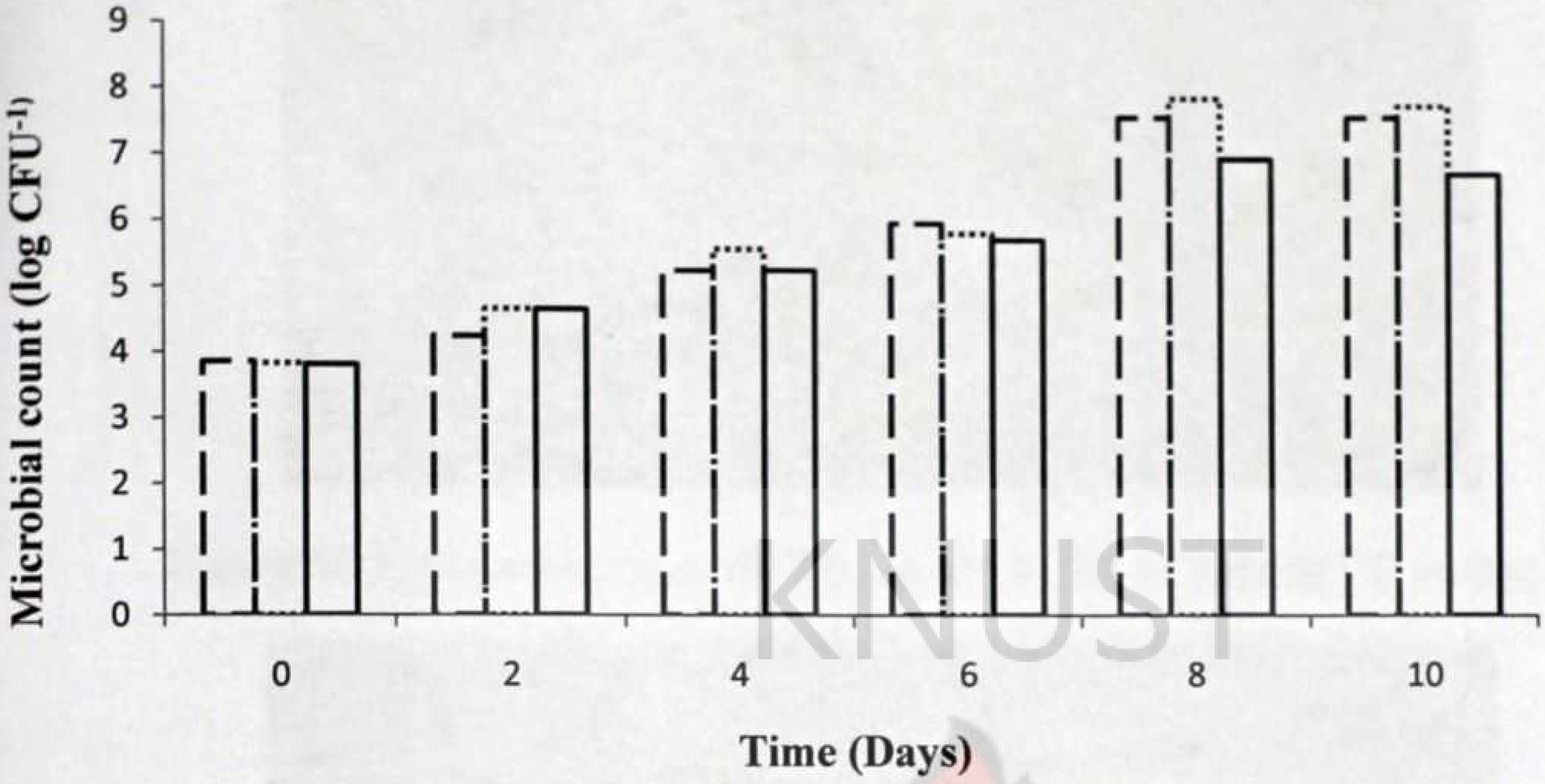

Figure 21: Interaction effect of packaging, HDPE ( ), LDPE ( ) and PVDC/PE ( ) and storage time on total microbial count of minimally processed carrots stored for 10days.

Figure 22: Photograph of carrots slices in PVDC/PE package at 3°C on day 10

Figure 23: Photograph of carrot slices in PVDC/PE package at 10°C on day 10

Figure 24: Photograph of carrot slices in PVDC/PE package at 20-25°C on day 10

4.9 Chemical analysis (Soluble solids, pH, and titratable acidity)

The content of soluble solids did not change during the 10 days of storage, and it was around 9-10°Brix, for all treatments.

