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THE COMBINED EFFECT OF MAP AND OTHER BARRIERS ON THE GROWTH OF *SALMONELLA ENTERITIDIS* IN PACKAGED CHICKEN THIGHS UNDER VARIOUS STORAGE CONDITIONS.

By

SAMEER .F. AL- ZENKI

Department of Food Science
and Agricultural Chemistry
Macdonald Campus
McGill University
Montreal, Quebéc

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ABSTRACT

The Combined Effect Of MAP, Additonal Hurdles And Storage Conditions On The Growth of *Salmonella enteritidis* In Packaged Chicken Thighs

During storage and handling of poultry products, strict temperature control is the most important parameter influencing microbial growth and subsequent spoilage. However, due to the potential of temperature abuse and the subsequent growth of pathogenic bacteria, the need for additional hurdles is essential to ensure the public health safety of food. *Salmonella enteritidis* has recently emerged as a potential pathogen in poultry products. The growth of *S. enteritidis* in poultry is affected by several factors such as storage temperature, pH, water activity, modified atmosphere and the presence of preservatives. All of these factors may act alone or in combination with each other resulting in a synergistic, antimicrobial effect.

In this research, initial storage studies were done to determine the effect of various atmospheres (air, vacuum, oxygen absorbent and gas packaging) on the microbial changes of packaged chicken thighs followed by challenge studies with a strain of *S. enteritidis* ^{NAST}. Chicken thighs were packaged in Cryovac bags and stored at 4 and 12°C for up to 28d. Changes in headspace gas composition, pH, drip loss, color and odor were monitored at each sampling day. Results showed that the final microbial composition of all packaging treatments comprised mainly of lactic acid bacteria and coliforms and to lesser extent the *Pseudomonas* spp. At 4°C, gas packaging (60% CO_2+40% N_2) extended the shelf-life of chicken thighs to 28d compared to only 7d for air packaged samples. A 14d extension of shelf-life was possible by vacuum packaging or packaging products with an oxygen absorbent. Major differences in treatments were observed at 12°C, again indicating that the rate of spoilage was directly proportional to storage temperature.

The effect of various packaging treatments, dipping solutions (chitosan (0.2%w/v) and potassium sorbate (0.2%w/v)) and low dose irradiation (1.5 & 3.0 kGy) on the growth of *S. enteritidis*^{NAST} and on the shelf-life of chicken thighs stored at 4 and 12°C was also investigated. Results showed that gas packaging resulted in increased shelf-life when chicken thighs were dipped in 0.2% potassium sorbate than 0.2% chitosan. Pretreatment with 0.2%

potassium sorbate prior to gas packaging and storage at 4°C extended shelf-life to more than 28d compared with 21d for similarly packaged chicken thighs treated with 0.2% chitosan prior to packaging and stored at the same temperature. Furthermore, gas packaging inhibited the growth of *S. enteritidis*^{NAST} with inhibition again being greater in thighs dipped in 0.2% potassium sorbate than 0.2% chitosan. Packaging of chicken thighs in higher concentrations of CO_2 (i.e. 100% CO_2) with or without potassium sorbate resulted in an increase in shelf-life and inhibited the growth of *S. enteritidis*^{NAST} throughout the 28d storage. Gas packaging samples (60 or 100% CO_2) followed by low dose irradiation with 1.5 or 3 kGy completely eliminated *S. enteritidis* and improved shelf-life to more than 28d compared to 6d for aerobically packaged samples at 4°C. From the results obtained, it can be concluded that inhibition of *S. enteritidis*^{NAST} and shelf-life extension of chicken thighs, can be achieved substantially by storage at low temperatures, and by packaging in 60 or 100% CO_2 with or without pre-dipping in potassium sorbate. For maximum shelf-life of packaged chicken thighs, thighs can be MAP packaged and irradiated with low dose irradiation (1.5 or 3 kGy) and stored at 4°C.

RESUME

Influence de L'emballage Sous Atmospheres Modifiees Combinee Avec D'autres Methodes De Controle Et D'entreposage Sur La Croissance De *Salmonelle Enteritidis* Utilisant Des Cuisses De Poulet

Durant l'entreposage et le transport des produits de volaille , le contrôle efficace de la temperature est un paramtre important qui influence la croissance des bacteries et ainsi que la détérioration du produit. *Salmonelle enteritidis* est un microorganisme pathogene isolé fréquemment à partir de plusieurs produits de volaille.

L'habilitée de la bacterie pathogène a se reproduire depend principalement de la température, de pH, de l'activité de l'eau, atmospheres modifiées et la présence d'autres préservatives. Tous ces facteurs peuvent agir individuellement ou ensemble pour aboutir a un effet de synergie et anti-microbien. Pour cela d'autres mesures de contrôles sont essentielles pour assurer la sûreté des aliments.

Dans ce projet, des études préliminaires utilisant différentes conditions d'entreposages ont été effectuées pour détérminer l'effet de plusieurs atmosphères gazeuses (l'air, sous-vide, absorbant d'oxygène et emballage gazeux) avec l'intention de détérminer les changements microbiens dans les cuisses de poulet emballées suivi des études de systemes modèles sur la croissance d'une souche de *S. enteritidis*^{NAST}. Des cuisses de poulet ont été emballées dans des sacs Cryovac et entreposées à des températures de 4 et 12°C pour une durée de 28 jours.A chaque jour d'échantillonage, les changements dans le milieu gazeux de l'emballage, du pH, écoulement liquide, couleurs et odeurs du produit ont été analysés. Les resultats montrent que la composition microbienne finale de tous les traitements contient principalement la bactérie acide lactique, des coliformes et une faible quantite de *Pseudomonas* spp.

L'emballage sous gaz (60% CO_2 + 20 % N_2) entreposé a 4°C a prolongé la durée de conservation des cuisses de poulet pour une durée de 28 jours comparé à une durée de 7 jours pour l'emballage entreposé sous air. Une prolongation de 14 jours de la durée de conservation a été possible avec l'emballage sous-vide ou un emballage contenant un absorbant d'oxygène. Les résultats des mêmes conditions d'entreposage à 12°C ont varié, montrant ainsi que la détérioration est directement proportionnelle a la température d'entreposage. En se basant sur ces etudes préliminaires, une méthode combinant l'utilisation de différent traitement d'emballage gazeux en conjonction avec du chitosan (0.2% w/v), sorbate de potassium (0.2% w/v) (comme solutionde trempage) et faible dose d'irradiation (1.5 et 3 kGy) ont été etudiés pour contrôler la croissance de *S. enteritidis*^{NAST} et leur effet sur la durée de conservation des cuisses de poulet entreposées a 4 et 12°C. Les resultats montrent que l'emballage gazeux combinée avec une solution de trempage de 0.2% de sorbate de potassium a prolonger la duree de conservation plus efficacement que la solution de 0.2 % de chitosan. Pré-traitement des cuisses de poulet avec 0.2% de sorbate de potassium et l'emballage et l'entreposage a 4°C furent prolongés la durée des conservation de plus de 28 jours, compare à la solution de trempage de 0.2% de chitosan qui furent prolongés la dureé de conservation de 21 jours seulement. En outre, l'emballage gazeux arrête la croissance de *S. enteritidis*^{NAST} avec une inhibition encore plus importante pour les cuisses de poulet trempées dans 0.2% de sorbate de potassium. L'emballage des cuisses de poulet sous une forte concentration de CO_2 (100% CO_2) avec ou sans sorbate de potassium démontre une augmentation de la durée de conservation et empêche la croissance de *S. enteritidis*^{NAST} durant toute la durée d'étude de 28 jours. Les echantillons emballés sous gaz (60 et 100% CO_2) soumis à une faible dose d'irradiation de 1.5 et 3 kGy ont montreés une complète inhibition de *S. enteritidis*^{NAST} et leur durée de vie a été améliorée pour une période depassant 28 jours d'entreposage.

Cependant, à partir de ces résultats on peut conclure que l'empêchement de la croissance de *S.enteritidis*^{NAST} et l'extension de la durée de conservation des cuisses de poulet peuvent être susbtantiellement achéves, en emballant sous un atmosphere de 60 ou 100% de CO_2 avec ou sans pré-immersion dans une solution de sorbate de potassium combiné avec une

temperature basse d'entreposage.

Ainsi pour assurer une durée maximale de conservation des cuisses de poulet. Elles doivent être emballees sous atmosphères modifieés combinée avec une faible dose d'irradiation (1.5 ou 3 kGy) et entreposées a 4°C.

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CHAPTER 1

LITERATURE REVIEW

1.1. INTRODUCTION

Increasing demand for poultry products by consumers has imposed a greater responsibility on the poultry industry to provide them with a safe and wholesome food product. Raw poultry is a highly perishable food commodity that is subject to both chemical and microbiological deterioration which can render it unacceptable for human consumption. While poultry meat is relatively sterile prior to slaughter, it soon becomes contaminated with both spoilage and food poisoning bacteria from sources such as processing equipment, knives and the hands of personnel. Thus, commercially processed poultry products are often implicated in outbreaks of *Salmonella* food poisoning.

Extending the shelf-life and safety of poultry meat continues to be an important priority to the poultry industry. The shelf-life of poultry depends on a number of interrelated factors such as storage temperature, the initial bacterial load, the packaging material and the gaseous environment surrounding the product. Improved methods of preservation and new packaging technologies have been introduced to reduce spoilage. However, for these preservative techniques to be effective, it is necessary to start with a high quality product, maintain strict time-temperature control throughout all stages of processing and distribution and ensure good manufacturing practices. All these factors must work in conjunction with each other to achieve the desired shelf life and safety.

In this research work, methods of extending the shelf-life of chicken using modified atmosphere packaging will be investigated. The effectiveness of this packaging technology in combination with other preservative techniques in controlling the growth of pathogenic bacteria, specifically *Salmonella enteritidis* will be addressed.

1.2. Types of poultry

Poultry is an important food item that is highly appreciated worldwide. Poultry is defined as " those species of birds, whether alive or dressed, that have been domesticated to reproduce and grow in captivity and that render products of economic value" (Ensminger, 1992). These species include chicken, turkey, duck, geese, swan and other game birds.

1.3. Importance of poultry

The consumption of poultry dates back to ancient times.Throughout history, its importance as a food source has increased both domestically and commercially. Year round availability of poultry was achieved when the broiler industry was established just after World War II . Scarcity of red meat and milk in Europe resulted in an increase in poultry production in North America to satisfy the demands for an economical source of food protein. As a result, production of poultry meat in the USA increased by 143% between 1940-1961 (Mountney, 1966). Since then, the poultry industry has continued to grow rapidly and dramatic improvements in all phases of production have ensured the availability of fresh and frozen poultry all year around and marketed at prices within the reach of the average consumer. In fact, poultry product consumption has increased at the expense of other sources of dietary protein, namely red meat and fish.

1.3.1. Nutritional quality

Poultry meat is palatable and is a source of essential nutrients. The nutritional importance of poultry meat and its preference by consumers, is due to its high quality protein and its low fat content (Table 1). It also contains all the essential amino acids required for health and development. Poultry is an excellent source of vitamins and minerals such as niacin, riboflavin,thiamine and ascorbic acid and of phosphorus and iron (Mountney,1976). All of these nutritional properties are an important reason why consumers choose to purchase poultry.

Table 1. Comparison of the nutrient content of cooked turkey, chicken and beef.

TYPE OF MEAT	MOISTURE (%)	PROTEIN (%)	FAT (%)
Turkey(white meat)	66.3	29.9	3.2
Chicken(white meat)	64.3	31.9	4.3
Beef	60-61.2	27.4-31.3	6.4 -11.3

Source: Ensminger (1992)

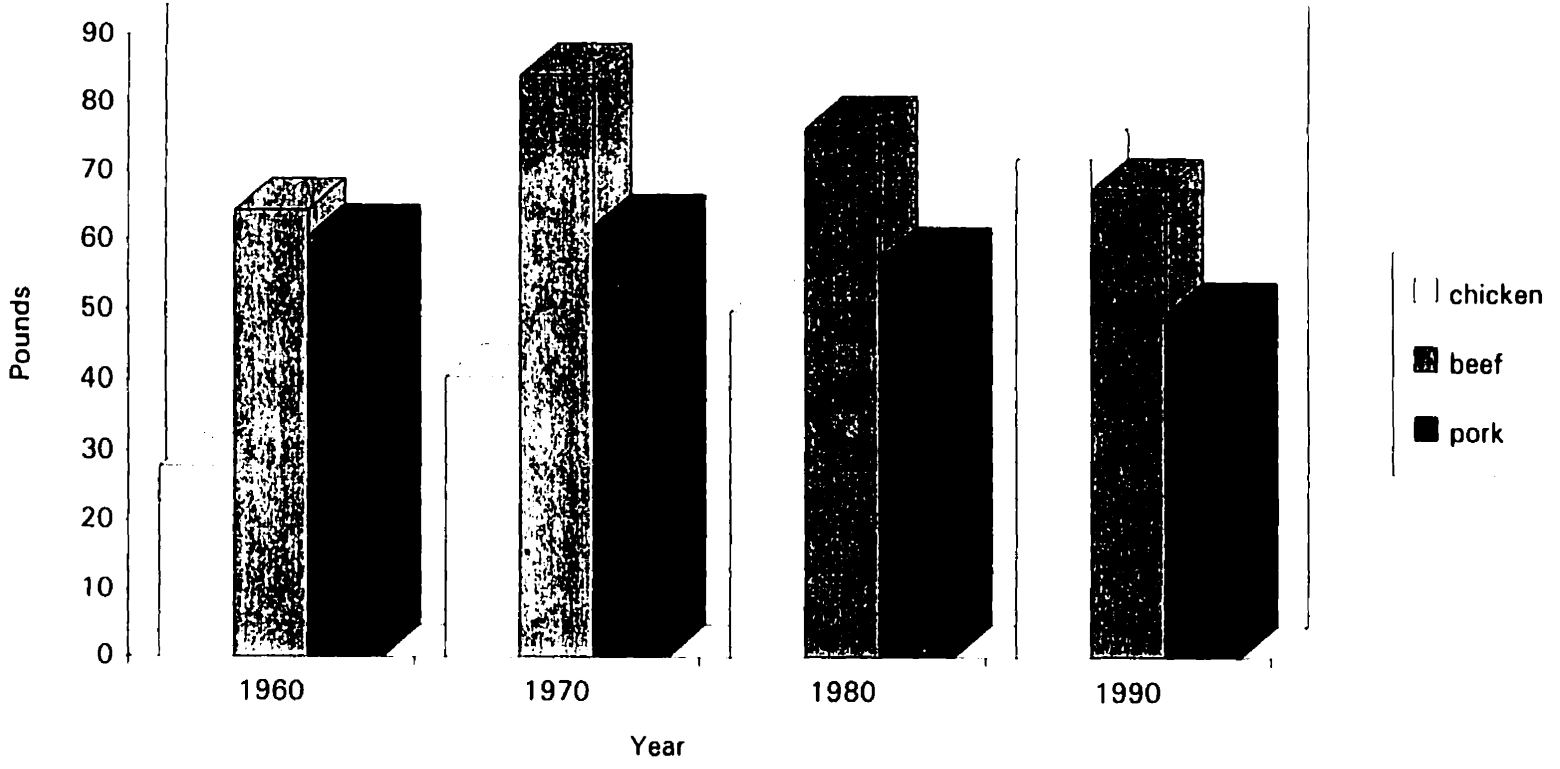
1.3.2. Economical aspect

The continued demand for fresh and frozen poultry has stimulated increased production by the poultry industry.This increase in demand has also resulted in the introduction of new production and marketing technologies to produce inexpensive and wholesome poultry meat. Poultry consumption per person has increased in the last decade and now ranks first or second in consumer expenditure on meats (Figure 1). The broiler industry accounted for over 8.8 billion dollars of the total 15.3 billion of annual income from poultry and eggs in the United States, (Ensminger, 1992).This increase is mainly due to consumer preference for chicken . Its nutritional quality, convenience and versatility to processed products has also created new markets and increased revenue for the poultry industry.

1.3.3. Public health concerns

Poultry are reared in an environment that supports contamination. Bacterial contamination from dust, food handlers, rodents, other animals and air all are important vectors for the spread of pathogenic microorganisms. Under abuse-temperature storage conditions, these microorganisms will multiply rapidly resulting in the deterioration of food and in some cases the transmission of food borne disease. Therefore, preventive measures have been adopted by the poultry industry, both at the farm level and the processing plant, to ensure good manufacturing practices which limit the level of contamination and growth of potential food spoilage and pathogenic bacteria.

Fig. 1. Trends per capita. consumption in poultry and meat



1.4. Processing of poultry

The key means to improving the quality and shelf-life of perishable products, such as poultry, is to minimize their initial microbiological contamination. In a poultry processing plant the major sources of contamination are the plant's environment and other poultry, which transmit both spoilage and pathogenic bacteria. Thus, it is important to improve the flow process at the slaughterhouse by modifying both its physical setup and processing parameters at the different steps in the process flow.

1.4.1. Critical control points of processing

Critical control points are defined as "The location, practice, processing step or procedure where control must be excerised to prevent one or more of the identified hazards" (Baird Parker,1987). This concept of prevention underlies the Hazard Analysis Critical Control Points (HACCP) system. This system provides a structure for anticipating foodborne, microbiological, chemical and physical hazards depending on their associated risk and on effective measures to prevent these hazards from occurring (Notermans et al., 1994). Nevertheless, even with the present advanced commercial processing procedures, contamination of chicken carcasses, with both spoilage and pathogenic bacteria, still occurs at different processing stages (Mead, 1989; Sawaya et al., 1993). The most important stages to monitor include: i) the transportation of poultry to the slaughter house ii) scalding and/or defeathering and iii) chilling.

The transport of poultry from the housing pen to the slaughterhouse is a very crucial stage for limiting the infection of live birds with pathogenic bacteria which would otherwise contribute to carcass contamination and cross-contamination in the plant. Poultry coexists with considerable amounts of microorganisms, dirt, dust and faeces that are present on their feathers, feet and skin (Lillard, 1990). Therefore, special attention should be made to minimize the crowded conditions of live birds during transportation This reduces the contamination of poultry by faeces and dirt, and thus reduces the bacterial count on the feathers and other body parts.

Scalding and defeathering present one of the earliest opportunities for carcass cross- contamination (Lillard et al., 1987). The freshly killed birds are immersed in a scald tank of hot water at temperatures ranging between 51-53.5 ^{0}C to loosen the feathers follicles for later removal by plucking (Mead, 1989). Due to the high scalding temperature, the continous overflow of contaminated scald water and the heat sensitivity of most bacteria, the bacterial load in the scalder remains relatively constant throughout processing. Indeed, low levels of *Salmonella* have been reported in immersion scalders (Lillard, 1990) and the level of *Salmonella* decreased from 19 % to 12% after scalding (Humphrey and Lanning, 1987; Mulder et al., 1978). High scalding temperatures of 60^{0}C are much more effective than soft scalding in reducing cross-contamination. Immersing the carcasses at 60^{0}C reduced the level of *Salmonella* contamination by between two to three fold (Mulder and Domesteijn, 1977). However, high temperature scalding impairs the appearance of the chicken by causing browning and by tightening its flesh. Bailey et al. (1987) also indicated that the shelf-life of broiler chickens was reduced by scalding at temperatures higher than 58^{0}C. The scalding step (58^0 C for 2.5 minutes) damages or removes the protective epidermal tissue exposing the smooth and hydrophilic dermal tissue to contamination from the scald tank, the defeathering machine, and subsequent processing steps (Thomas and McMeeckin, 1980). Microscopic studies have shown a heterogenous population of gram negative bacteria, mainly *Pseudomonas*, *Flavobacterium*, *Acinetobacter* and *Enterobacteriaceae*, within a thin film of water on the skin surface and inside deep skin channels (Bailey et al., 1987; Thomas and McMeeckin, 1980). This film is initially derived from the scald tank and appears to play an important role in the importation of bacteria onto the skin surface of the carcass. Although the number of these psychrotrophic bacteria decreases during scalding, those that survive may multiply very rapidly in subsequent steps and spoil the carcass or cause food borne disease. Under the conditions of hard scalding, (58^{0}C for 2.5 min.) the denatured skin becomes an ideal substrate for the growth of microorganisms (Bailey et al., 1987). Lillard (1984) demonstrated that both flagellated and non-flagellated bacteria contaminated the skin under both soft and hard scalding. Recently, Kim et al. (1992) showed that hard scalding increased the risk of *Salmonella* attaching to the skin and that these bacteria displayed greater heat resistance and tolerance to subsequent processing than did

unattached bacteria. These findings further stress the need for strict control of temperature and humidity during scalding and defeathering to yield a microbiologically sound product.

The USDA Food Safety And Quality Service Regulations (1973) states that "The carcass temperature must be below 40^{0}F (4.4^{0}C) within 4 hrs of slaughter. The poultry industry has been using immersion cold water chiller and air chilling systems to remove heat from carcasses and to prevent the growth of pathogenic and spoilage bacteria. However, chillers have often been implicated as a major site of cross-contamination by psychrotrophic bacteria. Thomas and McMeeckin (1980) reported a significant increase in psychrotrophic bacteria at both the immmersion washing and chilling stages. Mead and Impey (1970) observed high levels of *Clostridium perfringens* in immersion chillers of both turkey and chicken processing plants. Lillard (1990) reported high levels of *Salmonella* on 37 % of broiler carcasses after immersion chilling compared to 14.3% before chilling. In contrast to these findings, Busta et al. (1973) reported no substantial change in the bacterial count of chilled carcasses and Schmitt et al.(1988) reported a marginal increase in bacterial counts during chilling.

The washing effect of the immersion chiller is well documented (Lillard, 1990; Sawaya et al., 1993). A reduction in aerobic bacteria and *Enterobacteriaceae* has been observed, which is consistent with most literature reports (Thomas and McMeeckin, 1980; Lillard, 1990). However, this reduction is less consistently observed with counts of *Salmonella*. In fact, a significant increase in the level of *Salmonella* after chilling was observed, as reported earlier (Lillard, 1990). This suggests that *Salmonella* cross-contamination has occurred through direct contact with contaminated birds or through the water in the immersion chillers. There are numerous opportunities where cross-contamination may occur during processing. Recommendations for modifying the processing method to reduce the bacterial load include a counter-current scalding tank (Bailey et al., 1987), as well as a post-scald rinsing and chiller water chlorination. Lillard (1990) reported that 34 p.p.m. of chlorine and 5 p.p.m. chlorine dioxide in the immersion chillers would result in a significant improvement in the bacterial quality of chicken carcasses. Spraying chicken carcasses or immersing them in hot water, in solutions of various chemicals, including organic acids, phosphates and trisodium phosphate, will also

decrease the intial bacterial load (Lillard et al., 1987). Moreover, plant sanitation should also be stressed so that food handlers are aware of proper hygienic procedures and food handling practices that will improve the shelf-life and quality image of poultry meat.

1.5. Food poisoning outbreaks

Food poisoning outbreaks are defined as" the occurrence of two or more cases of a disease transmitted by a single food. There are two exceptions, botulism and chemical poisoning in which one case constitutes an outbreak" (Centre for Disease Control, CDC, 1990). The main sources of these food poisoning outbreaks in the USA and Canada are red meat, poultry and dairy products. Food poisoning outbreaks continue to be of major concern to producers, consumers and public health officials. Todd (1978) reported that poultry and poultry products ranked first or second in food associated disease in Canada, England, Australia and Wales and fourth in the USA. Bryan (1978) indicated that meat, poultry and poultry products accounted for over 50% of the reported food borne disease outbreaks between 1968 and 1977 in the United States. The etiological agents responsible for the transmission of food borne disease included bacteria, viruses, parasites and chemicals. The Communicable Disease Surveillance Centre (1986) attributed 98% of the total 1400 outbreaks over the period between 1970-1982 in England and Wales to bacterial pathogens. Between 1973 and 1987, the Center for Disease Control reported 7458 outbreaks of which 66% were caused by bacteria (Bean and Griffin, 1990). These statistics show that bacterial pathogens are a consistent public health hazard. To combat this trend, the food industry has adopted long term initiatives to reduce bacterial contamination of food. Evaluation of the factors contributing to food poisoning outbreaks provide important data on which the food industry can develop strategies to create industry wide improvements (Table 2). In addition, educating the consumer on the importance of preservation techniques, such as refrigeration, is also important since there is a tendency by consumers to buy products that are preservative free. These preservation techniques will be referred to later in this review. Outbreaks of food borne disease have also an impact on both the public and on the national economy. Mortality, morbidity and economic losses are the major outcome of such outbreaks. In the USA, 300 deaths were

Table 2. Contributing factors to outbreaks of food poisoning in food service establishments.

Factors	Outbreaks(%)
Inadequate refrigeration	63
Preparing food far in advance of planned service	29
Holding food in warming device at bacterial incubating temperature	27
Infected person touching cooked food	26
Inadequate reheating	25
Inadequate cleaning of kitchen equipment	9
Use of leftovers	7
Cross Contamination	6
Inadequate cooking or heating processes	5
Storing low acid food in toxic containers	4
Others	6

* The percentage value will exceed 100 because more than one factor frequently occurs in outbreaks
** Based on 235 outbreaks that occured between 1973-1976
*** Source: Bryan (1980)

reported from food borne disease during the period 1973 and 1984. Eight deaths were caused by the consumption of poultry products (Bean and Griffin, 1990). With respect to morbidity, 12.6 million cases are estimated to occur annually in the USA (Todd, 1989a) compared to 2.2 million in Canada (Todd, 1989b). The symptoms associated with food poisoning include diarrhoea, nausea, vomitting and fever. Food borne disease also results in economic losses. Todd (1989c) did extensive work on the economics of food borne illness in the US and Canada. He estimated annual costs in terms of cost production time and treatment of patients to be $ 1.1 billion for Canada and $ 7 billion in the USA. The cost of such outbreaks to different food establishments in Canada and elsewhere is illustrated in Table 3.

1.5.1. Food borne pathogens associated with poultry

Poultry and poultry products are often implicated in outbreaks of bacterial food poisoning. *Clostridium botulinum, Staphylococcus aureus, Campylobacter jejuni, Clostridium perfringens, Listeria monocytogenes and Salmonella spp.*, have all been identified as responsible for poultry associated food borne illness (Bean and Griffin, 1990). *Clostridium botulinum* is a gram-positive spore forming anaerobe which grows well in the absence of oxygen (Farber,1989). The strains of *C. botulinum* can be classified according to the toxin they produce into seven groups designated A through G. *C. botulinum* types A, B and E spores are responsible for botulism in humans. Botulism results from consumption of neurotoxin as the bacteria grow in food. Low acid, high proteinaceous foods, such as meat and fish have been shown to support toxin production by *C. botulinum* types A, B and E (Lambert et al., 1991; Post et al., 1985). However, the rate of botulism associated with poultry is very low. Only 1 outbreak was reported from 1973 to 1987 (Bean and Griffin, 1990). Recently, safety concerns have been raised about the potential of *C. botulinum* to grow and produce toxin in meat products packaged under modified atmosphere packaging (MAP) conditions (Lambert et al.,1992b). The MAP environment inhibits the growth of the normal aeorbic spoilage microorganisms due to the reduced level of oxygen and the antimicrobial effect of elevated levels of carbon dioxide (Blickstand and Mollin, 1983). Studies have shown that carbon dioxide also

Table 3. Cost associated with food borne outbreaks at food processing establishments.

Etiologic agent	Year	Country	Establishment	Cost per incident($)
	1970-1971	Sweden	Candy	89,640
Salmonella spp.	1981	Scotland	Dairy	551,865
other than typhi	1976	USA	Cheese processor	271,080
	1974	Australia	Chicken processor	1,897,136
	1978	Canada	Bakery	4,473,355
Staphylococcus	1985	Canada	Pasta processor	12,917
aureus	1965	USA	Cheese processor	529,632
	1979	Brazil	Meat canner	2,967,516
	1977	Canada	Cheese processor	875,836
Listeria monocytogenes	1983	USA	Dairy	565,582
Clostridium	1978	USA	Bean canner	8,859,240
botulinum	1978	USA	Fish canner	6,266,160
	1982	USA	Fish canner	160,106,380
	1963	USA	Fish canner	175,670,640

Source: Todd (1987)

stimulates the germination of *Clostridium* spores and enhances its growth under anaerobic packaging conditions (Enfors and Mollin, 1978). Furthermore, non-proteolytic strains of *C. botulinum* can grow at temperatures as low as 4°C. Therefore, the relationship between the inhibition of spoilage microorganisms and the growth of *C. botulinum* is a critical factor in the use of modified atmosphere for shelf-life extension of muscle foods and continues to be a subject of extensive investigation (Hintlian and Hotchkiss, 1987, Hotchkiss, 1988).

Staphylococcus aureus is another major food poisoning microorganism frequently involved in poultry associated food borne illnesses. It is a gram-positive, facultative anaerobic coccus with a temperature range from 7 to 45°C (Farber, 1989). Animal carcasses and cuts of meat become contaminated with *St. aureus* from noses, skin or infected lesions of food handlers (Bryan, 1980). The microorganism is also capable of producing different heat stable enterotoxins on the surface of poultry products (Bergdoll, 1989). These entertoxins are high molecular weight proteins and are produced in the lag phase of bacterial growth (Genigeorgis, 1989). *St. aureus*, grown on agar plates stored in 100 % CO_2 at 10°C, showed a decrease in bacterial counts when compared to plates stored in air (Mollin, 1983). While *St. aureus* is a facultative anaerobe, it grows best in the presence of oxygen or reduced low levels of CO_2. Cooking at 60°C will kill the organism but its entertoxin will not be destroyed. Numerous reports have cited the presence of *St. aureus* in poultry and poultry products (Genigeorgis, 1989; Mead, 1989) High levels of *St. aureus* in poultry indicates poor hygiene and improper storage conditions. Nevertheless, this microorganism is a poor competitor and does not grow well in the presence of psychrotrophic bacteria found in poultry.

Campylobacter jejuni has been recognized as the most common cause of bacterial diarrhoeal disease in humans (Griffiths and Park, 1990). It is a gram-negative, spiral-shaped, microaerophilic bacterium that belongs to the *Spirillaceae* family (Farber, 1989) It grows optimally in an atmosphere containing 3-5 % O_2 and 5-10 % CO_2 (Farber, 1989) Fresh poultry harbours detectable levels of *Campylobacter* with the caecum being the principal site (Stern et al., 1984; Fricker and Park, 1989). This microorganism will survive commercial processing and packaging procedures, such as modified atmosphere packaging (MAP), chlorination and refrigeration but not freezing (Christopher et al.,

1982; Stern et al., 1986). The microorganism is also sensitive to U.V., and gamma radiation where a 1 Kilo gray dose of gamma irradiation (1 kGy) was sufficient to destroy *C.jejuni*, in both culture medium and in chicken pasta (Tarjan, 1985).

Clostridium perfringens is an anaerobic, spore-forming, gram positive bacterium, that has been epidemiologically associated with foods such as meat, poultry, fish. Although this organism is considered to be an obligate anaerobe, certain strains can survive in low levels of oxygen (Hayes, 1993). This organism grows at temperatures from 10 to 52°C, the range of improper handling and storage (Farber, 1989). Low temperatures have been successful in reducing the growth of *C. perfringens* significantly. Food contaminated with *C. perfringens* will result in mild gastroenteritis. The symptoms include diarrhoea, abdominal cramps, nausea and fever (Farber, 1989).

Listeria monocytogenes is a gram-positive, rod shaped, motile bacterium that has been isolated from various foods including cheese, milk and fresh and processed meat. Great concerns have been expressed about poultry and meat as major vehicles of transmission of listeriosis to humans. However, the level of *Listeria* in raw poultry is low and raw poultry is rarely implicated in food poisoning outbreaks due to this pathogen (Bean and Griffin, 1990). In accordance with other psychrotrophic bacteria, *L. monocytogenes* exhibits a wide temperature range from 1 to 45°C with an optimum temperature of 35 - 37°C (Farber, 1989). Listeriosis is the disease contracted by the ingestion of contaminated food and water. Although the minimum infectious dose is still unknown a high number of viable cells ($>10^6$ CFU/g) is required to cause illness in healthy adults (Farber,1989). High risk groups include pregnant women and their fetuses, the elderly and immunocompromised individuals who will show clinical symptoms of listeriosis at around 10^3-10^4 CFU/g (Farber, 1989). The importance of *Listeria* as a causative food borne agent stems from the following i) the ability of the microorganism to withstand the minimum pasteurization heat treatment ii) the ubiquity of *Listeria* in the environment iii) the ability of the microorganism to grow in extended shelf-life refrigerated products at temperatures as low as 1°C iv) high mortality rates as high as 30% (Farber and Losos, 1988).

1.6. *Salmonella* and their common isolates

Non-typhoid *Salmonella* species continue to be the most reported foodborne disease with incidence rates varying from 17.4 to 187 cases per 100,000 population and an estimated number of 2 million cases per year worldwide (D'Aoust, 1991; Silliker, 1982).

Salmonella is a genus of the family *Enterobacteriaceae* which also contain other genera such as *Escherichia coli, Shigella* and *Proteus*. *Salmonella* species are high temperature mesophiles that grow at temperatures from 5.2-45°C (Farber, 1989). These organisms are ubiquitous in nature and have been isolated from several sources including sewage, rodents, animal feed and dust (Mackenzie and Bains, 1976; Oosterom, 1991).

Salmonellosis is the collective term used for the gastro-intestinal disease resulting from the infection by a species of *Salmonella*. Non-typhoid *Salmonella* such as *S. typhimurium* and *S. enteritidis* account for intestinal infection in both humans and animals (Suzuki, 1994).The mode of infection involves the bacterial invasion of the mucosa and the secretion of a heat labile enterotoxin that precipitates the effusion of water and electrolytes into the intestinal lumen (D'Aoust, 1990). The symptoms associated with this disease include abdominal pain, nausea, fever and watery diarrhoea (D'Aoust, 1990).

The levels of *Salmonella* species in poultry and poultry products in food poisoning outbreaks from these products have been studied extensively. Sawaya et al. (1993) reported high counts of *Enterobacteriaceae* in conventionally packed broiler carcasses stored at 7 and 9°C. *Salmonella spp.* represented 15 % of the total enteric isolates. Waldroup (1993) reported 21.8 % positive *Salmonella* isolates from pre-chilled broiler carcasses while Izat et al. (1991) reported 17-50 % positive strains of *Salmonella* from retail chicken.

Salmonella species consists of 2324 serovars with less than 10% being isolated from humans and animals. Kelterborn (1979) reported that the most common serovars isolated between 1934 and 1975 in 109 countries were *S. typhimurium, S. enteritidis, S.infantis, S.heidelberg, S.newport and S. dublin*. Both *S. typhimurium* and *S.enteritidis* ranked first and second as the most common isolated *Salmonella* serotypes (Tauxe,1991). However, recent increases in *Salmonella* outbreaks have been attributed mainly to *S.*

enteritidis (Table 4). Between 1973-1987, a four fold increase in outbreaks due to *S. enteritidis* has been reported in the United States (Bean and Griffin, 1990). In England and Wales, *S. enteritidis* accounted for 46 % of the total outbreaks caused by *Salmonella* species in 1988, and was the most prevalent serovar isolated from humans (Public Health Report Service, 1989; Rodriguez et al., 1990). In Canada, the prevalence of this microorganism is less dramatic, with incidence rates varying from 4.2 and 9.2 % between 1976 and 1989, and increasing to 12.5 % in 1991 (Poppe, 1994). *S. enteritidis* is an invasive microorganism with a pathogenicity for humans and animals. This organism has been commonly isolated from animal feed but rarely from poultry (Faddoul and Fellows, 1966). Recent studies have shown an increase in the contamination of poultry flocks with *S. enteritidis* (Dressen et al., 1992 ; Hopper and Mawer, 1988). The epidemiology of *S. enteritidis* has usually been associated with the consumption of contaminated eggs and egg products (Hedberg et al.,1993). Eggs are infected by penetration of the egg shell by faecal material containing the microorganism or by transovarian infection which normally leads to vertical transmission (Suzuki,1994). Hopper and Mawer (1988) isolated this organism from the internal organs of naturally infected chicken flocks. As *S. enteritidis* can be spread transovarially, it can be introduced into chicken flocks and then passed on to the offspring. Studies on the pathogenicity of *S. enteritidis* have shown it to cause clinical symptoms in young chickens leading to either high mortality rates or stunted growth, whereas infection in adults tends to show no clinical abnormalities (Humphrey, 1990). Thus, these asymptomic carriers present a public health hazard by spreading the infection through the breeder and commercial egg laying flocks resulting in the contamination of poultry meat and egg products.

Table 4. The level and incidence of S. enteritidis in poultry and poultry products.

Author	Year	Country	Food
Perales and Audicana	1989	Spain	Eggs
Van De Giessen et al.	1994	Netherlands	Eggs
Humphrey et al.	1989	U.K	Eggs
Poppe	1994	Canada	Layer flocks

Table 5. Summary of food borne pathogens associasted with poultry.

Miccroorganism	Characteristics of organism	Temp. Range For Growth	Symptoms Of Food Poisoning
Clostridium botulinum	Gram + anaerobic spore forming rod	3.3-45° C	Neuromuscular paralysis
Staphylococcus aureus	Gram + facultative anaerobic cocci	7-46° C	Nausea/vomitting
Listeria monocytogenes	Gram + pleomorphic rod	1-45° C	Meningitis,septicemia and gastrointestinal symptoms
Clostridium perfringens	Gram + anaerobic spore forming rod	10-52° C	Diarrhea
Salmonella spp.	Gram - non spore forming rod	5.2-45° C	Gastrointestinal symptoms

Source: Farber(1991)

1.7. Growth factors of *Salmonella*

Microbial growth in food is affected by a number of environmental factors such as temperature, pH, water activity (a_w), composition of the atmosphere surrounding the product and the presence of preservatives. Growth is controlled when one or more of these parameters is at a level inhibitory to the growth of *Salmonella* species. In most cases, these inhibitory factors act in combination to give a synergistic effect. These inhibitory factors have been called hurdles and their combined effect on bacterial growth underlies the hurdle concept developed by Leistner and Rodel (1976). This concept illustrates the complex interaction of inhibitory factors at levels where one factor fails to control microbial spoilage under poor food storage conditions.

1.7.1. Storage temperature

Storage temperature can be regarded as the main factor affecting the rate of microbial growth. *Salmonella* species are mesophilic microorganisms that can grow optimally at temperatures between 30 and 45°C but grow slowly in the 5 - 15 °C temperature range. Early studies demonstrated an extension of the lag phase and the generation time at decreased temperature. Smith (1985) observed an increase in the generation time from 9.65 hrs at 10°C to 0.43 hrs at 35°C in minced lamb inoculated with *S. typhimurium*. In a subsequent study, a mixed inoculum of *Salmonella* serovars in minced beef showed an increase in generation time from 4 hrs at 35°C to 10 hrs at 10°C. This increase in the generation time of *Salmonella* could be attributed to the competitive growth of the saprophytic bacteria that have a much lower optimum temperature than *Salmonella* and which thus can grow more rapidly than *Salmonella*.

The minimum temperature at which *Salmonella* can grow is a function of several conditions such as the growth medium, temperature, time and the optimum growth of the individual strain. Angelotti et al. (1961) examined the growth rate of a mixture of 3 *Salmonella* serovars, namely *S. thompson*, *S. stanley* and *S. infantis* in three food products. These species did not grow within 5d in custard and ham at 4-10°C. However, in chicken, growth of all three species occurred after 4d of incubation at 6.7°C. The

minimum growth temperature of *Salmonella* is also influenced by the incubation period. Catsaras and Grebot (1984) reported a one log increase of *S. typhimurium* (initial inoculum level 10^3 CFU/g) in minced meat, followed by a decrease in cell numbers throughout the remainder of the storage period. Comparable results were also reported by Farell and Upton (1978) who observed a significant increase of *S. typhimurium* in bacon during the first 48 hrs., and Baker et al. (1986), who detected growth of inoculated *S. typhimurium* in minced chicken at the level of 10^4 CFU/g within 2-6d of incubation at 2^0C. Recent reports on the growth of *Salmonella* serovars at low refrigerated temperatures are of concern.The psychrotrophic behaviour of *Salmonella* at chilled temperature clearly stresses the need to apply additional hurdles to control the growth of this pathogen in food stored at low temperatures i.e., $< 4^0$C.

1.7.2. pH

Generally, as the storage temperature deviates to the range permitting the growth of mesophilic bacteria, the impact of acidity alone or in combination with other inhibitory factors becomes increasingly important.The microbial growth of bacteria is primarily affected by the pH of the food. The minimum pH allowing the growth of *Salmonella* in food is between 6.5 and 7.5 but growth of *Salmonella* has been observed to occur at pH values as low as 4.05 (Chung and Goepfert, 1970). The behaviour of *Salmonella* at low pH is a function of the type of food, the incubation period, the level of inoculum, and the incubation temperature (Chung and Goepfert, 1970). In a recent study, Perales and Garcia (1990) demonstrated the survival of inoculated *S. enteritidis* (10^6 CFU/ml) in mayonnaise incubated at 4, 24, and 35^0C for five days. They concluded that the growth of *Salmonella* depended on the type of acidulant used and storage temperature. More recently, Humphrey et al. (1993) demonstrated that alkaline conditions can induce heat resistance in certain bacteria. In a growth study of *S. enteritidis* in a liquid medium at pH 9-9.5, they observed a significant increase in the heat resistance at temperatures of 25^0C, 30 and 37^0C. These findings causes great concern for eggborne outbreaks as egg albumen tends to be alkaline in nature (pH 9-9.5).

1.7.3. Water activity (A_w)

It has been established that decreasing water activity (a_w) has a variable effect on the growth of pathogenic microorganisms. Many microorganisms are highly sensitive to reduced a_w and have a maximum growth rate between a_w 0.99-0.995. Moreover, the minimum a_w for the growth of *Salmonella* depends on a number of factors including the strain, pH, temperature and the type of solute.

The reduction of the a_w has shown to have a preservative effect on *Salmonella spp.* which tend to have greater thermal resistance at low a_w. Consequently, decreasing the water activity tends to increase the decimal reduction time. Li and Torres (1993) concluded that the growth rate of *S. typhimurium* decreased as the temperature and a_w was reduced. In an investigation on the effect of different solutes on the lag phase of *S. typhimurium*, Li and Torres (1993) also demonstrated that sucrose was the most inhibitory of all solutes examined whereas glycerol was the least inhibitory. Indeed, glycerol has been shown to allow the growth of *S. typhimurium* at low a_w compared to sucrose and sodium chloride (NaCl). This is due to the fact that glycerol functions as a compatible solute capable of permeating the bacterial cell and does not cause osmotic stress like most other solutes.

1.7.4. Gaseous atmosphere

Since most spoilage bacteria are aerobic, packaging fresh foods in a reduced O_2 and enriched CO_2 environment may be used to inhibit their growth. This is the principle behind vacuum packaging or gas packaging where air is replaced by other gases, usually nitrogen and carbon dioxide. By modifying the gaseous environment, common psychrotrophic spoilage bacteria are retarded by the low concentration of oxygen and high concentration of CO_2. This synergistic effect between elevated levels of CO_2 and the reduced levels of O_2 have also been shown to inhibit the growth of *Salmonella*. Baker et al. (1986) found that an atmosphere of 80% CO_2 (balance air) inhibited the growth of *Salmonella* in naturally contaminated chicken held at 2-13^{0}C for 18 d. Gray et al. (1984) observed the growth of *S. entertidis* in chicken thighs in both modified atmospheres examined (20%CO_2 : 80%O_2 ; 60%CO_2: 40%O_2) but inhibition occurred when packaged in 100% CO_2. These findings are consistent with earlier reports on the effectiveness of high concentrations of CO_2 at temperatures below the optimum growth of *Salmonella*. However, Gill and Delacy (1991) observed no inhibitory effect on the growth of *S. typhimurium* in vacuum packaged beef held at 6^{0}C. Therefore, modified atmosphere alone is insufficient to provide a safe product. Furthermore, as the minimum growth temperature of *Salmonella* is 5.2^{0}C, the importance of using modified atmosphere in conjunction with low temperature refrigeration is apparent.

1.7.5. Chemical preservatives

Chemical preservatives have been one of the most effective treatment methods for the inhibition of pathogens. Chemical preservatives used in the poultry industry include chlorine, chlorine dioxide, ozone, trisodium phosphate and organic acids, such as potassium sorbate. These preservatives are used in spray washing, rinsing, and dipping applications at processing plants. For example, low levels of organic acids have been used very effectively to reduce the number of pathogenic microorganisms in many foods. These acids include sorbic acid, lactic acid and citric acid. Although these acids are "generally regarded as safe" (GRAS) food additives, the amounts permitted in food are

regulated by legislation and should have a negligible effect on food or consumers. These acids act by lowering the pH of the food. In their undissociated form, the acids penetrate the bacterial cell, releasing hydrogen ions and thus eliminating the proton gradient across the cell membrane. Therefore, they are more effective at acid pH levels. Several reports have shown the inhibitory effect of organic acids on *Salmonella* counts in poultry. Robach and Ivey (1978) found that a 10 % sorbate dip reduced the number of *Salmonella* in chicken breasts. Gray et al. (1984) reported that a 5% potassium sorbate dip was effective in inhibiting *S. entertidis* inoculated onto fresh chicken thighs. These researchers also found out that the level of *S.entertidis* was reduced significally when a 2.5 % sorbate dip was used in conjunction with 100 % CO_2 .

1.7.6. Chitosan

Chitosan is a natural biodegradable polymer currently manufactured by deacetylating chitin through an alkaline treatment. Chitin is a naturally-occuring linear polymer of acetylglucosamine residues linked together by a ß-1- 4 glycosidic linkage, and is obtained from the outershell of crustaceans (e.g., shrimps and crabs). Chitin is widely distributed in nature and Ye (1987) estimated the world production of chitin and its derivative to be over 10 tonnes annually. The commercial availability of chitosan has increased its potential for use in fields such as medicine, agriculture, biotechnology, water and juice clarification and waste management (Table 6).

Chitosan is also a promising antimicrobial agent, although, it has not been approved for use as a food preservative by the FDA. It is effective in inhibiting the growth of both spoilage and food borne pathogens. In Japan, several patents involving the use of chitosan as a food preservative have been approved. Wang (1992) studied the bacteriostatic effect of chitosan following the exposure of five food borne pathogens to five different concentrations (0.5, 1.0, 1.5, 2.0, and 2.5% w/w) of chitosan in a model broth system. This study confirmed that the bacteriostatic effect of chitosan increased with increasing concentration. Moreover, the inhibitory effect was greatly influenced by the system's pH with the antimicrobial effect being greater at pH 5.5 than at pH 6.5. Nevertheless, the antimicrobial action of chitosan varied greatly among the tested bacteria.

Among the five bacteria studied, *Y. enterocolitica* showed the highest resistance to the various chitosan concentrations, with *E. coli* and *S. typhimurium*, with *St. aureus* being the most sensitive. No inhibition of *L. monocytogenes* occurred. Recently, Darmadji and Izumimoto (1994) studied the bacteriostatic effect of chitosan in different substrates. In liquid medium, chitosan (0.01 %) inhibited the growth of *B. subtilis*, *E. coli*, *P. fragi*, and *St. aureus*. Furthermore, 0.5-1.0 % chitosan inhibited spoilage bacteria and extended the shelf-life of fresh meat in meat stored at 4°C for 10 days (Darmadjia and Izumimoto, 1994).

1.8. Preservative techniques

Poultry meat is classified as a potentially hazardous food because when it is contaminated or mishandled, it supports the growth of foodborne microorganisms. Thus, the food industry has adopted numerous treatments to ensure the safety and quality of the food product. These treatments include refrigeration, freezing, thermal processing, irradiation, chemical preservatives and modified atmosphere packaging.

1.8.1. Refrigeration

Mechanical refrigeration is a process used for the short term preservation of many perishable and semi-perishable foods such as fresh and processed meat, dairy products and fresh fruits and vegetables. Storage under refrigerated conditions is carried out at temperatures ranging between 1°C and 5°C without inducing any physical change to the food. These temperatures will prolong the products' shelf-life by delaying spoilage and slowing down the growth of foodborne pathogens.

The safety of refrigerated food deserves attention because of the emergence of minimally processed foods and potential contamination by psychrotrophic foodborne pathogens. Psychrotrophic microorganisms have an optimal growth temperature range between 25 and 30 °C but can grow between 0 and 7°C. Examples include *C. botulinum* type E, *Y. enterocolitica*, Enterotoxigenic *E. coli*, *L. monocytogenes*, and *Aeromonas hydrophilia*. Another concern is that any temperature abuse due to improper temperature

control will facilitate the growth of food borne mesophiles that have a minimum growth temperature between 5 and 12°C such as *Salmonella spp.* and *St. aureus*. Thus, the use of refrigeration alone is no longer sufficient for preventing the presence of pathogens in refrigerated foods and the incorporation of additional treatments prior to refrigeration has become necessary. Such treatments include heating, irradiation, salting, modified atmosphere packaging and acidification.

1.8.2. Freezing

Freezing is the most commonly used process for the long term preservation of food. Properly conducted freezing is generally carried out at -18°C (0°F) both to minimize food spoilage and to inactivate food borne pathogens. While some bacteria contained in the food are destroyed as a result of freezing, El Kest and Marth (1991) suggested that sublethal physical or metabolic injury occurs first. Thermal shock (cold shock) is a phenomenon often experienced by some microorganisms when the temperature decreases suddenly below their optimum growth temperature. This is mainly observed in gram negative bacteria such as *E. coli, Enterobacter aerogenes, S. typhimurium, and Serratia marcescens* (El Kest and Marth, 1991).This type of inactivation only occurs under certain conditions such as rapid cooling or when the cells are in the exponential growth phase. Microorganisms can also be injured mechanically by the formation of ice crystals either inside or outside the microbial cell. Rapid freezing will encourage the formation of small scattered intracellular ice crystals resulting in minimal damage to the bacterial cell. Slow freezing, on the otherhand, results in the formation of large extracellular ice crystals. However, although slow freezing is more lethal to bacterial cells, it is less effective in preserving the product's quality. Recent experimental results have shown that crystallization occurs primarily in the extracellular fluid, regardless of the rate of freezing, and that the extracellular ice formation indirectly affects microbial growth (El Kest and Marth, 1991). Thus, following the onset of extracellular crystallization, the intracellular water will migrate in response to the vapor pressure differential. This extracellular freezing, and the resultant intracellular dehydration increases the solute concentration in the unfrozen phase. Such an increase will damage the membranes of the bacterial cell by

altering the bacterial permeability characteristics and will also damage the enzymatic and metabolic processes. It is important to note that *Salmonella* species may survive freezing in an injured state. Upon thawing, the microorganisms may undergo repair and, if the poultry is not cooked properly, it may still be a potential vehicle of *Salmonella* food poisoning.

1.8.3. Thermal processing

Thermal processing has received renewed attention in recent years due to the emergence of food borne pathogens of unknown or increased thermal resistance. The aim of thermal processing is to ensure commercial sterility while maintaining the organoleptic acceptability and microbial quality of the product. For low acid foods (pH>4.6), the destruction of spores of *Clostridium botulinum* is generally accepted as the minimum standards for processing. Canned perishable meat (pH<4.6), however, usually receives mild heat treatment known as pasteurization because of the detrimental effect of high temperature on the product's quality. *Salmonella* spp. have a D_{10} value of 0.1-0.2 min at 65.5°C (International Commission on Microbiological Specification of Foods, ICMSF, 1980). Therefore, pasteurization will inactivate all pathogens including *Salmonella* spp., but several spore-forming thermophiles can survive this heat treatment. Under temperature abused conditions, these spores will germinate and outgrow, thereby spoiling the food product.

1.8.4. Irradiation

Treatment of poultry meat with low doses of ionized radiation is very effective in reducing the number of food borne pathogens and in extending its shelf-life while maintaining the product's nutritive and sensory qualities. This process involves exposing the food to an energy source in the form of gamma rays, X-rays or a beam of high-energy electrons. These rays penetrate the produce, damaging the genetic material of all living cells including bacteria, so that they cannot survive or multiply.

Irradiation at doses up to three kilogray (kGy) are sufficient to eliminate most

pathogens found in meat (Table 7). Low doses of irradiation will be more effective against pathogenic microorganisms when carried out at refrigerated temperatures. *Salmonella* species are sensitve to irradiation with a D_{10} value of 0.38-0.77 at 2°C (Thayer, 1993). Furthermore, Thayer (1995) reported that an irradiation dose of 1.5-3.0 kGy would inactivate between 10^2-10^4 CFU/g of *Salmonella*. Thayer et al. (1995) reported that a dose of 1.5 kGy would be sufficient to destroy ~10^3 CFU/g of *S. enteritidis* on mechanically deboned chicken meat. Thus, low doses of ionized irradiation in the range of 2-5 kGy will significantly reduce the level of most *Salmonella* species as well as most food borne pathogens

Food irradiation is currently approved for certain plant and animal products in over 30 countries (Table 8). In Canada, irradiation may be used to prolong the shelf-life, disinfect or decontaminate products including potatoes and onions (0.15 kGy); wheat, flour and whole wheat flour (0.75 kGy); and spices and dehydrated seasoning preparations (10 kGy). In the USA, products cleared for irradiation include wheat and wheat flour, potatoes, spices and dry vegetable seasonings, dry or dehydrated enzyme preparations, pork carcasses, and fresh fruits. Recently, the FDA approved a petition submitted by the Food Safety and Inspection Service (FSIS) and the radiation processing industry to permit the irradiation of poultry. Under the USDA's final ruling of September 21st 1992, poultry products which may be irradiated include: fresh and frozen uncooked whole carcasses, and parts including mechanically-separated poultry products.Poultry products with added ingredients or that have been cooked or cured may not be irradiated. The regulatory agencies have permitted an absorbed dose ranging between 1.5-3.0 kGy for reducing the potential of foodborne illness from poultry. Although this technology produces a microbiologically safe product, consumers' objection to irradiated foods may limit the commercial application of this technology and sales of irradiated products.

Table 6 Potential applications of chitosan.