The average values of pH found for the minimally processed carrots ranged between 6.35-6.45 for all treatments throughout the storage period.

The titratable acidity content of the minimally processed carrots was not significantly affected by experimental treatments throughout the storage period, Fig. 25 and Fig. 26.

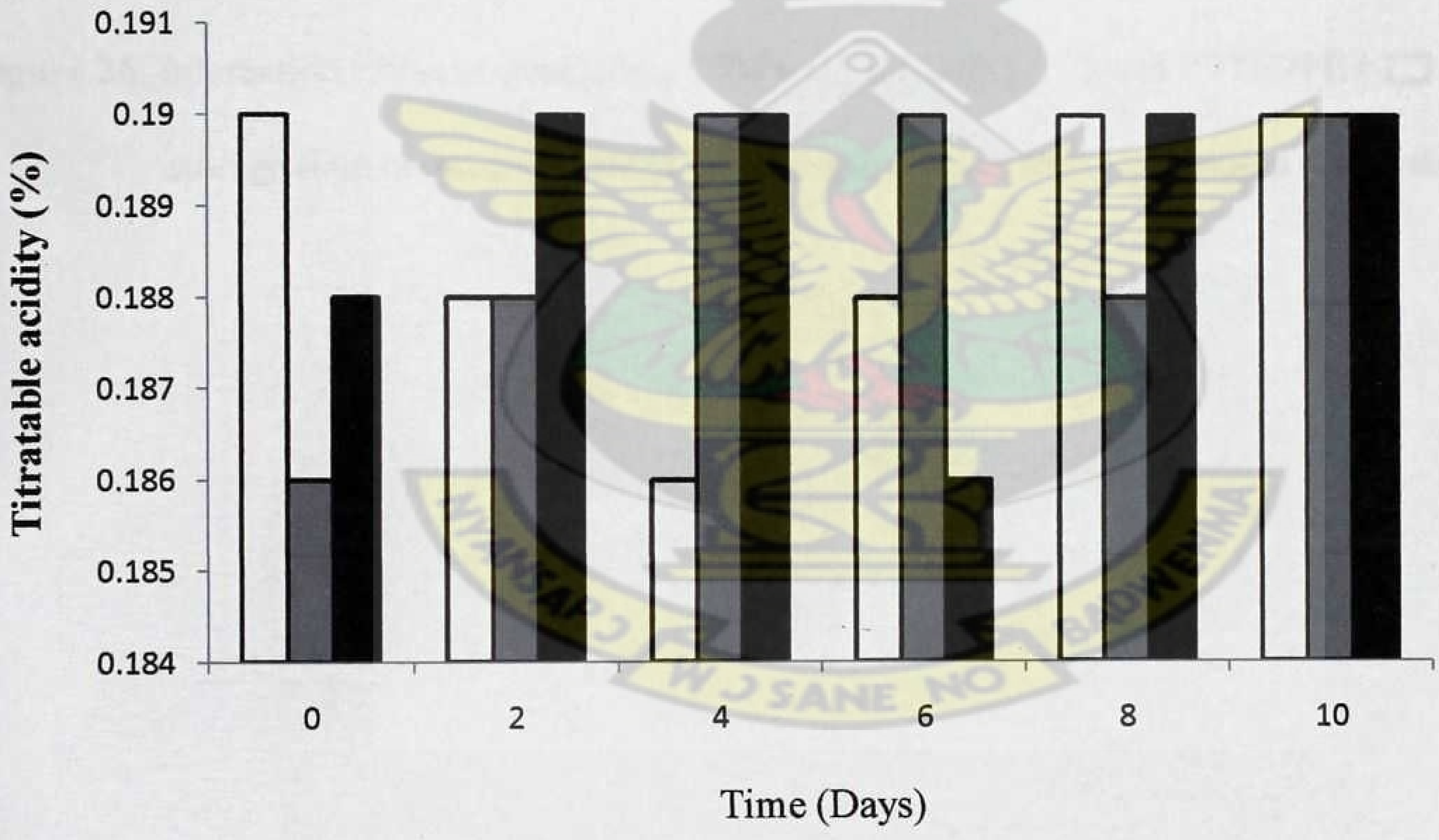

Figure 25: Interaction effect of storage temperature, 3°C (□), 10°C (■) and 20-25°C (■) and storage time on acidity content of minimally processed carrots.