Field	Key Properties	Key Uses
Agriculture	Flocculating agent and increase crop yield	Remove proteinaceous waste from animal feed and seed coating
Clarification and waste management	Non toxic cationic flocculent and metal chelator	Treatment of waste water and detoxification of hazardous waste
Cosmetic/ personal care	Non toxic cationic polymer and excellent tactile properties	Skin care and hair treatment
Food	Flocculent agent and metal chelator	Wine and juice clarification and protective fruit coating
Pharmeuctical	Biocompatibility, non toxic and hemostatic	Wound dressing and hypocholesterolemic agent

Source : Sandford (1989)

Table 7 D10 value of irradiation destruction of food borne pathogens in selected foods

Type of bacteria	Substrate	D10 Value (kGy)	Radiation Temp. (°C)
Aeormonas hydrophila	Beef	0.14-0.19	2 °C
Campylobacter jejuni	Ground turkey	0.19	0-5° C
Escherichia coli	Ground beef	0.27	5° C
Listeria monocytogenes	Chicken	0.77	2-4° C
Salmonella spp	Mechanically deboned chicken	0.38-0.77	2° C
Staphylococcus aureus	Mechanically deboned chicken	0.36	0° C

Source: Thayer (1993)

Table 8. Commercial applicaction of food irradiation in various countries.

Country	Type Of Food
Canada	Potatoes, onions, wheat, wheat flour, and spices
USA	Poultry, potatoes, onions, wheat, wheat flour, and spices
France	Poultry, strawberry, frog legs, spices and cereals
Italy	Potatoes , onions and garlic.
Germany	Onions
Japan	Potatoes
Netherland	Potatoes, onions, poultry, and mushrooms
England	Diets for hospital patients
Belgium	Deshelled and powdered eggs, poultry and rice

1.8.5. Hydrostatic pressure

The application of high hydrostatic pressure for the preservation of food is yet another process which is being re-evaluated. High hydrostatic pressure (HP) is a non-thermal process that involves exposing the food to high isostatic pressure while maintaining the product's sensory quality. As an alternative to thermal processing, HP offers potential improvements in the quality of specific food products. In HP, the food is aseptically filled, sealed in plastic containers, and then exposed to high pressures applied through a pressure-transfering medium (Mertens and Knorr, 1992). The pressure can be generated by direct or indirect compression, or by heating the pressure medium, which is usually water. Unlike thermal processing, the pressure is uniformly transmitted throughout the food, regardless of the size of the container. This processing method was first introduced in 1899, where Hite et al. (1914) focussed on the effect of hydrostatic pressure on milk and milk products among other foods. More recently, this technique has been commercialized in Japan, where pressure-preserved foods such as fruit yoghurt, fruit jellies, salad dressing and fruit sauces are available.

High hydrostatic pressure has a variety of effects on the food, depending on the magnitude of the pressure applied. These include: enzyme inactivation (Fukuda and Kunugi, 1985), protein denaturation (Bridgman, 1914), improving meat's textural quality (Ohmiri et al., 1991), maintaining organoleptic quality, especially flavor and texture and inactivating microorganisms of meat (Hoover et al., 1989).

Several researchers have examined HP to inactivate microorganisms and extend the shelf-life of food products. As with thermal processing, HP's effect on the bacterial cells result from a combination of factors, including changes in the cellular morphology, genetic mechanism and biochemical reactions (Hoover et al., 1989). Other factors that influence the growth of bacteria are the duration of the pressure treatment (Sale et al., 1970), the food's composition, the stage of microbial growth, and the type of microorganisms present (Zobell, 1970). In general, the incidence of microbial death increases with time and pressure. The pressures applied in food preservation range from 500 to 700 MPa, which is usually sufficient for destroying the vegetative cells of bacteria, yeast and molds. However, bacterial spores are much more resistant and their inactivation

requires the combined effect of higher pressure and temperature. The acidity of the food also plays an important role in the destruction of microorganisms by high pressure. Hite et al. (1914) observed that a pressure of 2,400-3000 atm had a greater effect on bacterial cells at acid pH than a neutral one. One explanation is that the acidity may have affected the bacteria's barosensitivity and hindered the outgrowth of spores. Zobell (1970) found that bacteria in their log phase were more sensitive to high pressure than cells in their stationary phase. Shigehisa et al. (1991) inoculated pork slurries with various microorganisms before subjecting them to pressures of 1000-6000 atm at 25°C and for 10 minutes. These microorganisms included *B. cereus, C. jejuni, Candida utilius, E. coli, Micrococcus luteus, P. aeruginosa, Sacchromyces cerevisiae, S. typhimurium, St. aureus, S. faecalis,* and *Y. enterocolitica.* Results showed that the gram-negative bacteria were more sensitive to high pressures than gram-positive bacteria. Moreover, all microorganisms examined (initial load 10^4 CFU/ml) were destroyed at pressure treatments of 3000-6000 atm except for the spores of *B. cereus.* The microorganisms, *C. jejuni, P. aeruginosa, S. typhimurium,* and *Y. enterocolitica* were destroyed at pressures > 3000 atm., while *E. coli, S. cerevisiae, C. utilius* were destroyed above 4000 atm. However, *M. luteus, St. aureus and S. faecalis* required higher pressures i.e., 6000 atm. for total destruction.

1.9. Alternatives - modified atmosphere packaging

Increasing consumer demand for fresh refrigerated food has renewed interest in modified atmosphere packaging (MAP) to extend the shelf-life of perishable products (Table 9). The term MAP refers to storage conditions where the atmospheric gas concentration is altered from that of air (air ca.= < 0.1 % CO_2 , 21 % O_2 , 78 % N_2).In MAP, the package atmosphere surrounding the product is modified to an initial new composition which then tends to alter because of tissue respiration, microbial action during storage or film permeability. MAP was defined by Young et al. (1988) as " the enclosure of food products in a high gas barrier material in which the gaseous environment has been changed or modified to slow down respiration, reduce microbial growth and retard enzymatic spoilage with the intent of extending shelf-life." Whereas in MAP the headspace gas composition changes during the storage period, controlled atmosphere storage (CAS) involves more precise gas compositional control.Thus, CAS is frequently used commercially for the shipment of chilled meat and the bulk storage of commodities, especially fruit and vegetables. Several methods have been used to modify the atmosphere within packages These include: i) vacuum packaging, ii) gas packaging, iii) oxygen absorbents, and iv) ethanol vapors.

1.9.1. Vacuum packaging

Vacuum packaging has been increasingly used to extend the shelf-life of poultry meat. Vacuum packaging is a form of MAP where the product is first placed in a package of polymeric film of low oxygen permeability ($<5ccm^{-1}$ day^{-1} atm^{-1}). Then, the air is removed and the package is heat sealed. Under good vacuum conditions, an atmosphere of less than 1 % O_2 and 10-20 % CO_2 is achieved (Lambert et al., 1991). This change in the gas composition will slow down the microbial spoilage of products and extend product shelf-life.

Table 9. Examples of food packaged under MAP in North America.

A. Fresh raw meat
e.g.Sliced bacon
Steak
Beef hearts
Pork kidneys
Ox tails
B. Cooked Meats
e.g. Hamburgers
Beef jerky
Sausage rolls
Sliced meat
Wieners
Cretons(head cheese)
C. Poultry
e.g. Whole carcasses
Nuggets
Chicken parts
Peeled hard cooked eggs
D. Fish (Canada only)
E. Cheese
F. Prepared salads
G. Pasta
H. Various types of sandwiches

Source: Farber (1991)

From a microbiological perspective, the antimicrobial effect of vacuum packaging effect is due to its inhibitory effect on the growth of the putrefactive species, such as *Pseudomonas* species, allowing the proliferation of facultative anaerobes, such as *Lactobacillus* and other anaerobic microorganisms. Although *Pseudomonas spp.*, were responsible for spoilage in unprocessed poultry meat stored under chilled conditions, *Lactobacillus* species were the predominant spoilage microorganisms in vacuum packaged eviscerated broilers (Sawaya et al., 1993). *Lactobacillus* species become the predominant spoilage microorganisms in vacuum packaged products because i) they are resistant to elevated levels of CO_2, ii) they can outcompete other microorganisms in anaerobic chilled conditions and iii) they produce bacteriocins that antagonize other competing microorganisms. However, *Lactobacillus* species grow at a slower rate and require a high population density to cause sour spoilage, thus delaying the deterioration of the product. The development of *Lactobacillus* in vacuum packaging is also accompanied by the growth of *Enterobacteriaceae* (Sawaya et al., 1993). However, the growth of the *Enterobacteriaceae* is greatly influenced by the storage temperature and the hygienic status of the product (Genigeorgis and Rieman, 1979; Sawaya et al., 1993). Thus, stringent temperature control and pre-packaging sanitation are important.

1.9.2. Gas packaging

As an alternative to vacuum packaging, gas packaging can also offer additional shelf-life extension to perishable products. Like most modified atmosphere techniques, gas packaging involves packaging a product in an impermeable film and then flushing an appropriate gas mixture into the evacuated package. It uses a single gas or a blend of gases. The most commonly used gases include nitrogen, oxygen and carbon dioxide.

Nitrogen is an inert filler that is added to the package to reduce the concentration of other gases and to prevent the package from collapsing as CO_2 dissolves in the product. Nitrogen displays no bacteriostatic effect, and thus contributes little to the product's shelf-life. Oxygen is added in modified atmosphere system for three main reasons. First, it is used to maintain a sufficient O_2 partial pressure in the package to preserve the red colour of meat or "bloom". Second, low concentrations are used in packaging fruits and

vegetables to prevent the onset of anaerobic respiration. And finally, it suppresses the growth of anaerobic pathogens that pose a public health hazard, especially *C. botulinum*. However, while substantial levels of oxygen have proven to be advantageous, high oxygen modified atmosphere systems begin to spoil much sooner as a result of the growth of aerobic bacteria (Newton et al., 1977).

As early as 1882, CO_2 was recognized as a potent inhibitor of microbial spoilage. Molds, yeasts, and highly aerobic bacteria have been shown to be sensitive to elevated levels of CO_2. For poultry, an anoxic CO_2 atmosphere is effective for suppressing surface microbial spoilage. Hotchkiss et al. (1985) demonstrated that an enriched CO_2 environment increased the shelf-life of chicken quarters by 21-23 d beyond that of conventionally packed chicken when stored at 2^0C. Baker et al. (1985) concluded that an elevated concentration of CO_2 (60-80 %) was required to extend the shelf-life of ground chicken to 28d. However, the use of elevated levels of CO_2 has also been shown to have a deterimental effect on the product's acceptability. High CO_2 concentrations can result in surface discolouration of meat and poultry, changes in the spoilage flora and denaturation of the muscle protein fibres. Thus, a gas mixture incorporating CO_2, O_2 and/or N_2 is often used to minimize such effects and to enhance the appearance acceptability of the product. In general, the bacteriostatic effect of CO_2 depends on a number of interrelated factors including concentration, storage temperature, type and initial load of the microorganisms and the time of application (Lambert et al.,1991).

1.9.3. The bacteriostatic effect of CO_2

The mechanism of carbon dioxide's antimicrobial action is still not fully understood. Early studies proposed the exclusion of oxygen by CO_2 as a mechanism for inhibition. This mechanism was later discounted by the fact that anaerobic bacteria were also inhibited by CO_2. Another theory postulated that the microbial inhibition of CO_2 involved the dissolution of CO_2 in the cell membrane with the disruption of the cell membrane and membrane functions (Dixon and Kell, 1989). Currently, the most referred hypothesis concerns the mass action inhibiton of certain bacterial decarboxylating enzymes, such as isocitrate and malate dehydrogenase, and non-decarboxylating enzymes by action at the nonpolar sites (Daniels et al., 1985). In conclusion, whatever the mechanism of inhibition, the overall effect of a CO_2 environment is the extension of the lag phase of the microorganism, a decrease of its growth rate in the log phase and an overall extension in shelf-life.

1.9.4. Oxygen absorbents

A novel approach to atmosphere modification involves using oxygen absorbents placed within a sealed package. These oxygen scavengers consists of a highly O_2 permeable sachet that contains reduced iron powder.The chemical reaction involves the oxidation of Fe^{2+} (in the ferrous state) in the sachet to iron III oxide which lowers the oxygen partial pressure.This reaction decreases the oxygen headspace to as low as 0.01 % as oxygen diffuses into the package providing a film of the correct gas permeability is used. The oxidation reaction mechanism can be summed up as follows (Smith et al., 1990).

$$Fe \rightarrow Fe^{2+} + 2e^-$$

$$1/2O_2 + H_2O + 2e^- \rightarrow 2OH^-$$

$$Fe^{2+} + 2OH^- \rightarrow Fe(OH)_2$$

$$Fe(OH)_2 + 1/2\ O_2 + 1/2\ H_2O \rightarrow Fe(OH)_3$$

The most widely used oxygen absorbent is the "Ageless" produced by the Mitsubishi Gas Chemical Co. It consists of a highly oxygen permeable sachet which contains an iron oxide powder that undergoes oxidation in the presence of oxygen and moisture to form the ferric state. These sachets come in a wide range of types and sizes that are capable of absorbing 200-2000ml of headspace oxygen. Ageless type SS is used extensively to extend the shelf-life of muscle foods, such as red meat and poultry. These absorbents are self reacting and immediately absorb oxygen on exposure to air. Furthermore, they can be used at refrigerated or freezing storage conditions. Other types of Ageless absorbents include type Z , S , FX , E , G , FM and SE These types have been approved by the FDA for use in foods such as beef jerky, dehydrated meat and poultry products (Labuza and Breene, 1989). Oxygen absorbents have also been used to extend the mold-free shelf-life of bakery products such as bread, pizza crust and most intermediate moisture confectionaires, to prevent surface discoloration of highly pigmented products, and to prevent rancidity in snack foods, fish, beef jerky and chocolates. The potential draw back to this technology is that the reduced oxygen partial pressure, coupled with elevated levels of CO_2, may enhance the growth of *C. botulinum*. Sasajama et al. (1978) demonstrated that these scavenging systems can induce botulism in Japanese cooked minced fish (kamaboko). The type of absorbent used also plays an important effect on the growth of *C. botulinum* in MAP foods. Oxygen/CO_2 absorbents will inhibit the growth of *C. botulinum* while O_2 absorbent-CO_2 generators have been shown to enhance the growth of *Clostridium* species (Smith, 1992). In recent challenge studies, pork samples were inoculated with non-proteolytic *C. botulinum* and stored at 15°C in air and modified atmosphere (100% N_2 and oxygen absorbent). Toxin production was detected in pork samples packaged in 100 % N_2 and an oxygen absorbent after 21d compared to 14d in air (Lambert et al., 1992b). This was attributed to the growth of aerobic spoilage bacteria in the air packaged products resulting in reduced oxygen levels and the production of CO_2 which may have enhanced toxin production by *C. botulinum*. More recently, Morris et al.(1995) reported the growth of *L. monocytogenes* at high CO_2 levels (>60% CO_2) held at 10-15°C. However, when an oxygen free environment was achieved using Ageless SS, growth of *L. monocytogenes* was completly inhibited, even at mild abusive temperatures. In conclusion, although these oxygen absorbents provide

an alternative method of modifying the gas atmosphere, further investigations are required into their effect on the microbiological shelf-life and safety of fresh and minimally processed foods.

1.9.5. Ethanol vapor emitters

Ethanol emitters are a recent technology introduced by a Japanese company to extend the mold-free shelf-life of bakery products. Freund Ltd. have introduced these sachets under the tradenames "Ethicap" and "Antimold 102". These sachets contain 55% ethyl alcohol (by weight) 35 % silicon dioxide, and 10 % moisture. Ethicap sachets come in various sizes ranging from 0.6 to 6g or 0.33 to 3.3g of ethanol evaporated.The size of the sachet used depends on **i)**the weight of the food **ii)** the a_w of the food, and **iii)** the desired shelf-life of the product. When a food is packed with a sachet of Ethicap, moisture is absorbed from the food while the ethanol vapor is released into the package headspace. The released ethanol vapors (0.5-2.5%) then condenses on the food surface and acts as a microbial inhibitor (Labuza and Breene, 1989).The main benefits of ethanol emitters include control of mold spoilage, delay of staling in bakery products, and the elimination of the need of the use of preservatives (Smith et al.,1987). The main disadvantage of using ethanol vapors is the absorbtion of ethanol by the product. However, recent studies have indicated that the levels of ethanol are within the maximum of 2 % permitted by the U.S. food ethanol regulations (Labuza and Breene, 1989). In addition, food products are usually packaged in a low or medium barrier film to ethanol vapors (ethanol permeability of <2g/m/day @30^0 C) to ensure compliance with the maximum acceptable limits set by regulatory agencices. Freund (1985) determined the level of ethanol of various bakery products to be less than 1 % after 20d, and Smith et al. (1987) reported a level of 1.45-1.52 w/w in apple turnovers. Smith et al.(1987) recommended that heating the product at 375^0F prior to consumption reduced the ethanol level to less than 0.1%. Another disadvantage is that the introduction of these emitters may incur extra costs on the processor posing a limiting factor for their expansion. Nevertheless, ethanol emitters improve the product quality and its shelf-life and the responsibility lies within the poultry industry to create awareness among consumers on the merits of modified atmosphere

packaging

There have been limited studies on the effect of ethanol on the growth of spoilage and pathogenic bacteria. Seiler and Russell (1993) reported that ethanol at a level of 1 % by product weight delayed the onset of rope by *Bacillus subtilis*. They also reported that low concentrations of ethanol (0.5-1.0% by product weight) inhibited the bacterial growth in both whipping cream and custard. More recently, Morris et al. (1995) examined the effect of Ethicap on the growth of *L. monocytogenes*. They observed that a 4 g sachet of Ethicap could control the growth of *L. monocytogenes* (Scott A) on agar media at 5, 10 and 15°C.

1.9.6. Objectives of research

Since poultry meat has a relatively short shelf-life, the food industry is constantly seeking additional alternatives to prolong the product's quality and safety. The most important parameter influencing microbial growth and subsequent spoilage in chilled poultry meat remains proper storage temperature. However, due to potential temperature abuse throughout the food chain from production to distribution and the emergence of pathogenic bacteria capable of growing at low temperatures, additional hurdles are necessary to control growth of pathogenic bacteria. In view of these comments, the objectives of this research are :

1. To monitor the physical, chemical and microbiological changes throughout storage conditions of chilled poultry meat packaged under various modified atmosphere packaging (MAP) conditions.

2. To evaluate the effectiveness of MAP to inhibit *S. enteritidis* inoculated onto chicken meat and stored at various commerical storage conditions;

3. To determine the effect of additional "hurdles" such as chitosan, sorbic acid dips and low dose irradiation alone or in conjuction with MAP to ensure the safety of the packaged product, particularly at temperature abuse storage conditions; and

4. To provide recommendations for the safe storage of poultry packaged under various modified atmospheres.

CHAPTER 2

STORAGE AND SHELF-LIFE STUDIES

2.1. INTRODUCTION

Proper temperature control of poultry meat throughout distribution and the application of good manufacturing practises (GMP) during processing is critical to minimize the growth of both spoilage and pathogenic bacteria during storage and to optimize the shelf-life of the pre-packaged product. The objectives of this study were:

(i) to determine the effect of modified atmosphere packaging on shelf-life extension of chicken thighs.

(ii) to determine the physical, chemical, microbiological and sensorial changes in the packaged products throughout storage.

(iii) to determine the optimum packaging conditions to give a 28d shelf-life at refrigerated temperature conditions (4°C)

2.2. MATERIALS AND METHODS

2.2.1. Storage experiments

Frozen, boneless, chicken thighs were used throughout this study and were obtained from Club Price, Montreal. Samples were stored frozen at -18°C until analysis. Prior to each storage trial, chicken thighs were removed from the freezer, placed (2 per bag) in 210 × 210 mm Cryovac bags (oxygen transmission rate of 3-6cc/m^2/day at 4° C and 0% relative humidity), weighed, and then thawed overnight in a refrigerator (5°C). Chicken thighs were packaged under the following gaseous conditions: air, vacuum packaging, gas packaging

(60%CO_2:40%N_2) and with an oxygen absorbent Ageless type SS (Mitsubishi Gas Chemical Co., Tokyo, Japan). Samples intended for air storage were heat sealed using an impulse heat sealer. Vacuum packaged samples were evacuated and heat sealed using a Multivac Chamber type heat seal packaging machine (Model A300/42 Multi-Vac D8941, Wafertwenden, Germany). Samples intended for gas packaging were evacuated and back flushed with a gas mixture of 60%CO_2 and 40% N_2 using a Smith's proportional gas mixture (Model 299-028, Tescom Corp., Minneapolis, MN). For the oxygen absorbent treatments, an Ageless type SS 100 oxygen absorbent, obtained from Mitsubishi Gas Chemical Co., Tokyo, Japan, was used. Oxygen absorbents were taped inside the packages prior to heat sealing with an impulse heat sealer. Triplicate samples per treatment per sampling time were then stored at 4°C and 12°C and analyzed after day 0, 3, 7, 14, 21 and 28.

2.2.2. Analyses

On the appropriate sampling day, three bags corresponding to each treatment were removed for physiochemical and microbiological analyses. The bags were aseptically opened and the samples blended by a stomacher (Lab Blender 400 BA 6021, Seward Medical, London). Approximately 25g were taken from each bag for pH determination. For microbiological analyses, a composite sample of 10g was made by combining subsamples from each bag. For the determination of the presence of *Salmonella*, several samples were taken from multiple points in each bag to make up 25g samples.

2.2.2.1. Headspace gas analysis

On each sampling day, the headspace gas composition was determined on appropriate packaged samples prior to opening. Gas samples for analysis were taken through an adhesive septum placed on the surface of the package using a gas tight syringe (Precision Sampling Corp., Baton Rouge, LA). Samples were injected into a Varian Gas chromatograph (Model 3400, Varian Canada Inc.) equipped with a thermal conductivity detector and using a Porapak

Q (80-100 mesh) and Molecular Sieve 5A (80-100 mesh) columns in series (Supelco, Canada Ltd). Helium was used as the carrier gas with a flow rate of 30 ml/minute. The oven temperature were set at 80°C, the injector port were at 100°C and the detector filament at 150°C. Gas concentrations of CO_2, N_2 and O_2 were determined by a Hewlett Packard Integrator (Model 3390 A, Hewlett Packard Co., Avondale PA).

2.2.2.2. Color analyses

Surface skin reflectance of the packaging treatments was analyzed using a Minolta CR100 colorimeter (Minolta Chromameter II, Minolta Corp, Ramsey N.J.) with a luminant C (6744) light source calibrated against a white reflector plate prior to colour measurements. Four to six measurements were made at selected locations on the meat surface and the results expressed as L* (lightness), a* (redness) and b* (yellowness).

2.2.2.3. Drip loss

On each sampling day, bags were weighed, opened the drip was poured into a sterile measuring cylinder and the bags were then reweighed. The loss in weight for each set of triplicate samples, due to drip loss, was expressed as a percentage of the initial sample weight.

2.2.2.4. pH measurement

The pH of chicken thighs was measured using a previously calibrated (pH 4.0 & pH 7.0) Corning pH meter (Model 2220, Corning Glass Works, Corning N.Y.). About 25 grams of the homogenated samples (prepared previously in section **2.2.2.)** were placed in a 100 ml beaker containing an equal weight of deionized water and mixed using a glass stirring rod. A fluted filter paper (Whatman no.1) was forced part way into the slurry and allowed to set for five minutes. A pH electrode (Fischer Scientific Model 13-620-104) was then immersed into

the filtered solution inside the fluted filter paper. The pH was recorded and the results were expressed as the mean of three samples per treatment.

2.2.2.5. Sensory analyses

At day 0 and each subsequent sampling time, the packaged chicken thighs were evaluated sensorially by 6 untrained panel members. Odor, color and overall acceptibility were evaluated using a hedonic scale from 0 to 10 (Larmond,1977). For each parameter, a score of six was considered the upper limit of acceptability, implying that the shelf-life was terminated when this sensory score was reached (Lambert et al., 1992a).

2.2.2.6. Microbiological analyses

Ten grams of the homogenized composite sample was placed in a sterile stomacher bag and stomached with 90 ml of 0.1 % buffered peptone water (Difco, Michigan, USA) for 2 mins. Appropriate dilutions were made again using 0.1 % peptone water and counts made using a surface plate or pour plate technique.

Total aerobic plate counts were determined by spreading 0.1 ml of the appropriate dilution in duplicate onto plate count agar (PCA, Difco). Plates were incubated aerobically and colonies were counted after 48 hours at 35°C (Health Protection Branch, HPB, 1989).

Psychrotrophic plate counts were also determined using a surface plate technique on PCA agar and incubating aerobically. Colonies were counted after10 days at 5°C.

Lactic acid bacteria were determined using Lactobacillus MRS agar (MRS broth (Difco) plus 1.5 % agar) and incubating plates aerobically at 35°C for 48 hours (Deman et al., 1960).

Coliforms were determined by pour plating 1ml of the appropriate dilutions using Violet Red Bile Agar (VRBA, Difco). After solidification of the agar, plates were overlayed with 5-10 ml of VRBA. Plates were then incubated at 35°C for 24 hours (HPB, 1989). At each sampling day, three colonies were picked for each experimental treatment and subjected

to biochemical tests (E20 API system, BioMerieux).

Total anaerobic counts were determined by plating the appropriate dilution onto PCA (Difco). Plates were then incubated in anaerobic jars (BBL, Gas Pak jar system, Cockeysville, MD) at 35°C for 48 hours.

Listeria monocytogenes counts were determined by spread plating 0.1 ml of the appropriate dilutions onto PALCAM agar (Oxoid). After 48 hours incubation at 35°C, several colonies were picked from countable plates and tested biochemically using the API Listeria system (Oxoid,Unipath, U.K.).

Faecal *Streptococcus* counts were determined by spreading 0.1 ml of the appropriate dilutions onto Kanamycin Esculin Azide Agar (Oxoid, Unipath, Basingstoke, U.K.) and then incubating the plates aerobically at 35°C for 48 hours.

Staphylococcus aureus counts were determined by spread plating 0.1 ml of the appropriate dilutions onto Baird Parker (Difco, Michigan, USA) and then incubating the plates at 35°C for 48 hours. Twenty five percent of colonies from a countable plates were picked and subjected to both catalase and oxidase test.

Pseudomonas counts were determined by spreading 0.1 ml of the appropriate dilutions onto Pseudomonas Agar (Difco, Michigan, USA) and then incubating the plates aerobically at 35°C. After 48 hours incubation, two or three colonies were picked and subjected to biochemical tests (E20 API system, BioMerieux) and the oxidase tests for confirmation of identity.

Aerobic and anaerobic sporeformers were determined by heat shocking the appropriate dilutions at 65°C for 20 minutes and then spread plating 0.1 ml onto Tryptic Soya Agar (Difco). For anaerobic sporeformer counts, plates were placed in anaerobic jars, and incubated at 35°C for 48 hours (HPB,1989).

Salmonella counts were determined by a series of pre-enrichment and selective enrichment techniques. Twenty five grams of the previously prepared homogenate (section **2.2.2.**) was placed in 225 ml of sterile 0.1 % buffered peptone water (Difco), stomached for 30 seconds, the pH adjusted to 6.8 (if necessary) and then incubated at 35°C for 18-24 hours. One ml of the pre-enriched sample was then inoculated into 10 ml Selenite Cysteine Broth

(Difco) and 0.1 ml into 10 ml Modified Rappaport Vassiliadis Broth (Difco). All broths were incubated for 24h at 37°C and 43°C respectively. After incubation, one loopful of each broth was streaked onto Bismuth Sulphite (Difco) and Modified Lysine Iron Agar (Bailey et al., 1988). Plates were then examined for the presence of presumptive *Salmonella* colonies after 24 hours incubation at 37°C. *Salmonella* species appear as purple colonies with black centers on the Modified Lysine Iron Agar and black colonies, with or without a metallic sheen on Bismuth Sulphite agar. At least two typical colonies were picked from each plate, streaked onto Mackonkey agar (Difco) and incubated for 24h at 35°C. The purified colonies were then inoculated on Lysine Iron Agar slants (Difco). Colonies showing positive results on Lysine Iron Agar Slants i.e., purple butt with blackening of the butt due to the production of hydrogen sulphide, were characterized biochemically using E20 API system (BioMerieux) and confirmed by the *Salmonella* Rapid Latex Test (Oxoid). A positive control of *S. typhimurium* ATCC# 14028 was carried out with each trial to show the appearance of *Salmonella* on the growth medium and to confirm positive *Salmonella* samples.

2.2.2.6.1. Microbial isolation and characterization

At the onset of spoilage, 20 colonies were selected from countable plates on PCA agar media corresponding to each experimental treatment. The colonies were purified by subculturing onto PCA agar and then incubating for 3 days at 20°C. The isolates were first examined for colonial morphology, gram stain reaction, motility and the production of catalase and oxidase as described by HPB (1989). Further additional tests were carried out for identification of the isolates. All gram negative, catalase positive bacteria were examined for the oxidative/fermentative dissimilation of glucose. The isolates were inoculated into two tubes of Bacto OF Basal Medium (Difco) containing 1% filter-sterilized glucose in which one tube was overlayed with mineral oil. All Gram positive catalase negative bacteria were examined for the ability to grow on Streptomycin Thallous Acetate Agar (STAA; Gardner, 1966). For Gram positive, catalase negative bacteria, the isolates were subcultured onto MRS agar (Difco) at 30°C for 24h and then further characterized using the API 50 CHL

Lactobacillus strip (BioMerieux Vitek). All test were performed according to methods outlined by McMullen and Stiles (1993).

2.2.2.7. Statistical analyses

Data were analyzed by the Statistical Analyses System (SAS Institute. Inc . 1988) using the General Linear Model Procedure. and the comparison of the means was done using the Duncan's multiple range test.

2.3. RESULTS & DISCUSSION

2.3.1. Changes in headspace gas composition

Changes in headspace gas composition for the various packaging treatments of chicken thighs stored at both 4°C and 12°C are shown in Figures 2-5 a,b respectively.

For air packaged chicken stored at 4°C, O_2 was rapidly depleted to < 1% after 7d with an increase in headspace CO_2 to ~ 40% after 28d (Figure 2a). A similar trend was observed in all packaged chicken thighs stored at 12°C. However, in this case, O_2 was depleted more rapidly reaching levels of <1% after 2-3d while CO_2 increased rapidly to ~90% after 21d resulting in all packages having a blown appearance (Figure 2b). These changes can be attributed to the growth and metabolism of aerobic and facultatively anaerobic spoilage bacteria found on chicken thighs during storage and reported in a subsequent section of this chapter.

Changes in headspace gas composition for the vacuum packaged chicken thighs are shown in Figures 3a,b respectively. Similar trends in headspace gas composition were observed for vacuum packaged chicken thighs at both 4°C and 12°C i.e., a rapid increase in headspace CO_2 from 10 % to ~ 90% at the end of the 28d (Figures 3a,b). These changes can be attributed to a shift in spoilage microorganisms from a predominately aerobic population at day 0 to a microaerophilic, facultative population comprising mainly of lactic acid bacteria & *E. aerogenes*. Similar shifts in microbial population have been reported in MAP studies on fish, pork and beef (Blickstand and Mollin, 1983; Christopher et al., 1979; Farber, 1991).

Similar trends were observed for chicken thighs packaged under a gas atmosphere (60%CO_2:40%N_2) or with an oxygen absorbent. In all cases, O_2 levels were <1% while CO_2 increased gradually throughout storage, particularly in products stored at 12°C (Figures 4-5 a,b respectively). These changes can again be attributed to the facultative nature of the spoilage microorganisms found on chicken thighs which are capable of growing under low concentrations of O_2 (<1%) and elevated CO_2 levels. Indeed, lactic acid bacteria have shown to grow in even 100% CO_2 (Kakouri and Nychas, 1994).

Thus, the trends and shifts in population and changes in headspace gas composition are consistent with previous studies on MAP muscle foods (Hotchkiss, 1988; Gill and Tan, 1980).

2.3.2. Color analyses

Changes in the L* (lightness), a* (redness) and b* (yellowness) color values for the different packaging treatments of chicken thighs are shown in Figures 6-8 a,b respectively

For most packaged products stored at 4°C, L* values decreased throughout storage i.e., they became darker. However, products stored with an oxygen absorbent became lighter This may be attributed to less oxidation of the myoglobin pigment to darker metmyoglobin or less drip loss and hence less solubilization of the myoglobin pigment in the product packaged with an oxygen absorbent. Similar changes in L* values were observed for treatments stored at 12°C. Furthermore, the L* values were significantly greater ($P<0.05$) at the higher storage temperature (12°C) than at 4°C.

Changes in both a* and b* values fluctuated throughout storage. Generally, chicken thighs had a consistently higher a* value than b* value (Figures 7-8) indicating that samples were more red than yellow. At 4°C, a* values tended to decrease throughout storage (Figure 7a-b) while b* values increased (Figure 8a-b) i.e., chicken thighs became less red and more yellow i.e., confirming the general observed decrease in L* values. This trend could again be attributed to formation of the brown metmyoglobin, less formation of carboxymyoglobin due to high CO_2 in packaged product or increased solubilizaion of myoglobin due to increased drip loss throughout storage. At 12°C, some a* values decreased (air & gas packaged samples) while a* values for the other treatments (vacuum package & oxygen absorbent) increased. Similar trends were observed for b* values at higher storage temperature

Fig.2a. Changes in headspace gas composition of chicken thighs stored in air at 4°C.

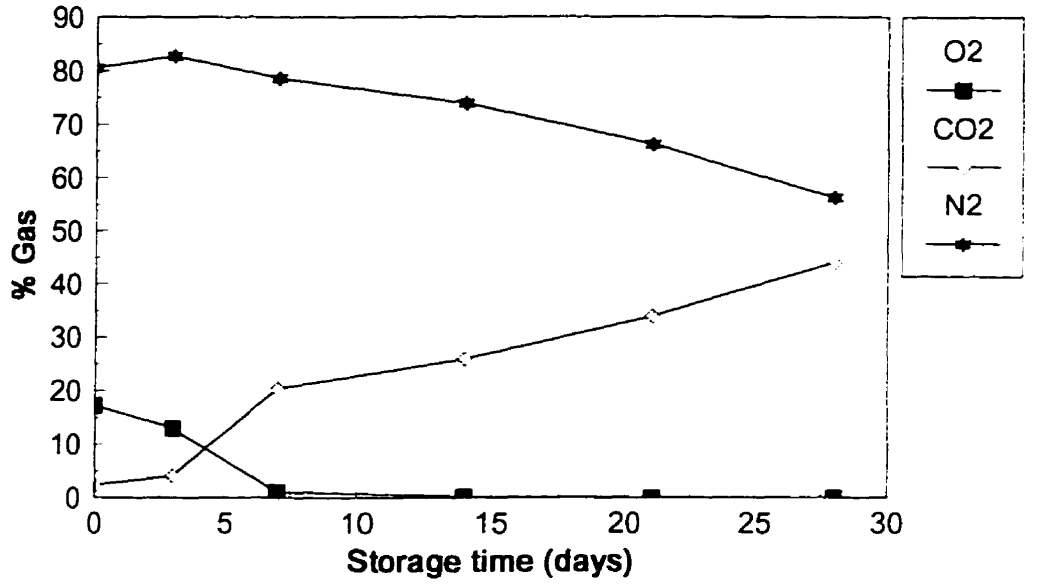


Fig.2b. Changes in headspace gas composition of chicken thighs stored in air at 12°C.

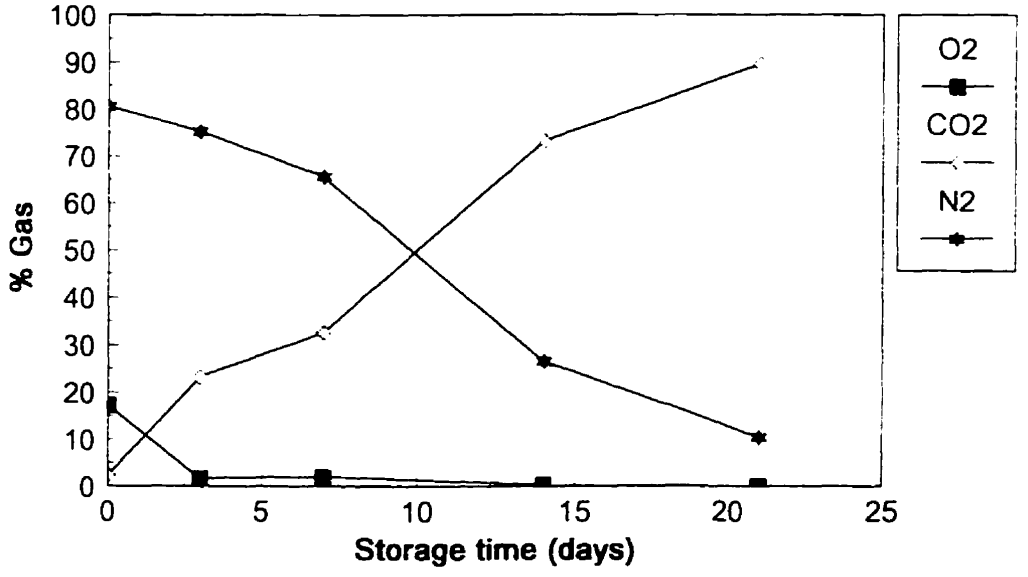


Fig.3a. Changes in headspace gas composition of chicken thighs stored under vacuum at 4°C.

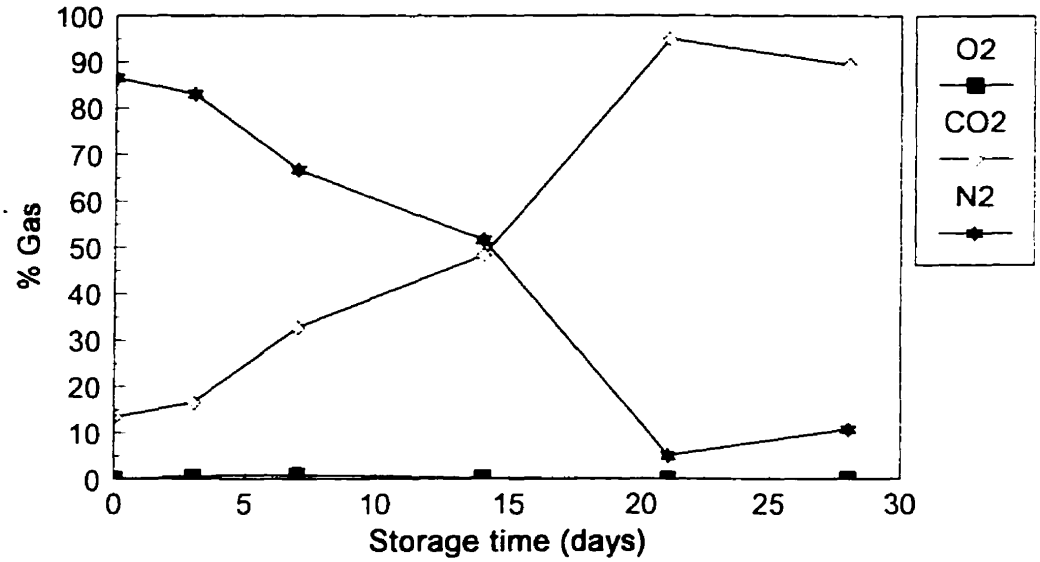


Fig.3b. Changes in headspace gas composition of chicken thighs stored under vacuum at 12°C.

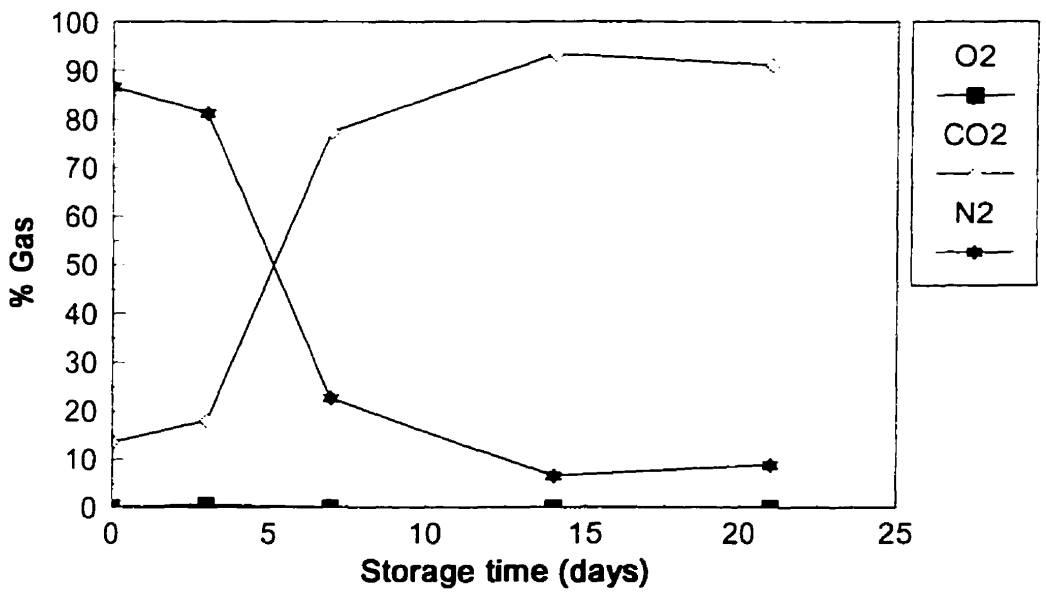


Fig.4a. Changes in headspace gas composition of chicken thighs stored in 60% CO2:40% N2 at 4°C.

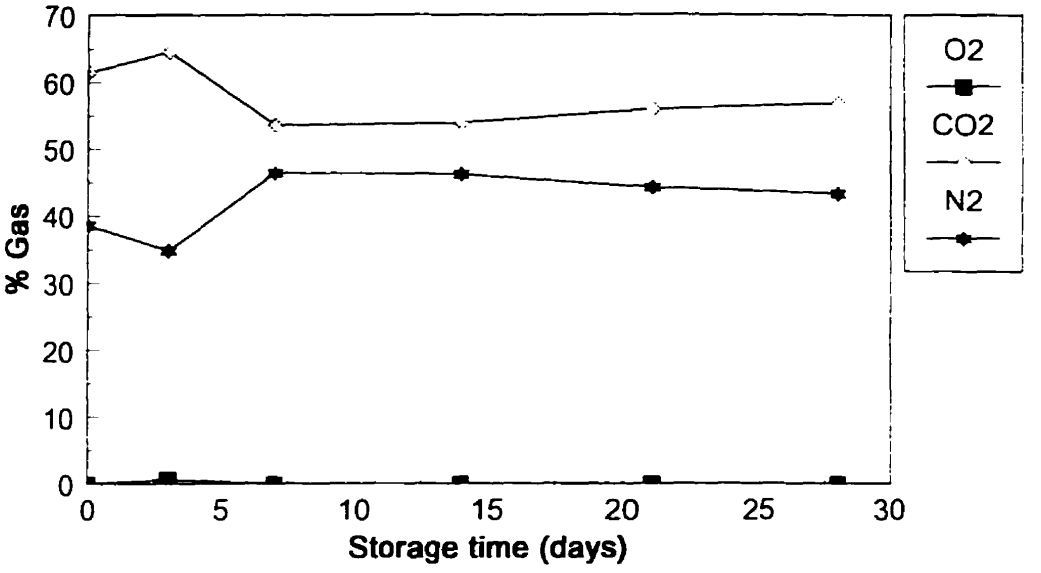


Fig.4b. Changes in headspace gas composition of chicken thighs stored in 60% CO2:40% N2 at 12°C.

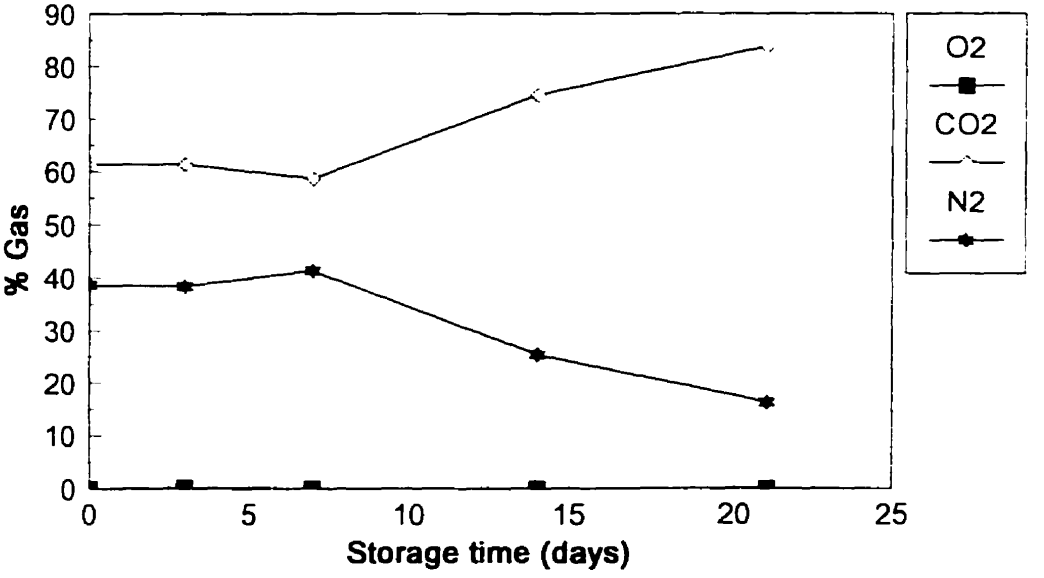


Fig.5a. Changes in headspace gas composition of chicken thighs stored with oxygen absorbent at 4°C.

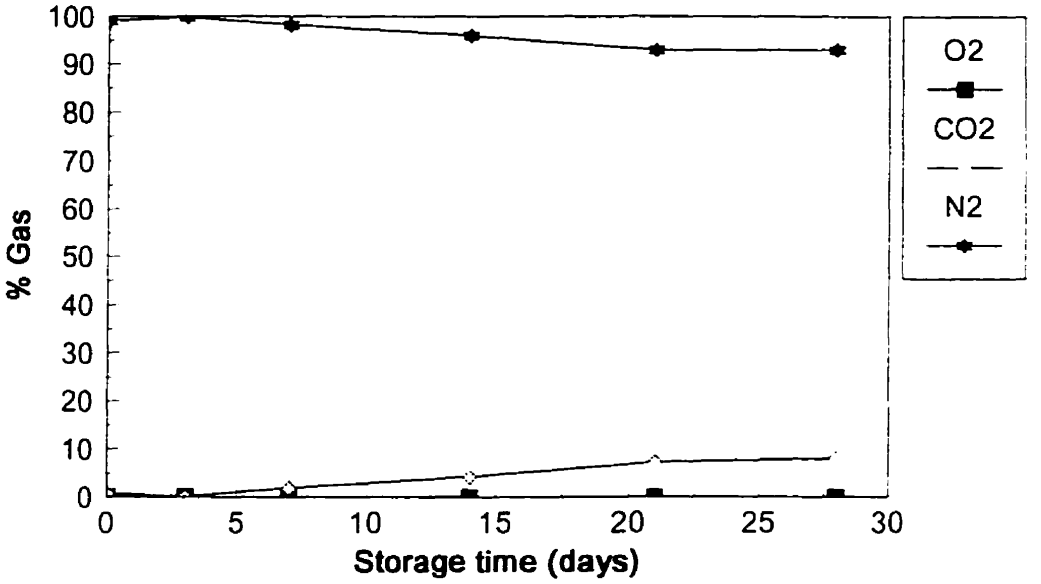


Fig.5b. Changes in headspace gas composition of chicken thighs stored with oxygen absorbent at 12°C.

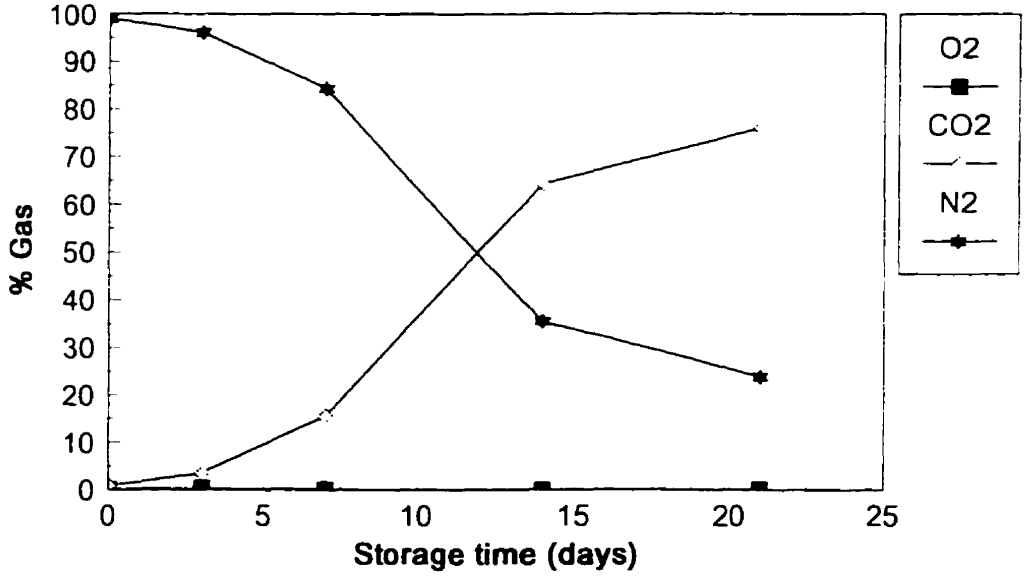


Fig.6a. Changes in L* coordinates of chicken thighs stored at 4°C.

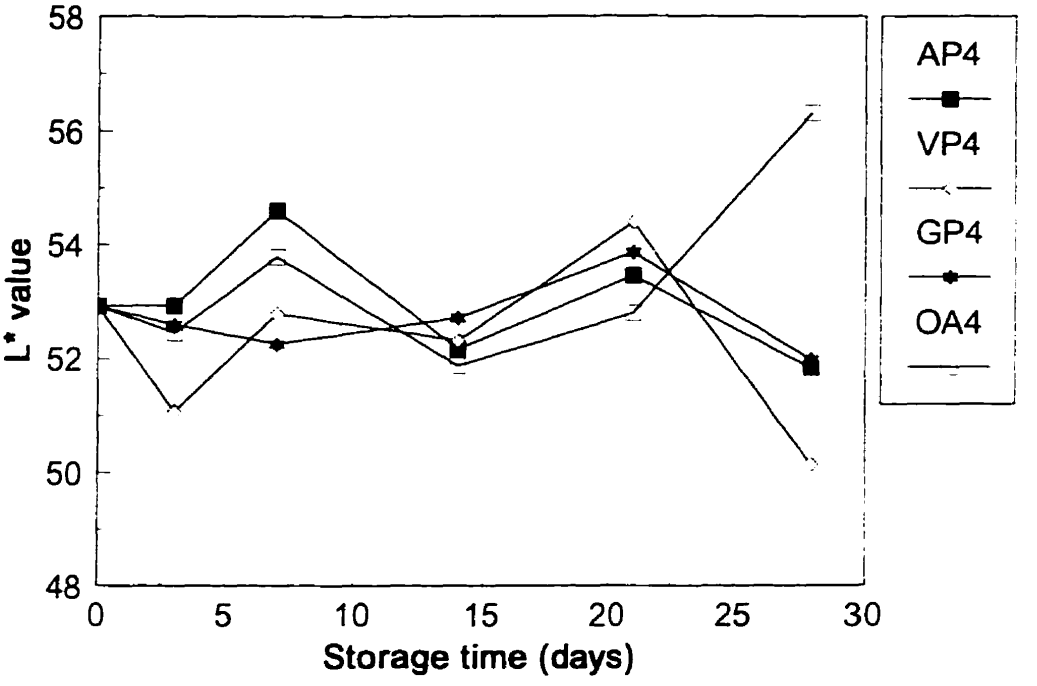
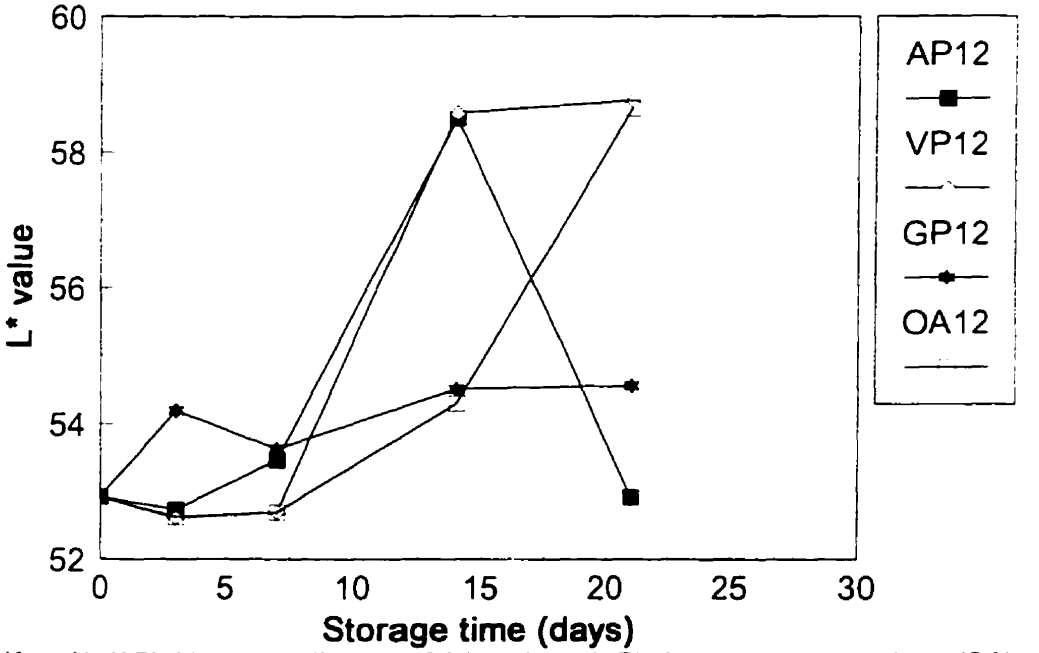


Fig.6b. Changes in L* coordinates of chicken thighs stored at 12°C.



Key: Air (AP), Vacuum (VP), 60% CO2+40%N2 (GP), And Oxygen Absorbent (OA)

Fig.7a. Changes in a* coordinates of chicken thighs stored at 4°C.