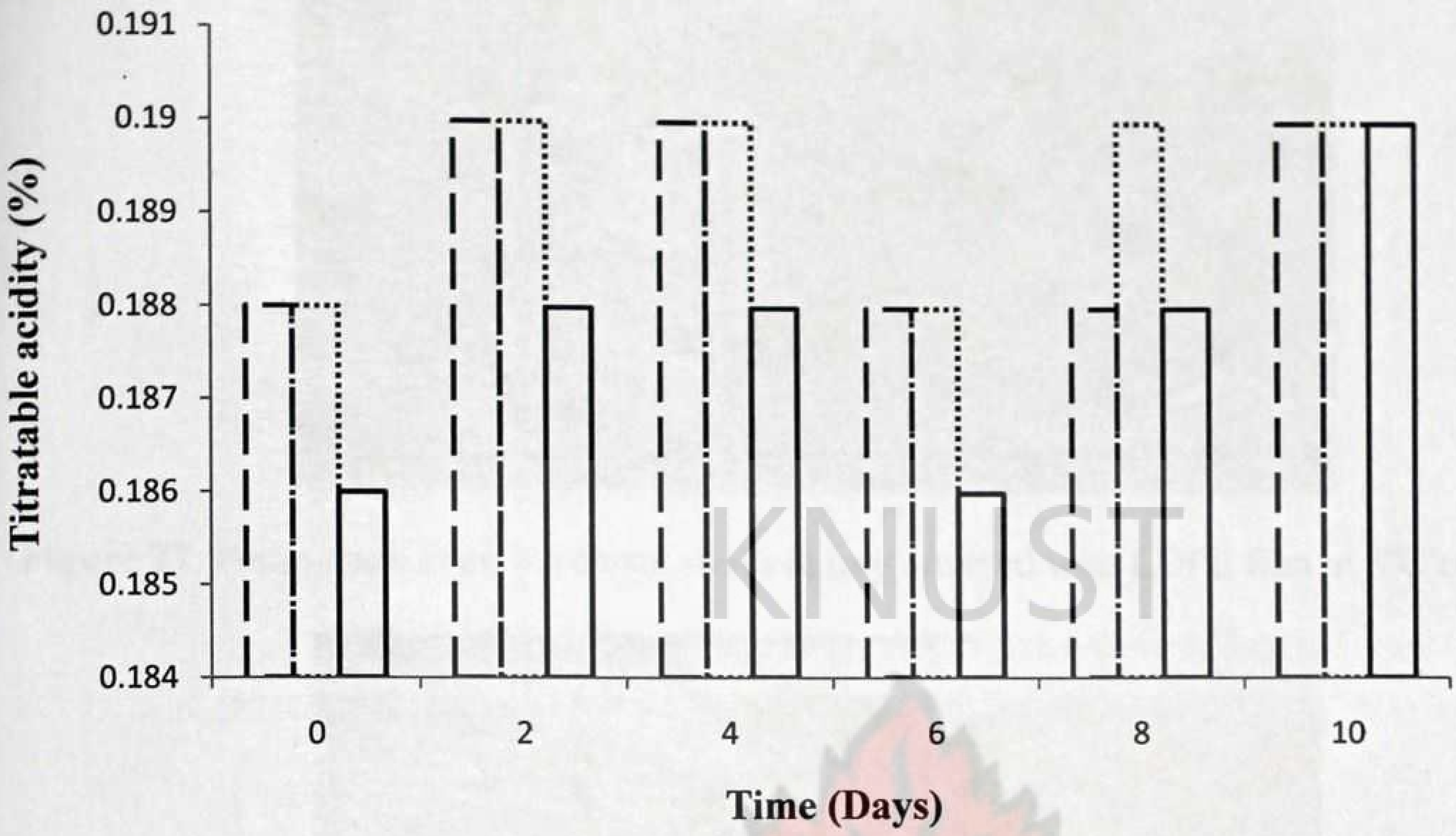

Figure 26: Interaction effect of packaging, HDPE ( ), LDPE ( ) and PVDC/PE ( ) and storage time on acidity content of minimally processed carrot stored for 10 days.

Figure 27: Photograph showing carrot slices in tray covered with LDPE film at 3°C on day 10

Figure 28: Photograph showing carrot slices in tray covered with LDPE film at 10°C on day 10

Figure 29: Photograph showing carrot slices in tray covered with LDPE film at ambient (20-25°C) on day 10

Figure 30: Photograph showing carrot slices in HDPE package at 3°C on day 10

Figure 31: Photograph showing carrots slices in HDPE package at 10°C on day 10

Figure 32: Photograph showing carrot slices in HDPE package at ambient (20-25°C) on day 10

5. Discussion

The results will be discussed in terms of the interaction effects of the experimental factors, temperature, packaging and time and also the two-way interaction effects of temperature and time and packaging and time on the shelf life quality of carrots.

5.1 Head space oxygen and carbon dioxide

The decrease in oxygen and increase in carbon dioxide concentrations in the headspace of minimally processed carrots in the PVDC/PE packages was as a result of the respiration rate of the carrots as well as the gas permeability of the polymeric packaging film. The change in the PVDC/PE packages showed the creation of a modified atmosphere that will decrease the metabolic activities. The PVDC/PE packages combined with reduced temperature gave the lowest oxygen and highest carbon dioxide levels in the head space atmosphere.

The lowest oxygen (0.00%) and highest carbon dioxide (44.4%) was achieved in the head space of PVDC/PE package and is indicative of anaerobic respiration. Carlin et al. (1989) suggested that the critical head space for anaerobic respiration was 1.5% oxygen and 30% carbon dioxide in a passive modified atmosphere for grated carrots. Sode and Kuhn (1998), however also recommended at least 5% oxygen in a controlled atmosphere for cut carrots to prevent anaerobic respiration. Both recommended limits were exceeded in this experiment for PVDC/PE packages at 10°C and 20°C.

Carlin et al. (1990b) found no clear relationship between the respiration rate and head space gas concentrations in packed carrots. They suggested that the diffusion rate of gases across the packaging film was of more importance than the head space. However it can be said that the respiration rate decreased because of the low concentration of oxygen in the pack. Sode and Kuhn (1998) reported that the respiration of cut carrots has a high dependency on oxygen concentration. They found that there was a decrease in energy consumption and a shift to glycolytic metabolism for rminimally processed carrots at 5% of oxygen under controlled atmospheres.

The PVDC/PE packaging material prevented moisture loss more successfully than creating optimum gas composition. The headspace oxygen and carbon dioxide concentration in the HDPE and LDPE packaging materials showed a rapid increase to atmospheric level on day 2 and plateau throughout the storage period. This occurrence was due to the high oxygen permeability of the HDPE and LDPE packaging materials as compared to the PVDC/PE packages, improper sealing or leakages in the films.

5.2 Colour Changes

One of the most important criteria in the extension of minimally processed carrots is whitening or white blush formation caused by drying at the surface of the peeled and sliced carrots (Emmambux and Minaar, 2003; Klaiber et al., 2004). A mechanism proposed for white discoloration on peeled carrots is related to physical and physiological responses to wounding.

The physical response is reflected as a color change because of the reversible surface dehydration and physiological response as a result of the activation of phenolic metabolism and the production of lignin resulting in an irreversible color change (Cisneros-Zevallos et al., 1995; Emmambux and Minaar, 2003). Emmambux and Minnaar (2003) reported that a polymeric packaging film maintaining a high relative humidity with a good moisture barrier should be considered to prevent white blush formation, which is the most important shelf life determinant for minimally processed carrots.

The surface cells became widespread and this allowed rapid dehydration that caused surface white discolouration as a result of scattering of reflected light (Cisneros-Zevallos et al., 1995). In addition, as a repair mechanism, wounding promotes lignifications of the cell walls by enzymatic processes (Howard and Griffin, 1993). Occurrence of lignin formation was shown to be a physiological response that led to surface white discolouration (Cisneros-Zevallos et al., 1995).

Tests conducted revealed that the water vapour transmission rate for PVDC/PE polymeric packaging material was 6.55g $(m^2.24h.atm)^{-1}$ at 37.5°C and 38%RH which maintained a high relative humidity inside the package at all temperatures preventing excessive water loss from the surface of the carrot slices thereby reducing the occurrence of whitish blush formation. The low water and gas permeability properties of the PVDC and the low vapour permeability property of the extruded PE material make the material a good moisture barrier which is most important in shelf life extension of minimally processed carrots.

A significant increase in the whiteness index was observed for both HDPE and LDPE packages and this was slightly higher for LDPE carrot samples from the third day of storage (Fig. 12). HDPE packages presented a slightly lower whitish blush formation than LDPE packages. This occurrence was attributed to the thickness and the lower water vapour transmission rate of the HDPE polymeric packaging compared with that of LDPE polymeric packaging film.

High Density Polyethylene (HDPE) bags are better for preventing moisture ingress than low-density polyethylene (LDPE) bags since the thicker the bag, the lower the water vapor transmission rate.

5.3 Vitamin C

Vitamin C content was retained throughout the 10-day storage period for the minimally processed carrots. Similar results were found by Pilon et al. (2006), in minimally processed carrots packed in multilayered laminated BOPP/LDPE (biaxially orientated polypropylene/low-density polyethylene) plastic bags under vacuum, air and modified atmosphere conditions (2% of O_2, 10% of CO_2 and 88% of N_2) at 1°C for 21 days.