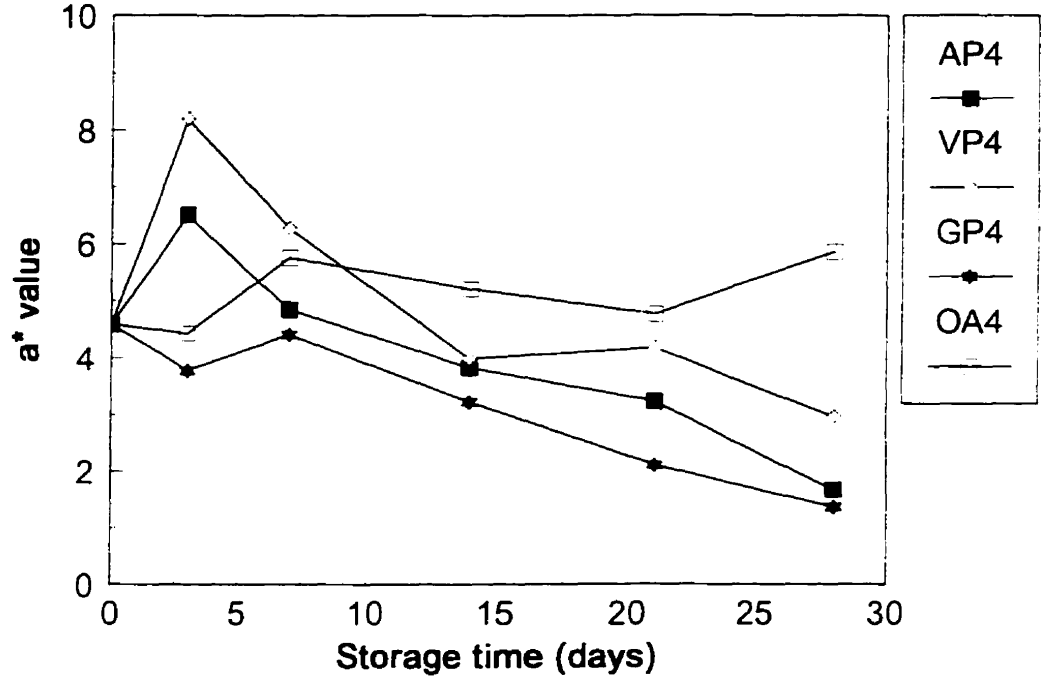
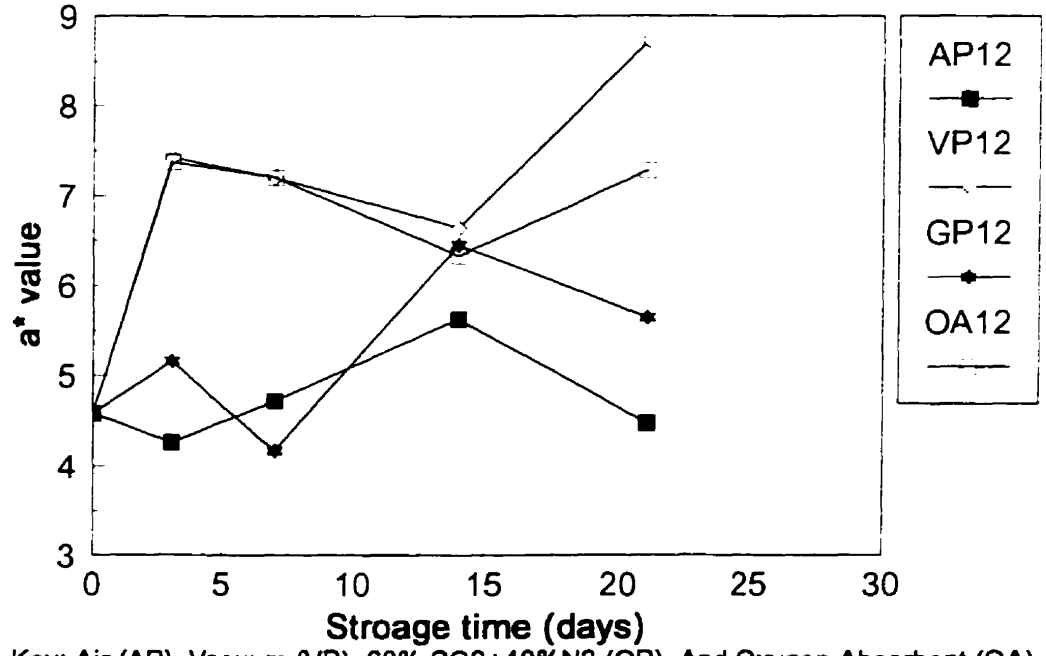


Fig.7b. Changes in a* coordinates of chicken thighs stored at 12°C.



Key: Air (AP), Vacuum (VP), 60% CO2+40%N2 (GP), And Oxygen Absorbent (OA)

Fig.8a. Changes in b* coordinates of chicken thighs stored at 4°C.

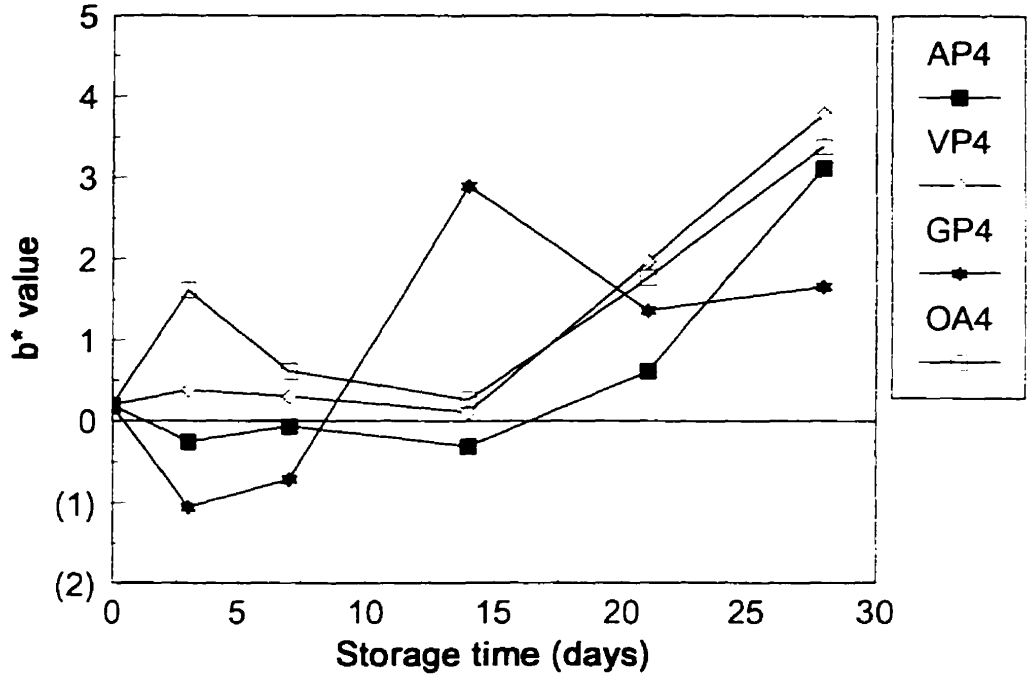
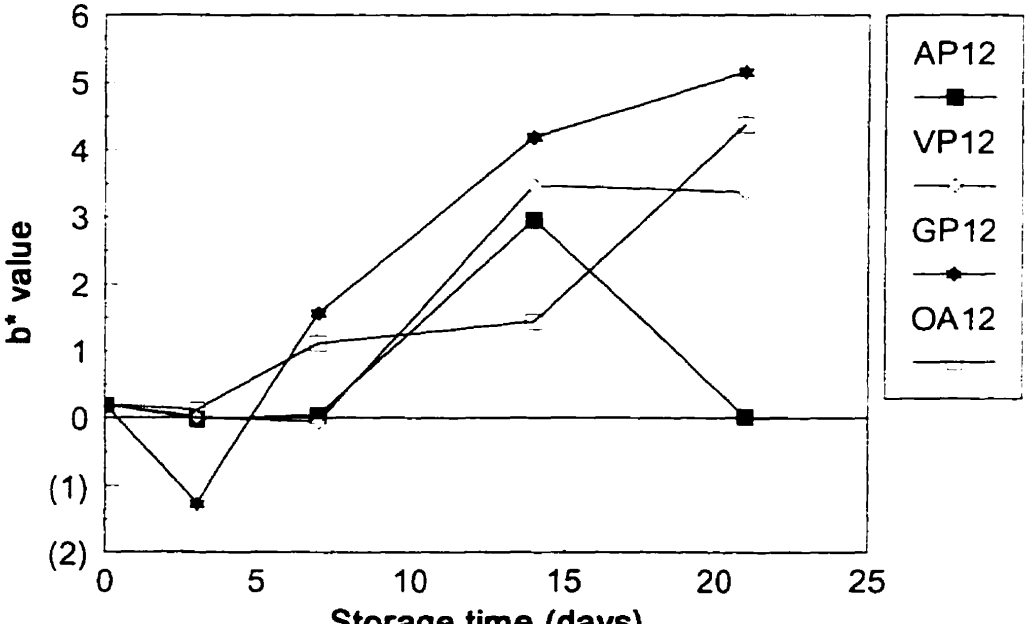


Fig.8b. Changes in b* coordinates of chicken thighs stored at 12°C.



Key: Air (AP), Vacuum (VP), 60% CO2+40%N2 (GP), And Oxygen Absorbent (OA)

2.3.3. Drip loss

The drip loss for different packaging treatments stored at 4 and 12°C are shown in Figure 9a,b. In general, the drip loss increased steadily throughout the storage period (Figure 9a,b). Both the packaging treatment and the storage temperature significantly ($P<0.05$) affected the % drip loss. The highest drip loss occurred in air packaged samples at 12°C after 21 days storage. Gas packaged samples stored both at 4°C and 12°C showed significantly less drip loss ($P<0.05$) compared to the other packaging treatments at day 14 and 21 respectively. Seideman et al. (1979) also recommended the use of a packaging atmosphere containing CO_2 and N_2 to reduce weight loss and extend the shelf-life of meats as drip loss correlates well with the bacterial spoilage. Lower *Pseudomonas* counts were observed in this study for chicken thighs initially packaged in 60:40 (CO_2:N_2). This probably resulted in decreased proteolysis and hence less drip compared to the other packaging treatments.

2.3.4. pH

Changes in pH of chicken thighs stored under various modified atmospheres are shown in Figure 10a,b. The initial pH of chicken thighs was ~ 6.4 and between 6.6 and 6.8 for products stored at 12°C. Nychas and Board (1991) reported that the initial pH of chicken thighs varied between 6.3-6.6. The most dramatic decrease in pH at 4°C was observed in air packaged products. In these products, pH decreased from pH 6.6 to 5.8 at day 14 and then increased to ~ 6.2 after 28 days. These changes in pH could be attributed to the rapid growth of spoilage bacteria in air packaged samples and subsequent production of CO_2 and lactic acid. Gas packaged samples stored at 12°C also showed a decrease in pH (probably due to the dissolution of CO_2 in the aqueous phase of the product followed by a gradual increase to pH~6.0. This data is generally consistent with those previously reported by other investigators who showed that elevated levels of CO_2 did not lower the final pH of the meat and that storage temperature did not affect the pH of meat kept under modified atmosphere

(Baker et al.,1985). Furthermore, since chicken thighs represent the red meat of poultry, the presence of high quantities of soluble proteins compared to chicken breast have been previously reported (Kakouri and Nychas, 1994). Hence, the lack of change in pH throughout storage could be also attributed to the buffering effect of these muscle proteins.

Fig.9a. Changes in drip loss (%w/w) of chicken thighs stored at 4°C.

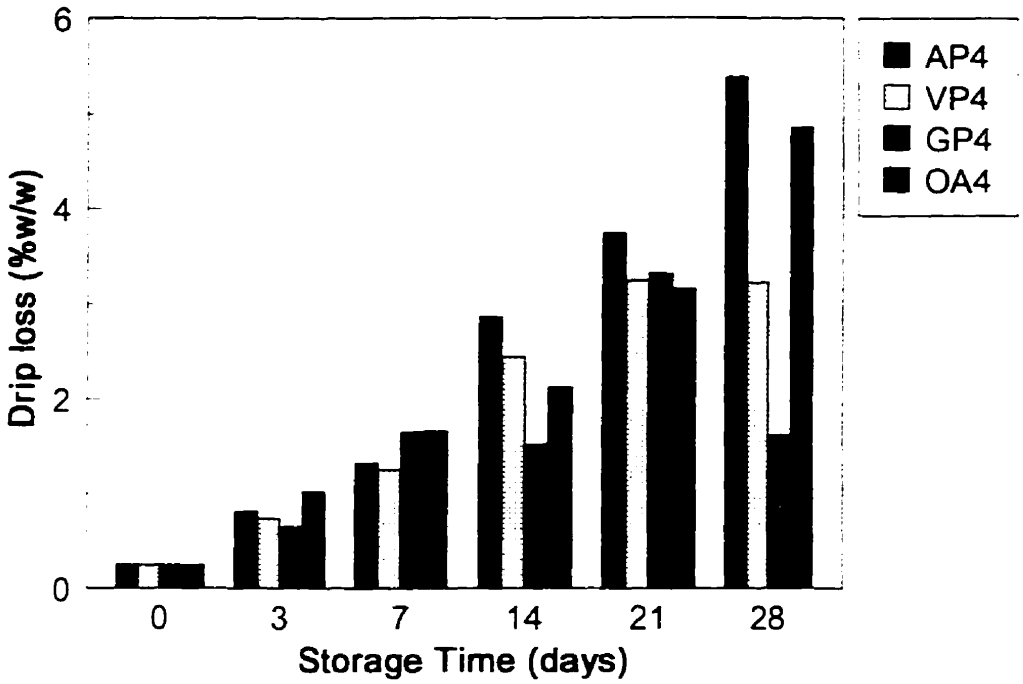
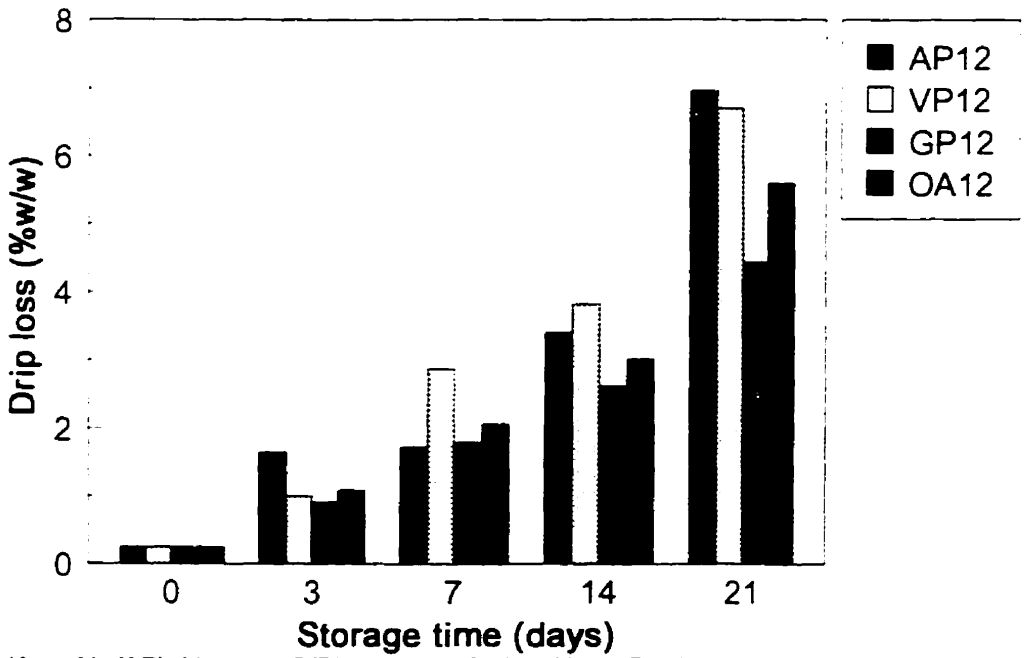


Fig.9b. Changes in drip loss (%w/w) of chicken thighs stored at 12°C.



Key: Air (AP), Vacuum (VP), 60% CO2+40%N2 (GP), And Oxygen Absorbent (OA)

Fig.10a. Changes in pH of chicken thighs stored at 4°C.

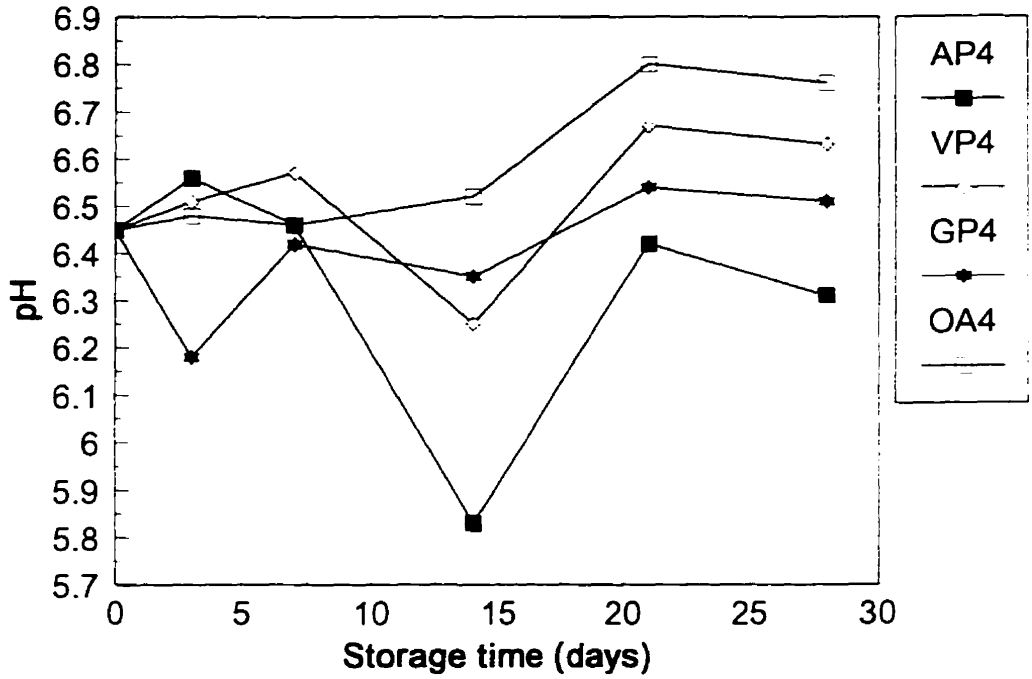
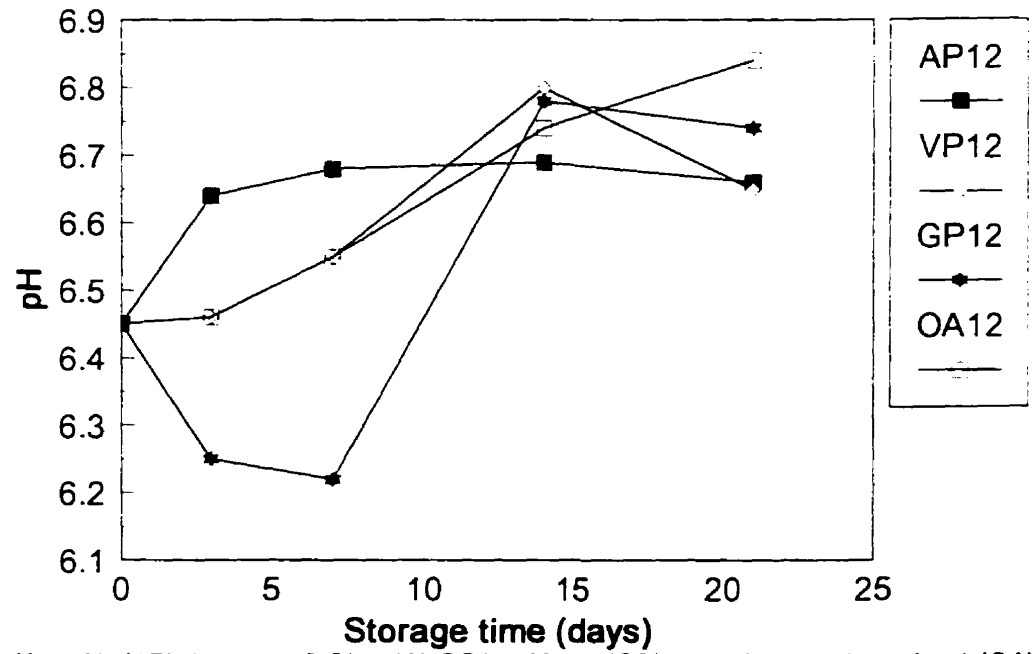


Fig.10b. Changes in pH of chicken thighs stored at 12°C.



Key: Air (AP), Vacuum (VP), 60% CO2+40%N2 (GP), And Oxygen Absorbent (OA)

2.3.5. Sensory analyses

Sensory analysis scores for the various packaging treatments are shown in Figures 11-12 a,b respectively. Products were regarded as unacceptable when a score of 6 on a scale of 10 was reached. Both odor and color scores were significantly higher ($P<0.05$) at 12°C than 4°C. Optimum results were obtained by gas packaging as chicken thighs had acceptable odor and color scores even after 26d at 4°C. In general, there was no significant difference ($P<0.05$) in the color of chicken thighs among the three modified atmosphere treatments. The air packaged chicken thighs were significantly more discolored ($P<0.05$) than all the other treatments between day 14-28 of storage at 4°C at after day 7 at 12 °C. The rapid discoloration in air packaged samples could be explained by the rapid increase in microbial growth and subsequent formation of slime on the meat surface.

Odor scores varied significantly ($P<0.05$) according to treatment and storage temperature. Chicken thighs stored in 60:40(CO_2:N_2) had significantly lower odor scores than air packaged samples during 21d storage at 4°C again indicating that modified atmosphere packaging played an effect on the odor of chicken thighs. A sour type of spoilage, due mainly to growth of LAB, was detected by the panellists in the gas packaged samples as early as 14d at 12°C and after 28d at 4°C. Similar off-odors have been reported in the vacuum packaged samples in the early stages of the storage period. However, these odors quickly dissipated after the packages were opened and were not regarded to have any impact on the sensory quality of the product. A rancid flavor was also detected by the panellists in both the air and oxygen absorbent treatments at 4°C and at higher storage temperatures (12°C). This was probably be due to oxidative rancidity in air packaged samples and to hydrolytic rancidity in the presence of low concentrations of O_2 .

Fig.11a. Changes in sensory color of chicken thighs stored at 4°C.

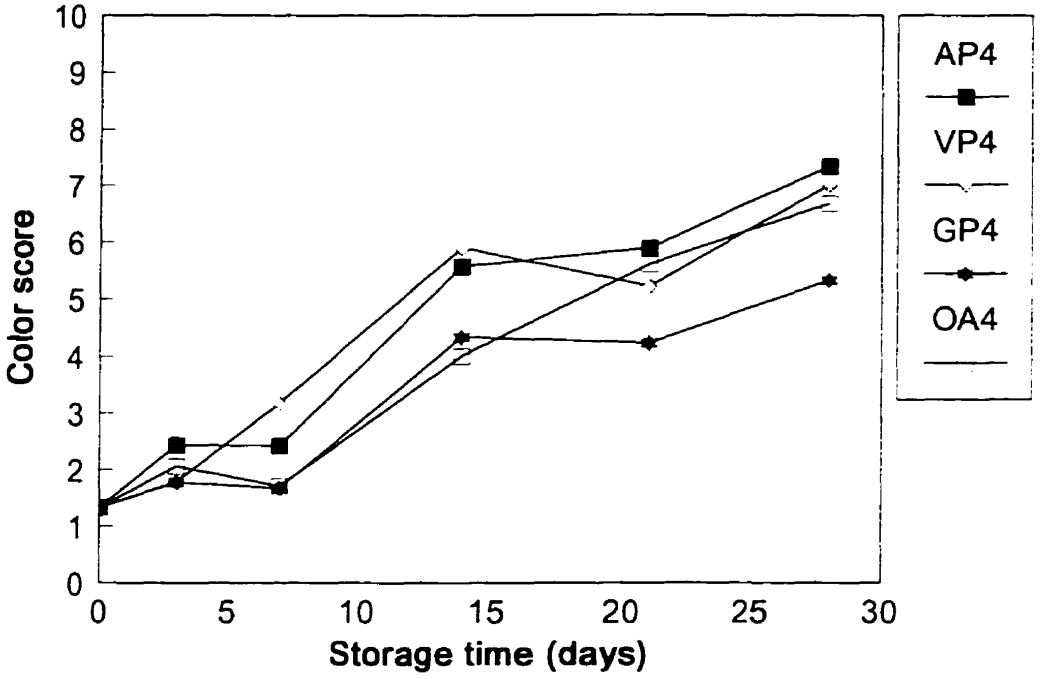
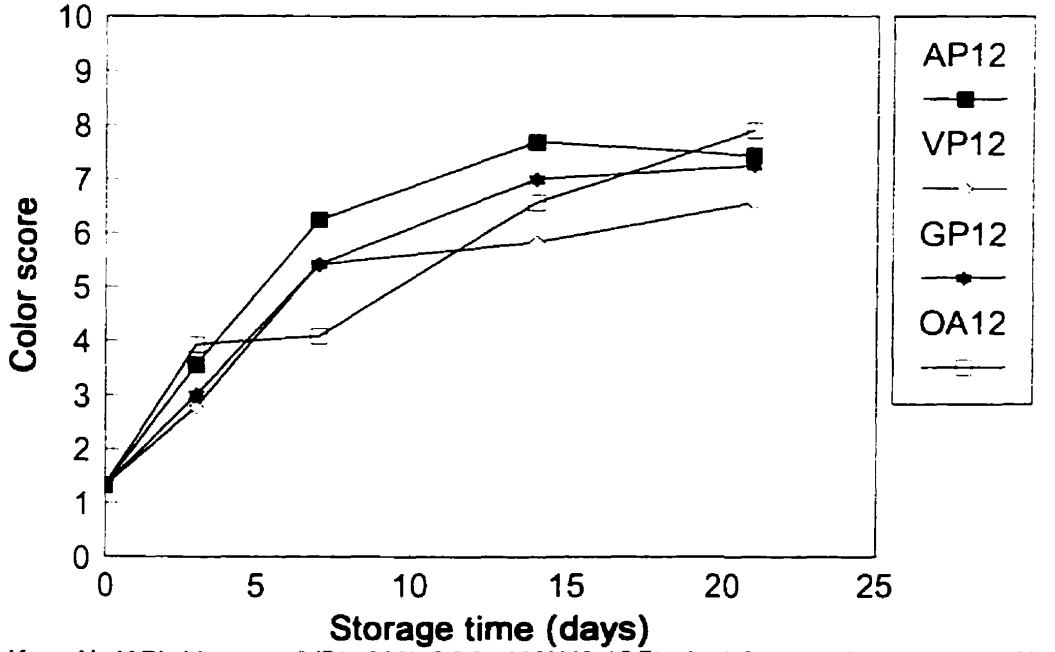


Fig.11b. Changes in sensory color of chicken thighs stored at 12°C.



Key: Air (AP), Vacuum (VP), 60% CO2+40%N2 (GP), And Oxygen Absorbent (OA)

Fig.12a. Changes in sensory odor of chicken thighs stored at 4°C.

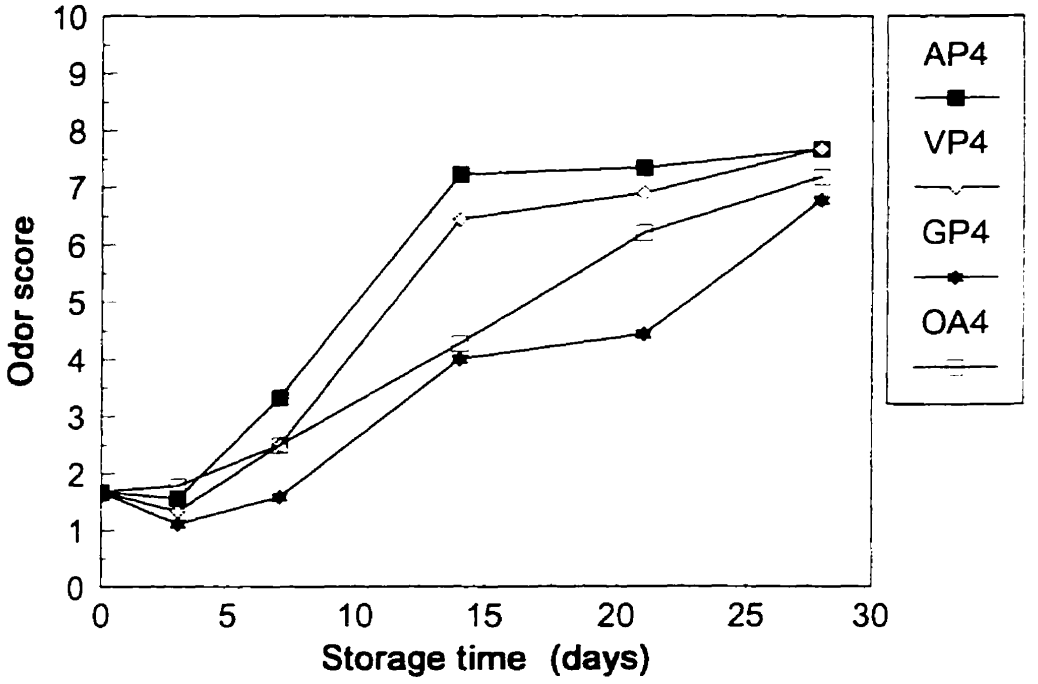
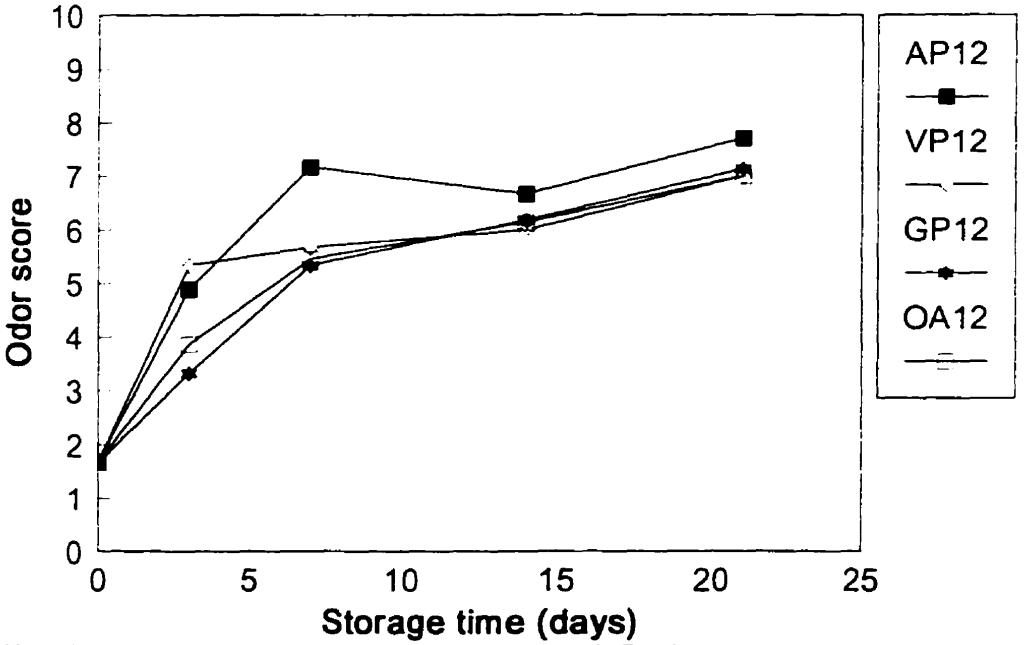


Fig.12b. Changes in sensory odor of chicken thighs stored at 12°C.



Key: Air (AP), Vacuum (VP), 60% CO2+40%N2 (GP), And Oxygen Absorbent (OA)

2.3.6. Microbial analyses

Changes in microbial plate counts in chicken thighs packaged under various gas atmospheres after four weeks of storage at 4 and 12°C are shown in Figures 13-20 a,b respectively. Initial bacterial counts ranges between 2.86 and 3.86 CFU/g for all chicken pieces i.e., of acceptable quality prior to packaging. Chicken pieces were regarded as unacceptable from a microbiological viewpoint when the recommended upper limit of acceptability of 10^7 CFU/g was reached (International Commission on Microbiological Specifications of Foods, ICMSF,1986). Changes in aerobic plate counts (APC) for all packaging treatments of chicken thighs stored at 4 and 12°C are shown in Figure 13a,b. For products stored at 4°C, counts reached 10^7 CFU/g after 7.5d in air packaged thighs and after 12-13d and 18-19d in thighs packaged with an oxygen absorbent or under vacuum. While the APC increased in gas packaged products (60%CO_2:40%N_2), it never reached unacceptable levels (10^7 CFU/g) even after 28d at 4 °C. For all packaging treatments, the APC increased significantly ($P<0.05$) as storage temperature increased. With the exception of gas packaged product, APC increased to 10^7-10^8 CFU/g at 12°C after 6-7d respectively and then decreased throughout storage. A similar trend was observed for gas packaged chicken thighs. However, the upper level of acceptability i.e., 10^7 CFU/g was only reached after 15d. These results stress the importance of strict temperature control if MAP is to be used successfully to extend the shelf-life and keeping quality of fresh poultry products.

Changes in psychrotrophic plate counts are shown in Figure 14a,b. At both 4 and 12°C, counts increased gradually throughout storage, especially in aerobically packaged product compared to products stored under a modified atmosphere. Again, this is due to the fact that a major population of fresh chicken thighs was composed of *Pseudomonas* species (Figure 15a,b) which are favoured by aerobic conditions and low temperature storage (Gill et al., 1990). The fact that counts increased throughout storage, even under MAP conditions, confirms previous observations that psychrotrophic *Pseudomonas* strains can grow at very low levels of residual oxygen (<1%) even in the presence of elevated levels of CO_2 (Marshall et al., 1991). However, all psychrotrophic counts failed to reach critical levels of $\geq 10^7$ CFU/g

throughout storage, irrespective of packaging treatment.

Counts of *Pseudomonas* and lactic acid bacteria (LAB) in chicken thighs packaged in air and various modified atmospheres and stored at 4 & 12°C are shown in Figures 15-16 a,b respectively. *Pseudomonas* counts increased in all treatments and this increase was proportional to storage temperature. After day 14, *Pseudomonas* counts reached maximum numbers (10^7 CFU/g) in air packaged product at 4°C (Figure 15a) and in products packaged in air, under vacuum and with an O_2 absorbent and stored at 12°C (Figure 15b). Gas packaged chicken thighs had lower *Pseudomonas* counts ($P<0.05$) than any of the packaging treatments at both storage temperatures. This could be due to changes in the headspace gas concentration during packaging or increased CO_2 levels and reduced O_2 gaseous conditions at the onset of packaging inhibiting the growth of *Pseudomonas*. However, storage in CO_2 enriched atmosphere and under other modified atmospheres did not have as a pronounced effect on the growth rate of *Pseudomonas* as expected. It has been shown that modified atmospheres containing CO_2 have been effective in reducing the growth rate and extending the lag phase of Gram negative bacteria (Genigeorgis, 1985). However, in this study, *Pseudomonas* grew without a significant lag period in all treatments at 4°C and 12°C. The absence of this extension in lag period for all modified atmospheres conditions i.e., gas and vacuum packaging or packaging with an oxygen absorbent may be a result of the presence of residual oxygen in the package. Low concentrations of O_2 in the package may be a result of improper evacuation and gas flushing or sealing allowing O_2 penetration into the package (Eyles et al., 1993). Kakouri and Nychas (1994) concluded that modified atmosphere packaging cannot inhibit the growth of *Pseudomonas* because complete anaerobic conditions could not be achieved. Furthermore, Marshall et al. (1991) reported that *Pseudomonas* can grow in pre-cooked chicken containing O_2 as low as 0.1% (v/v).

LAB counts were higher than *Pseudomonas* counts irrespective of the packaging treatment with growth being consistently higher at 12°C compared to 4°C. In air packaged samples, the rate of increase of LAB was less than that of *Pseudomonas* but increased rapidly after day 7. Furthermore, LAB counts in products packaged with an oxygen absorbent and stored at 4°C were significantly greater ($P<0.05$) than counts obtained from products

packaged in all other atmospheres. Despite the high concentrations of CO_2 in the gas packaged samples, increase in LAB counts were significantly ($P<0.05$) less than in other atmospheres. This was unexpected as lactic acid bacteria are CO_2 resistant and tend to proliferate under CO_2 enriched atmospheres. Previous studies have reported that storage temperature was an important factor influencing the prevalence of lactic acid bacteria in the microflora of modified atmosphere chicken (McMullen and Stiles, 1991). At temperature abuse conditions, the LAB did not grow and spoilage was mainly due to Enterobacteriaceae. Gill and Reichel (1989) reported that the prevalent microflora of vacuum packaged beef was composed of lactic acid bacteria and coliforms in equal numbers but comprised mainly of lactic acid bacteria in CO_2 packaged beef. However, the results of our study show that the spoilage microflora in all four treatments consisted mainly of lactic acid bacteria, *Pseudomonas* spp. and coliforms at the two temperatures used throughout this study (Table 12).

The growth of coliforms, and faecal *Streptococcus* i.e., indicators of faecal contamination and poor manufacturing practices are shown in Figures 17-18 a,b respectively. While the growth of coliforms increased more rapidly at 12°C than at 4° C, there was no significant difference ($P>0.05$) in coliform counts between the treatments stored either at 4°C or 12°C. Coliforms were less effected by the limited availability of O_2. In fact, their numbers generally increased throughout the storage period, irrespective of the packaging atmosphere. Coliforms are facultative anaerobes and have been shown to be less affected by elevated levels of CO_2 (Erichsen and Mollin, 1981). Similar results were observed for *faecal Streptococcus*. However, there was no significant difference ($P>0.05$) between packaging treatments and storage temperature, with counts being greater at 12°C than at 4°C (Figure 17a,b). The presence of high numbers of coliforms and faecal *Streptococcus* in this study are indicators of faecal contamination. Abu Ruwaida et al. (1994) reported that inadequate spray washing after evisceration and poultry processing equipments were the main sources of faecal contamination in poultry.

For the potentially hazardous microorganisms, growth appeared at various stages of the storage period. No significant treatment difference ($P>0.05$) was observed for counts of

Staphylococcus aureus counts indicating that this microorganism can grow under a whole range of packaging conditions (Figure 19a,b). However, counts increased significantly ($P<0.05$) in samples stored at 12°C, again indicating the importance of strict temperature control to minimize growth of this pathogen if present in raw products. The presence of *S. aureus* in processed poultry is usually an indicator of post handling contamination. *S. aureus* is a facultative anaerobe that can grow and produce an enterotoxin under both aerobic and anaerobic conditions with the amount of toxin being greater under aerobic conditions (Geingeorgis, 1985). While products were not analyzed for toxin, it is unlikely toxin would be present since counts of *S. aureus* need to be $>10^6$ CFU/g for sufficient toxin to be produced to cause food poisoning. Furthermore, Nychas (1994) reported that no enterotoxin was produced in chicken thighs inoculated with *S. aureus* and packaged under vacuum or 100% CO_2 after 1 week of storage at both 3 and 22°C.

With the exception of air packaged samples at day 7, *L. monocytogenes* was not detected in any of the packaging treatments during 28d storage at 4°C (Figure 20a). Counts of *L. monocytogenes* never reached more than 10^2 CFU/g after 28d. Gas packaging (60% CO_2:40% N_2) appeared to be more inhibitory to the growth of *L. monocytogenes* than the other packaging conditions. However, at abuse temperatures, *L. monocytogenes* was present in higher numbers (10^4 CFU/g) under most packaging conditions. The presence of *L. monocytogenes*, particularly in products stored at 12°C, presents a major safety concern. If temperature abuse should occur during storage, there is a possibility that *L. monocytogenes* may reach high numbers before incipient spoilage becomes evident.

It is interesting to note that *Salmonella*, aerobic and anaerobic spores were not detected in any of the packaged samples throughout the storage period. The fact that these microorganisms were absent may be due to the fact that they were below detectable numbers with the methodology employed in this study or they were inhibited by other bacteria particularly lactic acid bacteria, the predominant spoilage isolates in both air or modified atmosphere packaged chicken thighs.

Fig.13a. Changes in aerobic plate counts (APC) of chicken thighs packaged under various gas atmospheres and stored at 4°C.

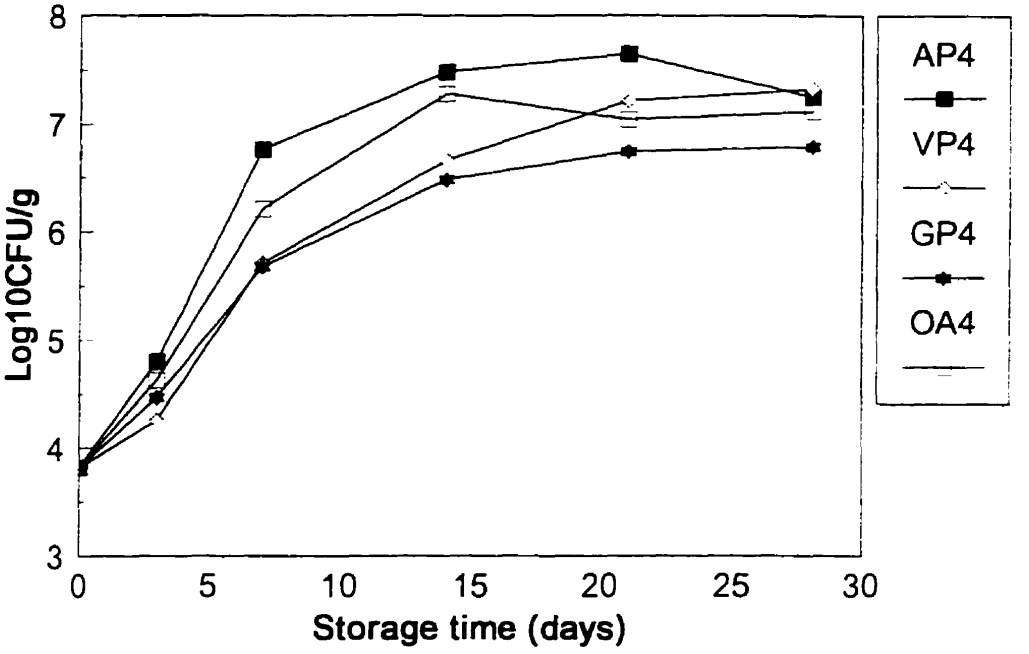
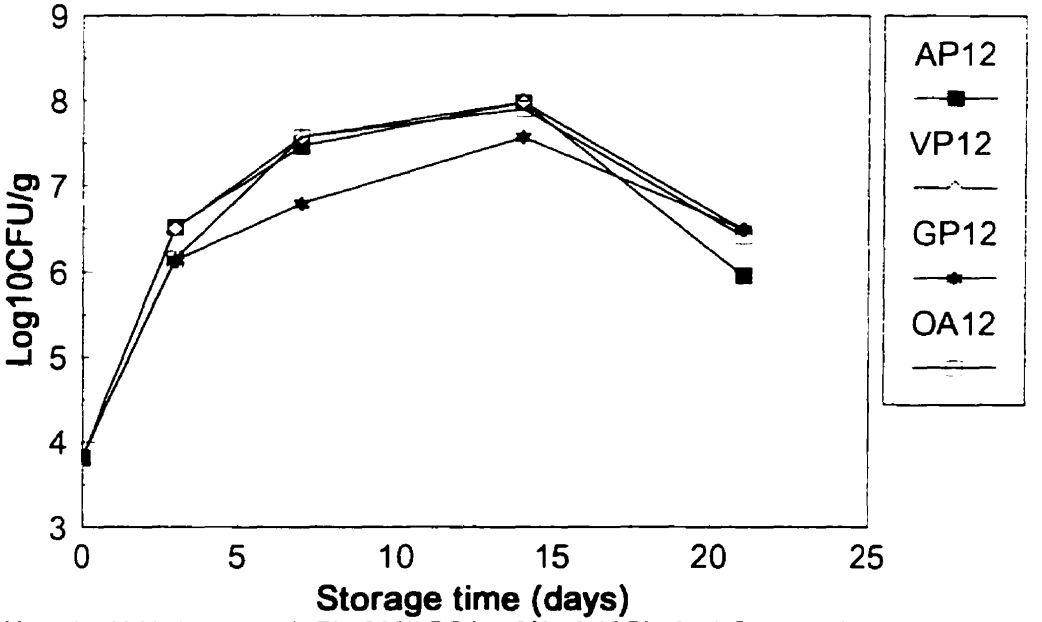


Fig.13b. Changes in aerobic plate counts (APC) of chicken thighs packaged under various gas atmospheres and stored at 12°C.



Key: Air (AP), Vacuum (VP), 60% CO2+40%N2 (GP), And Oxygen Absorbent (OA)

Fig.14a. Growth of psychrotrophic bacteria in chicken thighs packaged under various gas atmospheres and stored at 4°C.

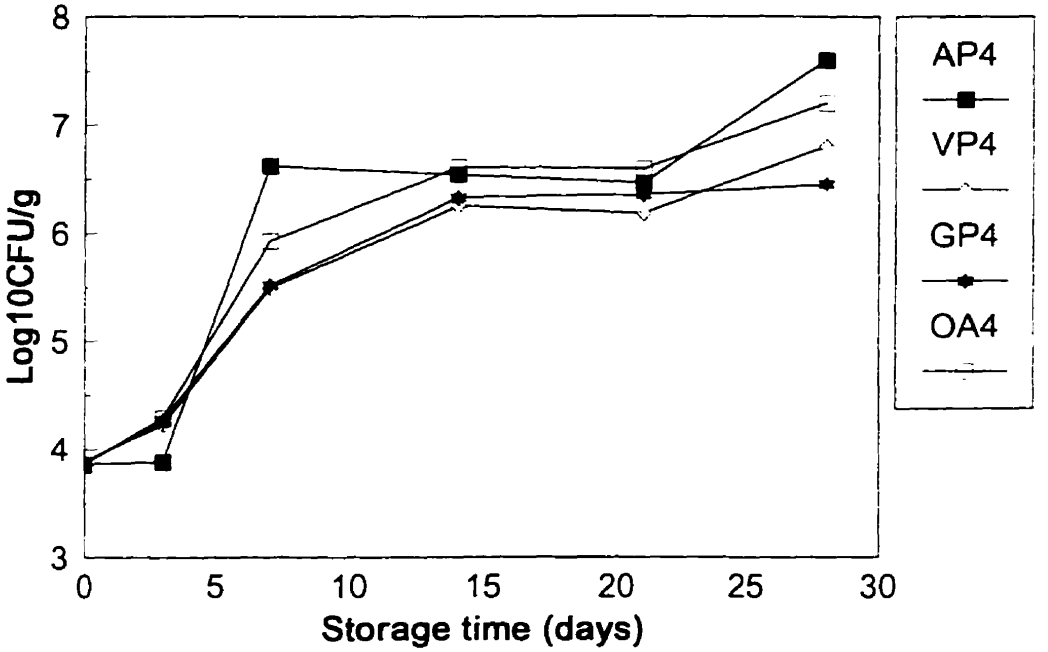
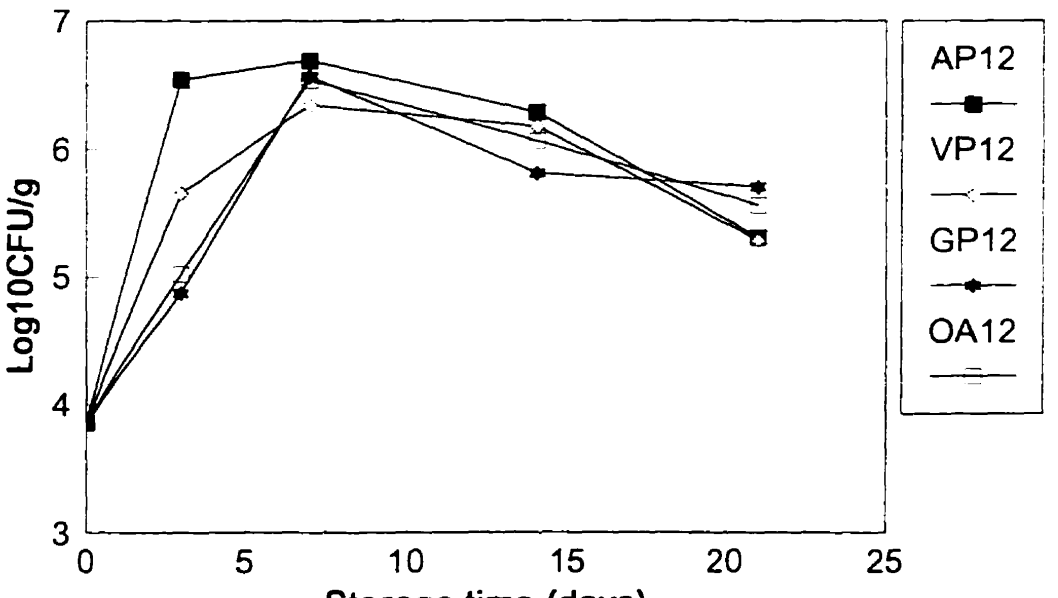


Fig.14b. Growth of psychrotrophic bacteria in chicken thighs packaged under various gas atmospheres and stored at 12°C.



Key: Air (AP), Vacuum (VP), 60% CO2+40%N2 (GP), And Oxygen Absorbent (OA)

Fig.15a. Growth of Pseudomonas in chicken thighs packaged under various gas atmospheres and stored at 4°C.

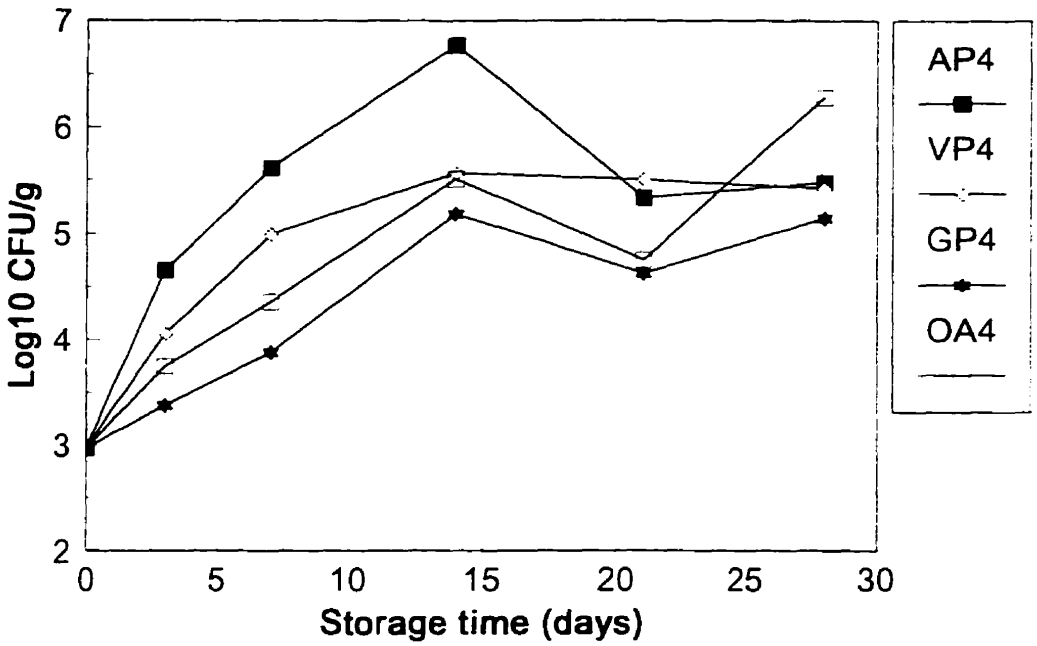
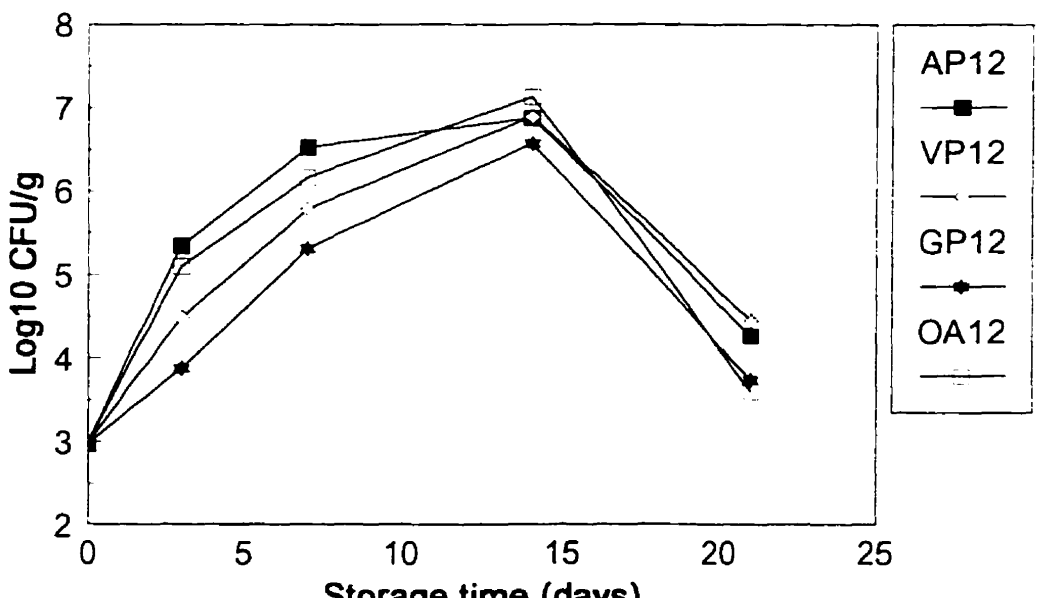


Fig.15b. Growth of Pseudomonas in chicken thighs packaged under various gas atmospheres and stored at 12°C.



Key: Air (AP), Vacuum (VP), 60% CO2+40%N2 (GP), And Oxygen Absorbent (OA)

Fig.16a. Growth of lactic acid bacteria in chicken thighs packaged under various gas atmospheres and stored at 4°C.