5.4 Texture

In general, significant differences were observed in terms of texture values for all treatments during the 10-day storage. The texture values representing the resistance to shear or firmness oscillated with a slight decrease and this was attributed to differences in thickness and size of the carrots slices rather than increase in metabolic activities.

5.5 Chemical analysis (Soluble solids, pH, and titratable acidity)

The results of the titratable acidity content of the minimally processed carrots are given in Fig. 25 and Fig. 26. There was no significant difference observed between the treatments at any given day during storage in terms of the acidity content.

pH values ranged between 6.35-6.45 throughout the storage period and no statistical differences were observed amongst the treatments.

Soluble solids content did not present differences in all treatments during the 10-day storage period.

5.6 Decay

The black patches and spots, tissue softening and exudates production as rotting symptoms for the minimally processed carrots resulted from microbiological growth on the minimally processed carrots. The typical black patches and spots were explained by oxidation of the carrots phenols found to increase during storage (Amanatidou et al., 2000). They found that oxidation and polymerization causing surface browning were catalysed by microbial enzymes (Howard et al., 1994). Enzymatic activities are catalyzed at higher temperatures and this explains the higher decay incidence observed in all packages at the ambient storage temperature, 20-25°C.

5.7 Weight Loss

Significant differences were found among weight loss of carrot samples in all treatments. This loss was reduced in PVDC/PE and HDPE polymeric packages because of their comparatively lower water vapour transmission rates which prevented the faster ingress of water vapor from within the package consequently reducing the weight loss. The LDPE polymeric film has a higher water vapor transmission rate and this contributed to the increase in water loss from the carrot slices reducing the weight. Evaporation of moisture from surfaces reducing water content and subsequently weight loss at higher temperatures can also explain the increased weight loss of carrot slices at ambient temperature (20°C-25°C) in all packages during storage. Thus the highest weight loss was recorded in carrot slices packed in LDPE polymeric packaging at ambient temperature (20-25°C).

5.8 Microbiology

Significant differences were observed for all treatments in the total bacterial counts of stored carrots after the second day of storage. The change in total aerobic microorganisms is shown in Table 4. The number of total aerobic microorganisms was higher in packages stored under ambient conditions (20-25°C) than in those stored at 10°C and 3°C. The lowest counts were observed in the least permeable packaging films (PVDC/PE and HDPE) up to day 10. Using a value of 5 for the log CFU g^{-1} as the acceptable maximum limit (Jacxsens et al., 2002), PVDC/PE with comparatively higher gas permeability seems to be the most useful package to minimize the microbial effect on the shelf life of the product. The total counts of micro-organisms for all treatments at 37°C never exceeded the maximum acceptable limit of

log CFU g^{-1}=8 (Jacxsens et al., 2002).

6. Conclusions and Recommendations

Polymeric packaging films and temperature impact differently on the quality parameters of minimally processed carrots.

From the results, it can be concluded that:

1. Packaging has a more pronounced effect on the head space gas concentrations than temperature as different packages under the same storage temperature exhibited different headspace gas composition according to their gas permeability rates throughout the storage period. Packages with relatively higher permeability to O_2 and CO_2 are preferred for maintaining normal respiration of carrots.

2. The polymeric packaging films do not prevent whitish blush formation, however polymeric packaging films with low water vapor permeability are able to keep the surface of the carrot slices from dehydration and thus are able to create a high relative humidity inside the package, thereby reducing whitish blush formation on the surfaces.

3. Temperature has an effect on the whitish blush formation on the surface of stored carrots. The higher the temperature, the higher the whitish index recorded. This may be attributed to the vaporization of moisture from the surface of the sliced carrots at higher temperatures causing the rapid formation of whitish blush. It is recommended that lower storage temperatures be employed in the storage of minimally processed carrots.

4. Temperature has a more pronounced effect on incidence of decay than polymeric packaging films. Lower storage temperatures are recommended for the storage of minimally processed carrots to prevent excessive decay incidence.

5. Weight loss is affected by both temperature and packaging. Higher temperatures and high water vapour transmission film packages cause increased weight loss.

6. The polymeric packaging films are able to control the microbiological growth in minimally processed carrots because of the generation of modified atmosphere in the packs. Higher temperatures, however, do not.

7. Polymeric packaging films and temperature are able to maintain the vitamin C, texture and the chemical parameters (acidity, pH and brix) of minimally processed carrots during storage.
8. Packaging films with relatively higher permeability to O_2 and CO_2 are preferred for maintaining normal respiration of carrots without the occurrence of secondary decomposition during storage. Modified atmosphere packaging combined with low temperature storage of carrots reduces both biological and biochemical activities resulting in improved quality.

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