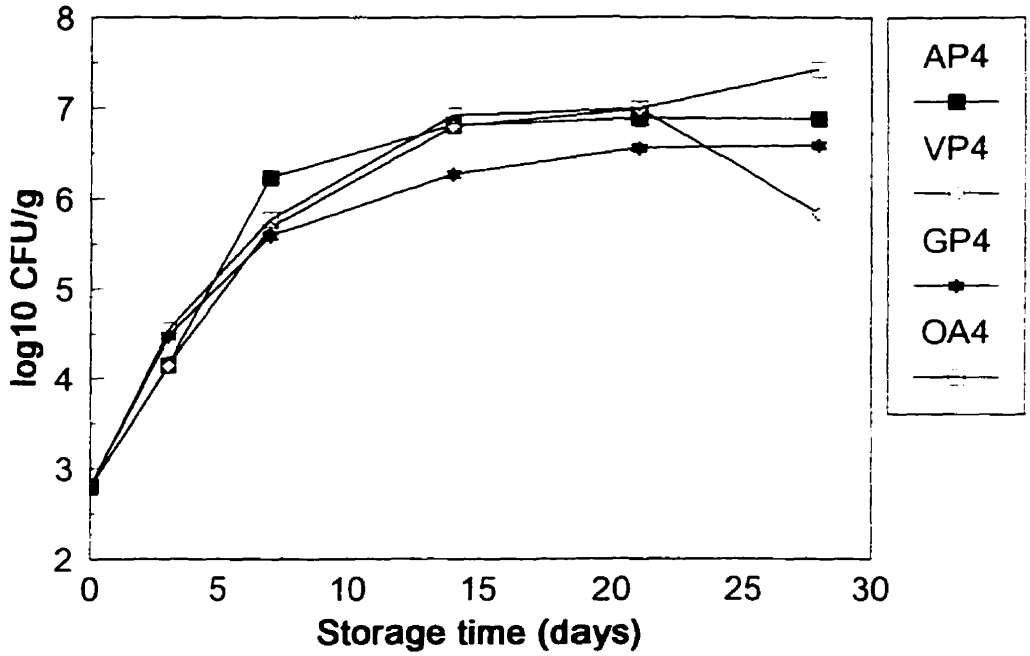
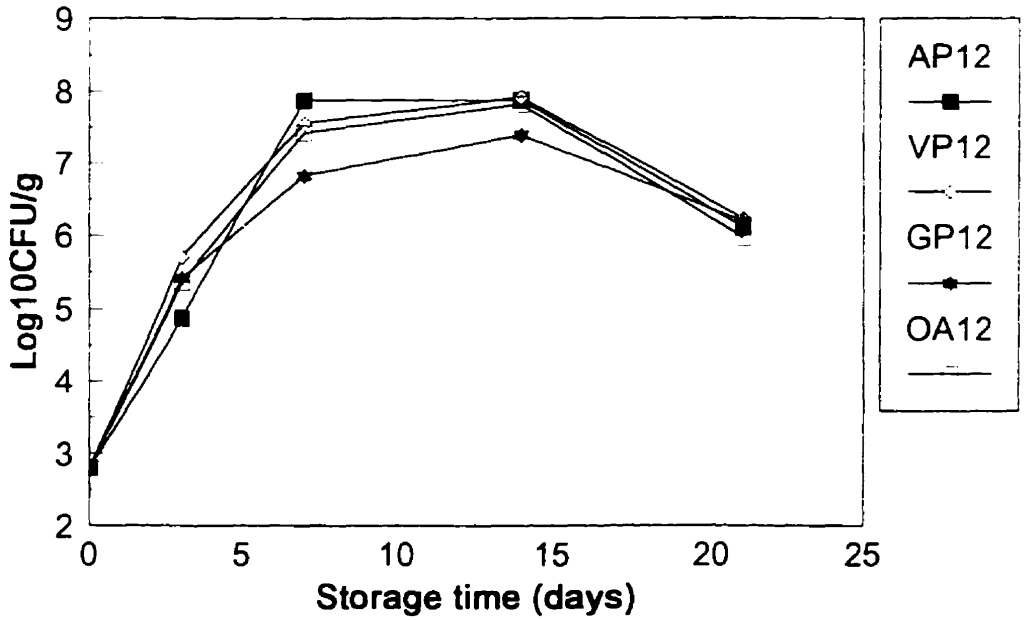


Fig.16b. Growth of lactic acid bacteria in chicken thighs packaged under various gas atmospheres and stored at 12°C.



Key: Air (AP), Vacuum (VP), 60% CO2+40%N2 (GP), And Oxygen Absorbent (OA)

Fig.17a. Growth of Coliforms in chicken thighs packaged under various gas atmospheres and stored at 4°C.

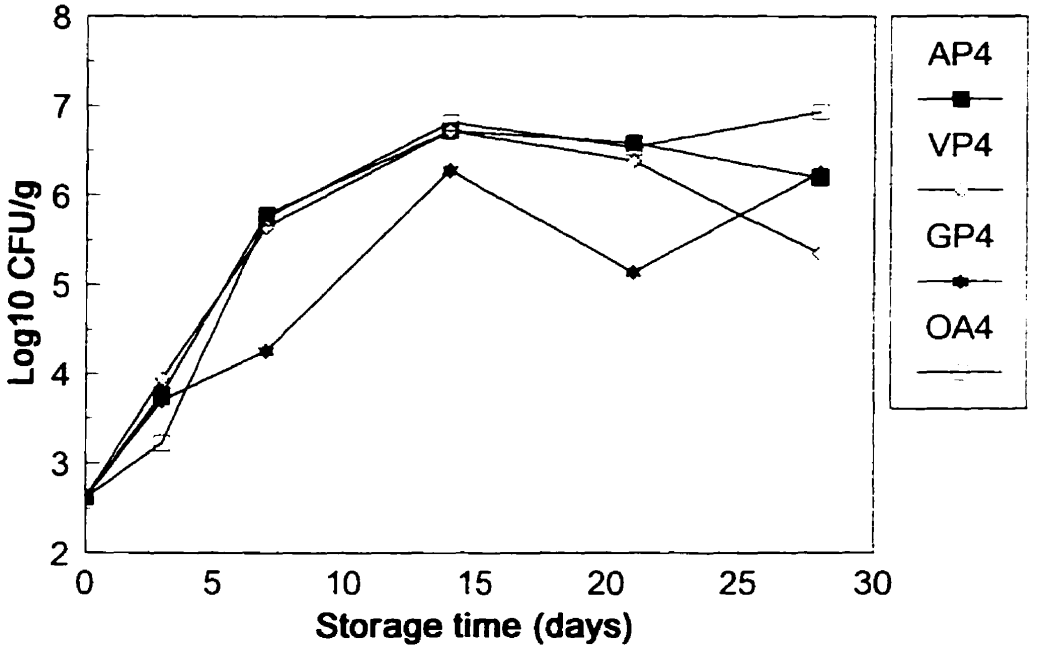
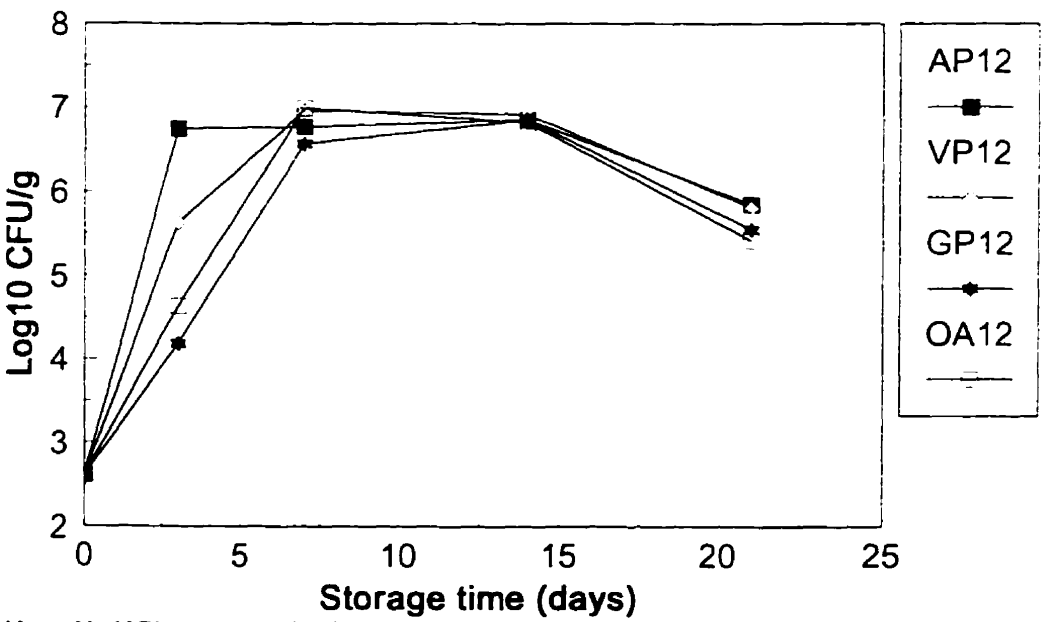


Fig.17b. Growth of Coliforms in chicken thighs packaged under various gas atmospheres and stored at 12°C.



Key: Air (AP), Vacuum (VP), 60% CO2+40%N2 (GP), And Oxygen Absorbent (OA)

Fig.18a. Growth of faecal Streptococcus in chicken thighs packaged under various gas atmospheres and stored at 4°C.

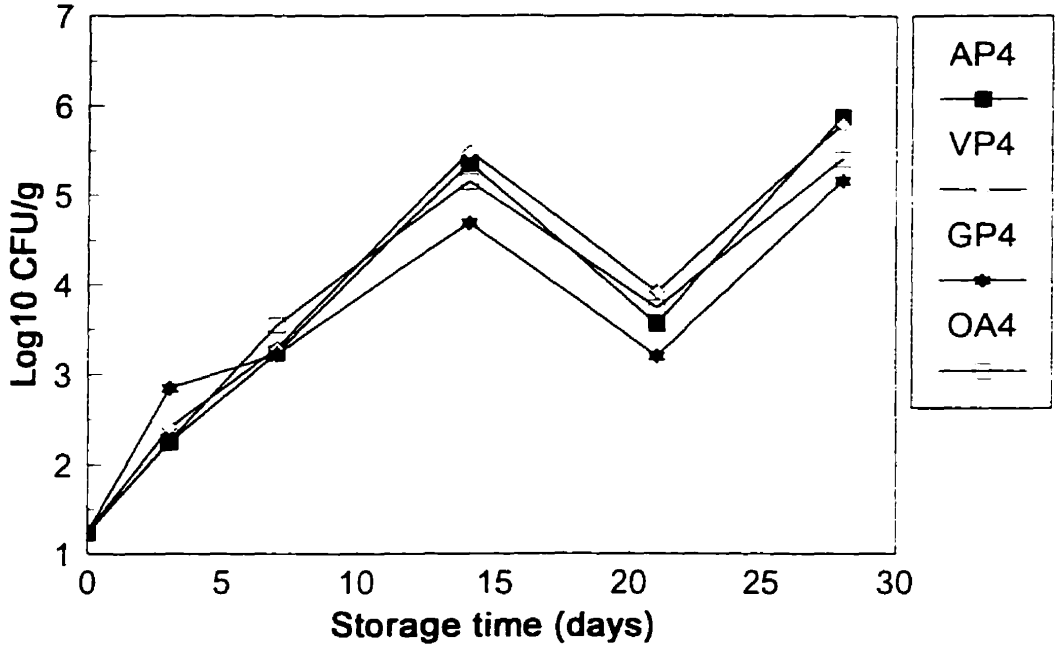
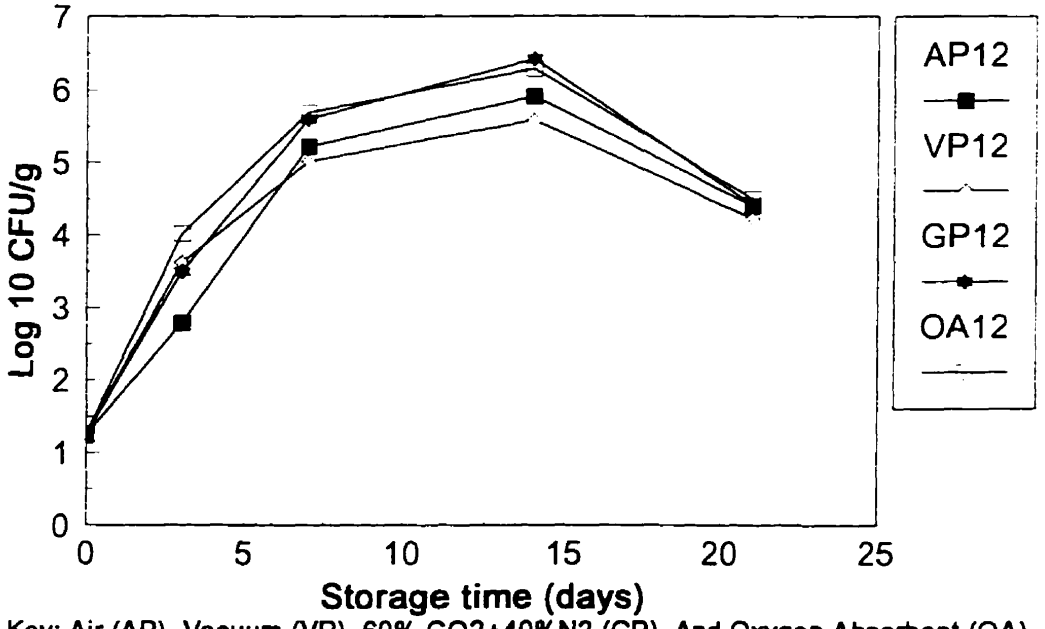


Fig.18b. Growth of faecal Streptococcus in chicken thighs packaged under various gas atmospheres and stored at 12°C.



Key: Air (AP), Vacuum (VP), 60% CO2+40%N2 (GP), And Oxygen Absorbent (OA)

Fig.19a. Growth of Staphylococcus aureus in chicken thighs packaged under various gas atmospheres and stored at 4°C.

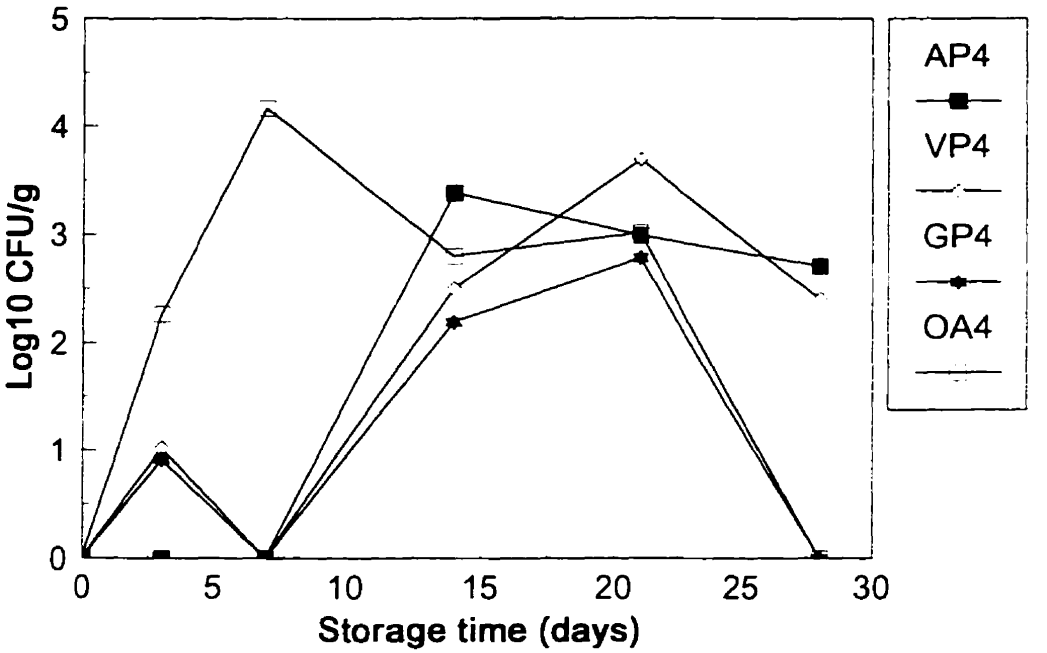
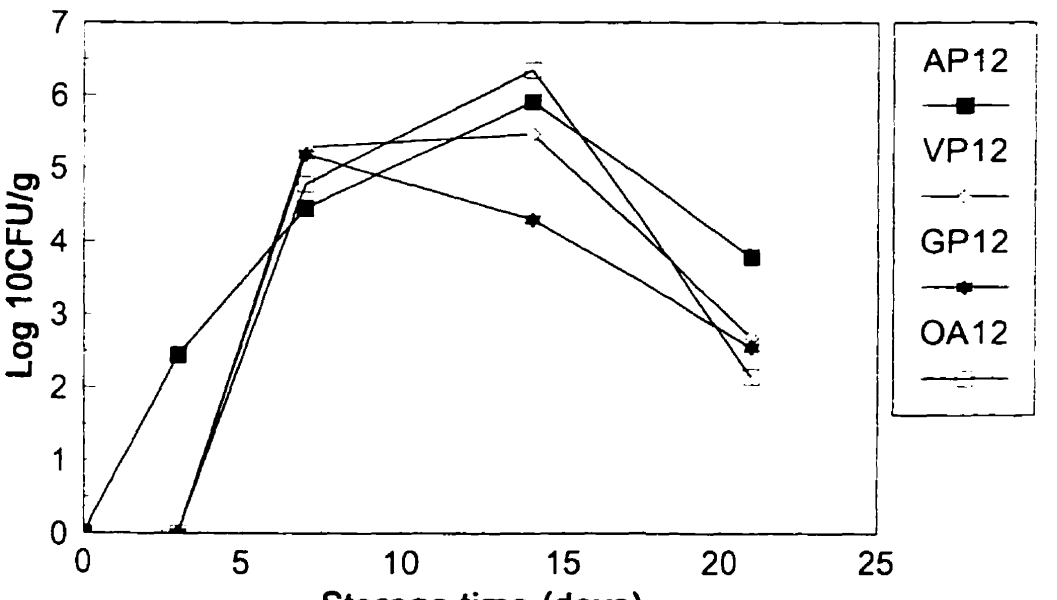


Fig.19b. Growth of Staphylococcus aureus in chicken thighs packaged under various gas atmospheres and stored at 12°C.



Key: Air (AP), Vacuum (VP), 60% CO2+40%N2 (GP), And Oxygen Absorbent (OA)

Fig.20a. Growth of Listeria monocytogenes in chicken thighs packaged under various gas atmospheres and stored at 4°C.

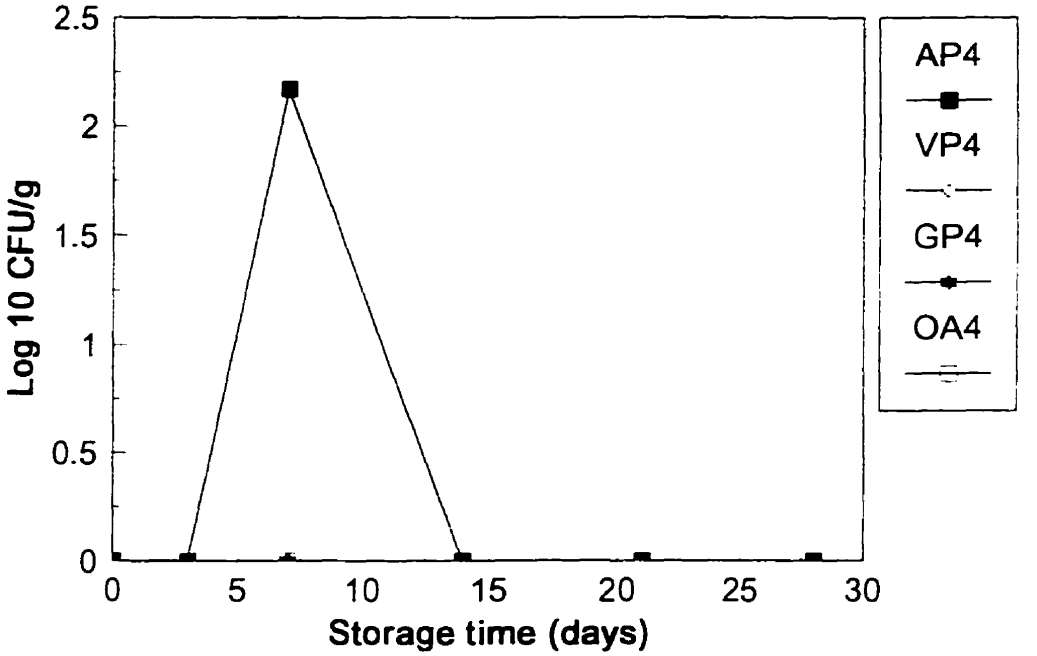
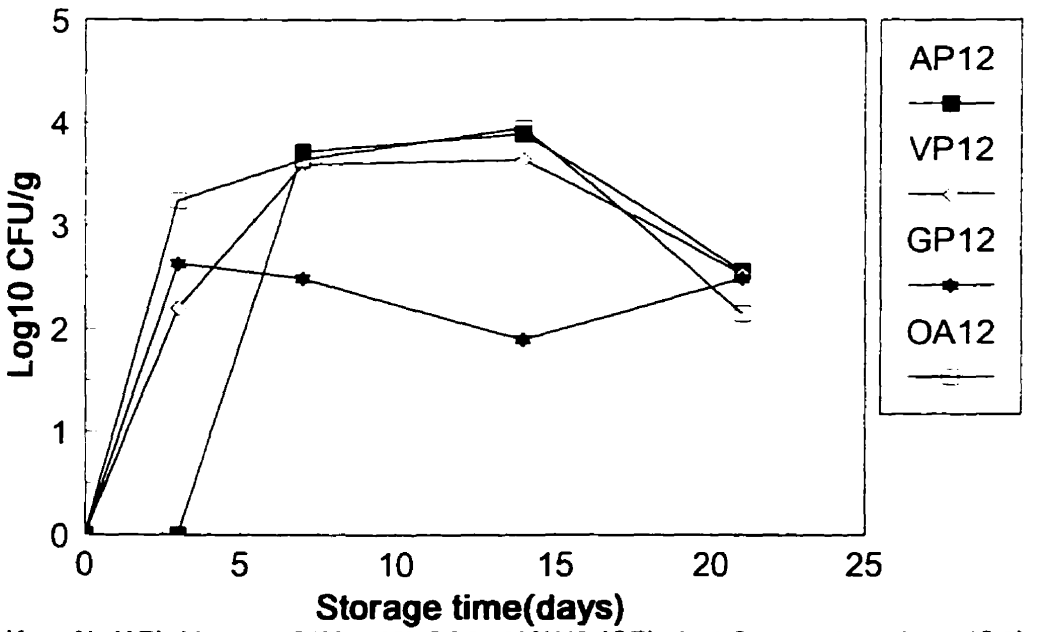


Fig.20b. Growth of Listeria monocytogenes in chicken thighs packaged under various gas atmospheres and stored at 12°C.



Key: Air (AP), Vacuum (VP), 60% CO2+40%N2 (GP), And Oxygen Absorbent (OA)

2.3.6.1. Bacterial isolation and identification

Since APC were used as the indicator of shelf-life acceptability i.e., when counts reached 10^7 CFU/g, these plates were used to isolate and identify the predominant spoilage bacteria and the termination of shelf-life and to monitor shifts in bacterial populations throughout storage. Approximately 20 colonies, representative of the major colony types were isolated from countable plates and examined for their morphological characteristics. The results shown in Table 10, indicates a heterogenous population of gram negative bacteria comprising of *Pseudomonas spp.*, *Flavobacterium spp.*, *Enterobacter aerogenes*, *Klebsiella oxytoca*, *Aeromonas spp.* and *Acinetobacter spp.* The remaining isolates were all gram positive bacteria identified as *Micrococcus spp.* and lactic acid bacteria .The main lactic acid bacteria were subsequently identified as homofermentative strains of *Lactobacillus plantarum* and *Lactobacillus curvatus* and a heterofermentative strain, *Leuconostoc mesenteriodes* (Table 11).

Changes in the microbial populations for the various packaging treatments are shown in Table 12. It is evident that at the onset of spoilage APC's comprised mainly of *Pseudomonas*, *Aeromonas*, *Flavobacterium and Acinetobacter spp.* Indeed these species accounted for ~ 70% of all bacterial types. However, at the termination of shelf-life i.e., APC of $\geq 10^7$ CFU/g, the bacterial population comprised mainly of LAB ranging ~ 45-70% of total bacterial populations depending on storage temperature with homofermentative *Lactobacillus* species being in greater numbers than heterofermentative *L. mesenteriodes*. The shifts in bacterial population can be attributed to depletion of headspace O_2 by aerobic, psychrotrophic strains and the growth of microaerophilic-facultative strains of bacteria tolerant of elevated levels of CO_2.

Table.10. Identification of predominant bacteria Isolated from APC's of chicken thighs stored under various packaging conditions at 4 &12°C.

Characteristics	Bacterial Isolates								
	Enterobacter aerogenes	*Escherichia* spp.	*Pseudomonas* spp.	*Serratia liquefaciens*	*Serratia rubidaea*	*Flavobacterium* spp.	*Acinetobacter* spp.	*Aeromonas* spp.	*Micrococcus* spp.
Gram stain	Gram ve rods	Gram ve rods	Gram ve rods	Gram ve rods	Gram ve rods	Gram ve rods	Gram ve rods	Gram ve rods	Gram ve cocci
Catalase	+	+	+	+	+	+	+	+	+
Oxidase	-	-	+	-	-	+	-	+	+
Motility	+	-	+	+	+	-	-	+	-
ONPG Test	+	+		+	+	+		+	NT
Arginine Dihydrolase	-	-		-	-			+	NT
Lysine Decarboxylase	+	+		+	+			+	NT
Ornithine Decarboxylase	+	-		+	+			-	NT
Tryptophan Deaminase	-	-		-	-			-	NT
Utilization of Citrate	+	+		+	+			+	NT
Production Of									
Hydrogen Sulphide	-	-		-	-			-	NT
Indole	-	+		-	-	+		+	NT
Acetoin	+	+	-	+	-	-	-	+	NT
Urease	-	+	-	-	-	+	-	-	NT
Gelatin Liquefaction	-	-	-	+	-	+	-	+	NT
Fermentation Of									
Glucose	+	+	-	+	+	-	+	+	NT
Mannitol	+	+	-	+	+	-	-	+	NT
Inositol	+	+		+	+		-	-	NT
Sorbitol	+	+		+	+			-	NT
Rhamnose	+	+		+	-			-	NT
Sucrose	+	+	-	+	+			-	NT
Melibiose	+	+		+	+	-	+	+	NT
Amygdalin	+	+	-	+	+	-	-	-	NT
Arabinose	+	+	-	+	+		+	+	NT
Nitrate reduction to NO2	+	+	+	+	+		-	+	NT
Acid From Glucose									
OF O Oxidative	+	+	+	+	+	+	+	+	+
OF F Fermentative	+	+		+	+			+	
Growth On STAA'	NT	NT	NT	NT	NT	NT	NT	NT	-

' Symbols +, positive reaction, -negative reaction

[b] Not Tested

' Streptomycin Thallous Acetate Agar (STAA; Gardner,1966)

Table.11. Identification of lactic acid bacteria isolated from chicken thighs stored under various packaging conditions at 4 & 12°C.

Characteristics	*Lactobacillus plantarum*	*Lactobacillus curvatus*	*Leuconstoc mesenteroides*
Growth on MRS	+*	+	+
Fermentation Of			
Temoin	+		
Glycerol			
Erthythritol			
D Arabinose			
L Arabinose	+		+
Ribose	+	+	
D Xylose			+
L Xylose			
Adonitol			
Beta Methyl D Xyloside			
Galactose	+	+	+
Glucose	+	+	+
Fructose	+	+	+
Mannose	+	+	+
Sorbose			
Rhamnose			
Dulcitol			
Inositol			
Mannitol	+		
Sorbitol	+		
Alpha Methyl D Mannoside	+		
Alpha Methyl D Glucoside	+		+
N Acetyl Glucosamine	+	+	+
Amygdaline	+		+
Arbutine	+		+
Esculine	+		+
Salicine	+	+	+
Cellobiose	+		+
Maltose	+	+	+
Lactose	+	+	+
Melibiose	+		+
Saccharose	+		+
Trehalose	+		+
Inuline			
Melezitose	+		
Raffinose	+		+
Amidon			
Glycogene			
Xylitol			
Gentibiose	+		+
D Turanose	+		+
D Lyxose			
D Tagatose			
D Fucose			
L Fucose			
D Arabitol			
L Arabitol			
Gluconate	+		
2 Keto Gluconate			
5 Keto Gluconate			

* Symbols +, positive reaction, -, negative reaction

Table 12. Shifts in bacterial populations in chicken thighs stored under various packaging treatments at 4 & 12°C.

	Packaging Treatment[b]							
	A		B		C		D	
Bacterial group	Day0	Day 14[c]	Day0	Day 21[c]	Day0	Day 28[c]	Day0	Day 14[c]
Storage at 4°C								
Pseudomonas spp	35	25	35	25	35	30	35	20
Flavobacterium spp	20	0	20	0	20	0	20	0
Lactobacillus spp	5	40	5	55	5	45	5	35
Micrococcus spp.	10	0	10	0	10	0	10	0
Enterobacter aerogenes	15	20	15	0	15	20	15	20
Leuconstoc mesenteriodes	0	5	0	10	0	0	0	15
Aeromonas spp	10	0	10	0	10	0	10	0
Acinetobacter spp	5	0	5	0	5	0	5	0
Serratia spp.	0	5	0	10	0	5	0	0
Klebsiella spp	0	5	0	0	0	0	0	5
Bacterial group	**Day0**	**Day 7**	**Day0**	**Day 7**	**Day0**	**Day 14**	**Day0**	**Day 7**
Storage at 12°C								
Pseudomonas spp	35	25	35	20	35	20	35	15
Lactobacillus spp	20	40	20	45	20	35	20	55
Micrococcus spp	5	0	5	0	5	0	5	0
Flavobacterium spp	10	5	10	0	10	5	10	0
Enterobacter aerogenes	15	10	15	5	15	10	15	5
Leuconstoc mesenteriodes	0	10	0	15	0	15	0	15
Aeromonas spp.	10	0	10	0	10	0	10	0
Acinetobacter spp	5	0	5	0	5	0	5	0
Serratia spp	0	0	0	10	0	10	0	10
Klebsiella spp.	0	10	0	5	0	5	0	0

[a] Each percentage is based on 20 isolates from Aerobic Plate Count 35°C (APC 35°C)

[b] A, Air B, Vacuum C, 60%CO_2:40%N_2 D, Ageless SS.

[c] Storage day of shelf-life termination

2.3.7. Shelf-life

The shelf-life of chicken thighs, estimated from sensory evaluation scores for color and odor i.e., two of the most important factors consumers associate with meat quality and freshness is shown in Table 13. The time (in days) to reach either a color or odor score of 6 (rejection point) was used as an indicator of shelf-life of chicken thighs packaged under various gas atmospheres. It is evident from Table 13, that there is not a good correlation between subjective methods of quality assessment and sensory evaluation and objective methods (microbiological counts). At both 4°C and 12°C, chicken thighs had acceptable odor scores even though most products would be rejected on the basis of microbial counts. This is due to the fact the predominant spoilage microorganisms in all treatments were LAB which produced a distinct, sharp, but not unpleasant, " lactic" odor. Therefore, although counts of APC were high i.e. $\geq 10^7$ CFU/g, and products would be rejected on this basis, products were still regarded as acceptable from an odor viewpoint. Similar results were observed for color. Again, LAB are not as proteolytic as common aerobic psychrotrophic spoilage microorganisms of muscle foods. The color stability of these products may be due to less proteolysis and hence less solubilization of myoglobin pigment. Whatever the reason, these results showed that there was a significant difference between sensory and subjective measurements of shelf-life and objective microbiological tests. In this study, these both tests were taken as a reliable indicator of shelf-life of chicken thighs packaged under various gas atmospheres.

Table 13. Estimated shelf-life of chicken thighs stored under various packaging treatments at 4 and 12°C.

Packaging Treatment[a]	Rejection point/color[b]	Rejection point/odor[b]	Microbial Shelf-life (Days)[c]	Overall Shelf-life (Days)[d]
Storage at 4°C				
A	20	11	7.5	7.5
B	14	14	18 19	14
C	>28	20	>28	28
D	25	28	12.5	12.5
Storage at 12°C				
A	7	5	5	5
B	21	14	5	5
C	11	14	14	14
D	14	14	5	5

[a] A, Air B, Vacuum C, 60%CO_2:40%N_2 D, Ageless SS.

[b] Time (Days) to reach a score of six

[c] Time (Days) to reach a APC count of 10^7 CFU/g.

[d] Earliest rejection point on terms of odor,color and microbial load

Conclusion

From the data obtained, it appears that gas packaging using elevated levels of CO_2 can be used substantially to improve the shelf-life of chicken thighs with minimal changes in their organoleptic properties, i.e., as odor and discoloration. This increase in shelf-life was substantially greater in gas packaged thighs (60% CO_2–40% N_2) than compared to the other two methods of atmosphere modification i.e., vacuum packaging and the use of oxygen absorbent technology. Indeed, packaging of chicken thighs with an oxygen absorbent did not significantly increase in shelf-life over storage in air at both 4°C and 12°C, and may not be considered as a possible alternative to gas packaging. These results were surprising and warrant further investigation perhaps with a larger oxygen absorbent sachet.

The microbiological analyses of chicken thighs stored under different packaging treatments showed that modified atmosphere packaging involving gas flushing inhibited microbial spoilage. The microbial population was lower at 4°C than at 12° C. However, storage of chicken thighs under gas packaging conditions showed that the relatively initial low coliform counts did increase at both storage temperatures throughout the storage period indicating their ability to proliferate under modified atmosphere conditions. Growth of pathogens e.g. *L. monocytogenes* was observed at higher temperatures (i.e., 12°C). Although growth was less in gas packaged thighs, the growth of *L. monocytogenes* was independent of the packaging treatment indicating that the microbiological safety of such products were primarily associated with storage temperatures.

In conclusion, this study has shown that MAP, involving gas flushing could be used to significantly increase the shelf-life and keeping quality of fresh chicken thighs. However, to be effective, strict temperature control must be maintained or products may be a potential public health risk due to the growth of pathogenic bacteria. The safety of MAP products is therefore an issue which must be seriously addressed prior to consumer acceptance confidence in MAP technology of muscle foods.

CHAPTER 3

CHALLENGE STUDIES WITH *SALMONELLA ENTERITIDIS*

3.1. INTRODUCTION

Modified atmosphere packaging (MAP) has been used extensively by the food industry in the preservation of perishable and semi-perishable products. In this research, the packaging technique has shown, to be very effective in the prolonging of the storage shelf life of chicken thighs, particularly at refrigerated temperature. However, *Salmonella* species have consistently been involved in outbreaks of food poisoning involving poultry products. The ability of this microorganism to grow in a microaerophilic environment and, at temperatures as low as 5.2°C, make it a potential public health hazard in refrigerated, MAP products. Therefore, this study was undertaken to study the effect of different modified atmosphere systems on the growth of *S. enteritidis* inoculated onto chicken thighs.

3.2. MATERIALS & METHODS

3.2.1. Microorganism and media preparation

A strain of *Salmonella enteritidis* (*S. enteritidis*^{NAST}) resistant to 100 parts per million (p.p.m.) nalidixic acid and streptomycin sulphate was used for this study. The *S. enteritidis*^{NAST} culture was obtained from Dr. Stephen Knabel, Department of Food Science, Penn State University, U.S.A. The culture was maintained at 4°C on Tryptic Soya Broth with Yeast Extract (TSBYE) (Difco, Michigan, USA) supplemented with 100 p.p.m. nalidixic acid (ICN, Biomedicals, Ohio) and streptomycin sulphate (Sigma, St.Louis, USA). The strain was subcultured every 21d at 35-37°C to ensure its viability. For the preparation of antibiotic TSBYE^{NAST}, a stock solution of both nalidixic acid and streptomycin sulphate (1%w/v) was

prepared by adding 0.2 grams of nalidixic acid and streptomycin sulphate to 20 ml of sterile water. The stock solution of each antibiotic was then sterilized using a pre-sterilized membrane filtration unit (Nalgene Co., Rochester, NY). Appropriate amounts of the antibiotic stock solutions were added at 45°C to pre-sterilized TSBYE broth to give a final concentration of 100 p.p.m. of each antibiotic in the media. Both stock solutions of nalidixic acid and streptomycin sulphate were covered in aluminium foil and stored in the dark at 4°C.

3.2.2. Growth curve

Prior to the start of the growth study, a fresh inoculum of the stock culture was prepared by inoculating *S. enteritidis* in a test tube of TSBYE broth containing 100 p.p.m. nalidixic acid and streptomycin sulphate and incubated at 37°C for 18-24h. To initiate the growth study, 0.1 ml of the stock culture was transferred into freshly prepared 10 ml TSBYE broth supplemented with the appropriate amounts of antibiotics and incubated at 37°C. Growth was determined by removing the cultures from the incubator every hour for ~11h and determining the optical density using a spectrophotometer (LKB, Novopake 4049, England) set at a wavelengh of 625 nm. Serial dilutions and enumeration of growth using a pour plate method on Tryptic Soya Agar (Difco, Michigan, USA) was as previously described **(See Section 2.2.2.6.)**. Plates were incubated at 37°C and colonies enumerated after 18-24h using a Darkfield Quebec Colony Counter (AO Scientific Instruments, Quebec, Canada). A plot of microbial growth versus time and optical density versus bacterial growth are shown in Figures 21-22 respectively.

3.2.3. Preparation of inoculum

A nalidixic acid & streptomycin sulphate resistant strain of *Salmonella enteritidis* (*S. enteritidis*^{NAST}) was used in this study. The stock cultures were kept frozen (-18°C) in Tryptic Soya Broth supplemented with 0.6% yeast extract (TSBYE), and with 50 % (v/v) glycerol. A working stock culture was prepared by inoculating *S. enteritidis*^{NAST} into TSBYE broth at

37°C for 24h and then maintained on TSA^{NAST} slants stored at 4 C. The cultures were routinely checked for purity by streaking onto TSA^{NAST} media and random colonies were selected and confirmed by gram stain and by the *Salmonella* rapid test (Oxoid, London, U.K.). When required for this study, cultures were transferred onto TSBYE and grown at 37°C for 24h, and then subcultured onto fresh TSBYE broth for 10 h at 37 °C. Cultures were then serially diluted in 0.1% buffered peptone water to give an initial inoculum level of approximately 10^5 CFU/ml.

3.2.4. Inoculation/ packaging of chicken thighs

Frozen chicken thighs were obtained from Club Price, Montreal. After thawing at 4°C overnight, thighs were placed separately (~50g) in 210 × 210 mm high gas barrier bags (Cryovac, Canada). The chicken thighs were then inoculated with 0.5 ml of *S. enteritidis*^{NAST} as prepared previously to give a final inoculum level of 10^3 CFU/g **(See Section 3.2.3.)**. All samples were packaged as described previously **(See Section 2.2.3.)** and stored at 4°C and 12°C for up to 28d.

3.2.5. Headspace gas composition

On each sampling day, headspace gas composition was determined on packages prior to opening. Gas samples for analysis were taken through an adhesive septum placed on the surface of the package using a gas tight probe. Samples were then injected into a previously calibrated Servomex O_2/CO_2 gas analyzer (Norwood, MA , USA).

3.2.6. Color analyses

Color analysis of packaged samples was measured as previously described **(See Section 2.2.2.2.)**.

3.2.7. Drip loss

Drip loss was measured as previously described **(See Section 2.2.2.3.)**.

3.2.8. Sensory, pH and microbiological analyses

Triplicate samples from each treatment were examined after 0,3,7,14,21 and 28 d. After the packages were opened aseptically, the appearance (color) and odor of each sample was assessed as previously described **(See Section 2.2.2.5.)**. Chicken thighs, including drip loss, were placed into a sterile stomacher bag containing 450 ml of sterile 0.1% buffered peptone water and stomached for 1 min. The pH of each resultant mixture was measured using a previously calibrated pH meter (Model 2220, Corning Glass Works, Corning N.Y.). Serial dilutions were prepared from the homogenate and 0.1 ml of the appropriate dilutions were plated (in duplicate) using a spread plate technique onto TSA media supplemented with 100ug/ml of nalidixic acid and streptomycin sulphate. All plates were enumerated for *S. enteritidis*^{NAST} after 48 h at 37°C.

3.2.9. Statistical analyses

Data were analyzed in the General Linear Model procedure (SAS,1988) using a split plot design in which the storage time was the main plot effect and the storage temperature and the packaging treatment were the subplot effect. Bacterial counts were expressed as $\log_{10}$ CFU/g and the reported means were the average of three replicates per treatment. Differences between means were analyzed using the least significant difference (LSD) and the Duncan multiple range test. A probability (P) of <0.05 were considered to be significantly different.

3.3. RESULTS AND DISCUSSION

3.3.1. Growth curve of *S. enteritidis*

The growth curve for *S. enteritidis* in $TSBYE^{NAST}$ is shown in Figure 21. It is evident that the maximum growth (~10^9 CFU/ml) was reached after 10h at 37°C. Furthermore, there was a significant correlation between optical density (O.D.) and growth (Figure 22). Thus, an O.D. of 0.7 at 625nm would result in a stock culture of *S. enteritidis* containing ~10^9 CFU/ml. This method was used continously throughout this study to produce a stock culture containing ~10^9 CFU/ml.

3.3.2. Changes in headspace gas composition

Changes in headspace gas composition for chicken thighs packaged in various atmospheres and stored at 4°C and 12°C are shown in figures 23-26 a,b respectively.

For air packaged samples at 4°C, headspace O_2 decreased to less than 2% and headspace CO_2 increased to approximately 25% after 21d (Figure 23a). Similar changes were also observed for air packaged samples stored at 12°C (Figure 23b). However, in this case, headspace O_2 decreased rapidly to less an 1% and CO_2 increased to 23% after 14d. These changes in gas composition can be largely attributed to meat tissue and/or microbial respiration (Gill and Tan, 1980).

For gas packaged samples, the initial headspace O_2 was negligible and remained unchanged at both 4°C and 12°C throughout the storage trial. However, the initial headspace CO_2 declined to approximately 46% and then gradually increased to approximately 56% after 21d at 4°C (Figure 24a). Similar trends were also observed for the gas packaged samples at higher temperatures (12°C). These changes in CO_2 concentration during the initial stages of the storage period are due to the solubility of CO_2 into the meat tissue (Seideman et al., 1979).

For samples packaged under vacuum or with an Ageless SS oxygen absorbent, similar changes in headspace gas composition occurred. Headspace O_2 rapidly decreased to less than 1% and remained at this low level at both 4°C and 12°C throughout storage (Figures 25-26 a,b). Headspace CO_2 increased to 4.13 % after 14d of storage at 4°C. Similar but more dramatic trends, were observed for headspace CO_2 at 12°C which increased to approximately 13.8% after 14d. This increase in CO_2 can again be attributed to the growth of microaerophilic/facultative anaerobes that utilize O_2 and produce CO_2 in the packaged product.

Fig.21. Growth curve of nalidixic acid & streptomycin sulphate resistant S. enteritidis.

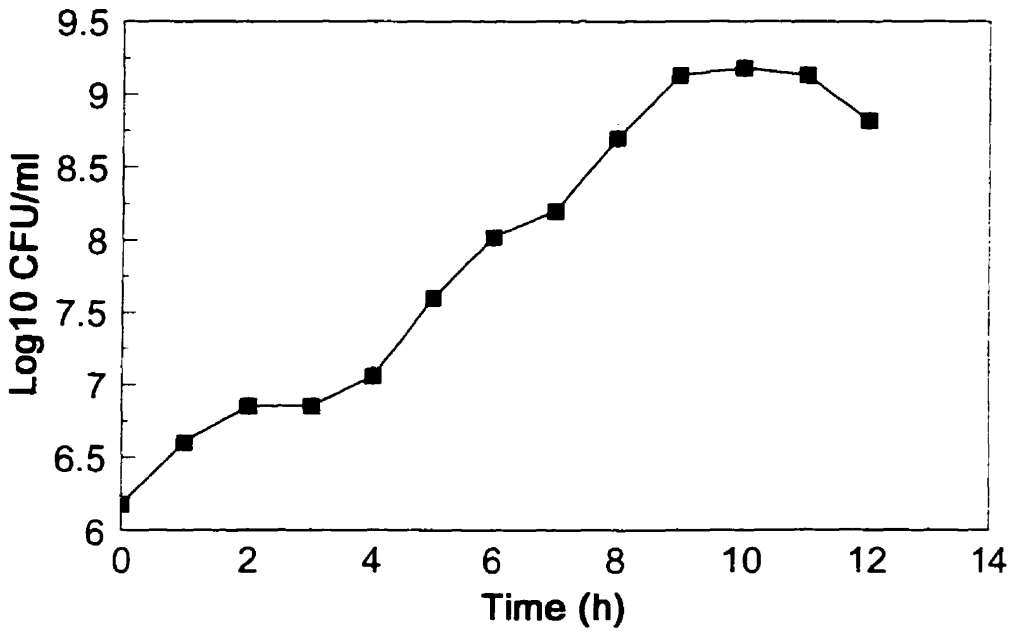


Fig.22. Comparison of optical density (O.D.) versus Log 10 CFU/ml for nalidixic acid & streptomycin resistant S. enteritidis.

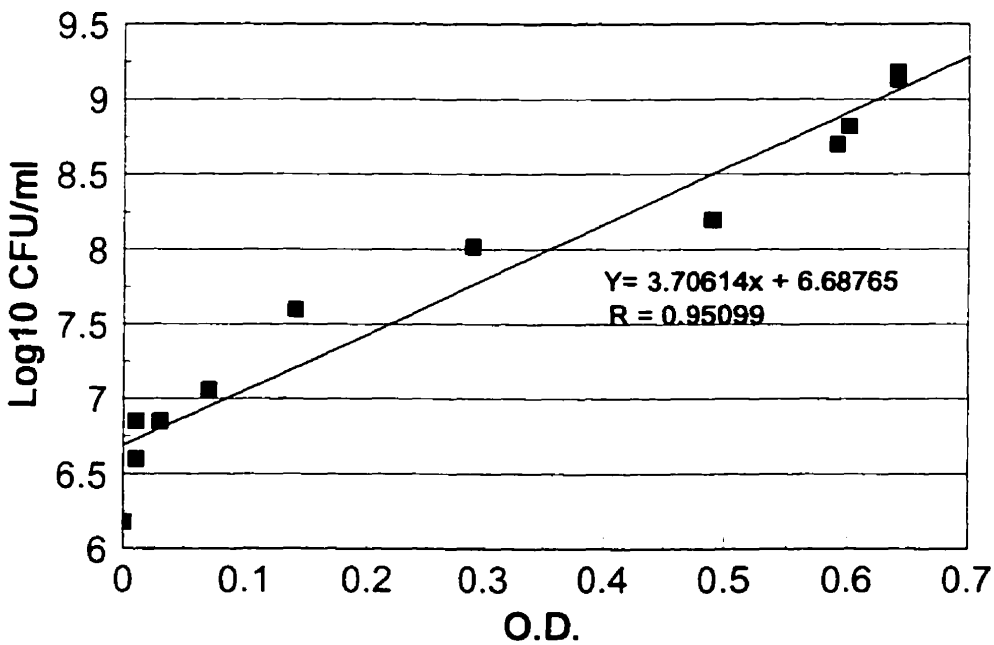


Fig.23a. Changes in headspace gas composition of chicken thighs inoculated with S. enteritidis stored in air at 4°C.

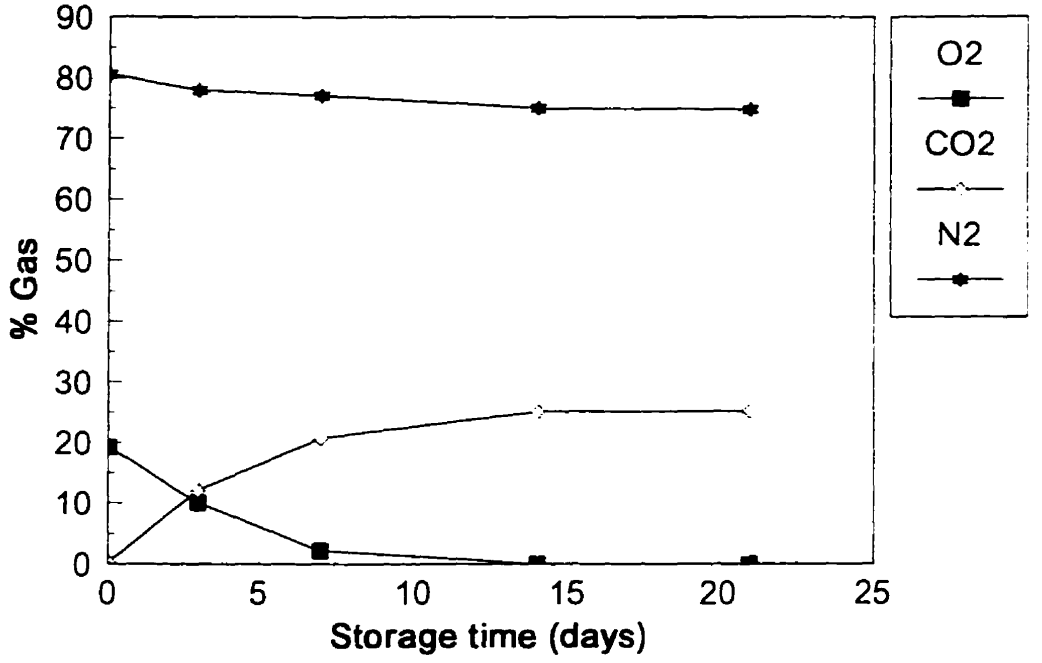


Fig.23b. Changes in headspace gas composition of chicken thighs inoculated with S. enteritidis stored in air at 12°C.

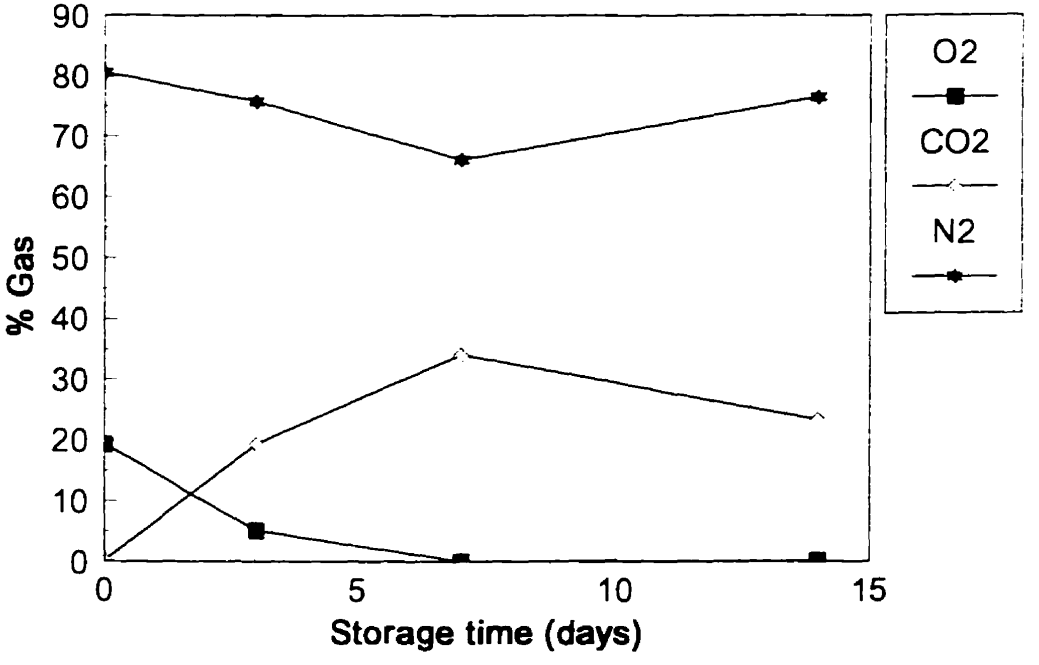


Fig.24a. Changes in headspace gas composition of chicken thighs inoculated with S. enteritidis stored in 60% CO2+ 40%N2 at 4°C.

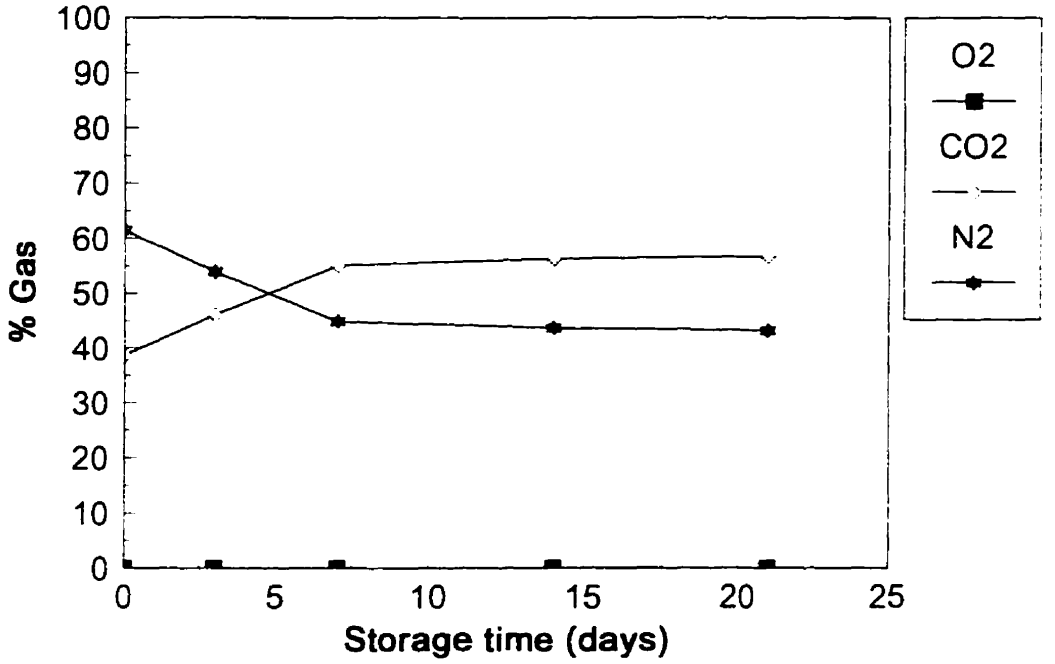


Fig.24b. Changes in headspace gas composition of chicken thighs inoculated with S. enteritidis stored in 60% CO2+ 40%N2 at 12°C.

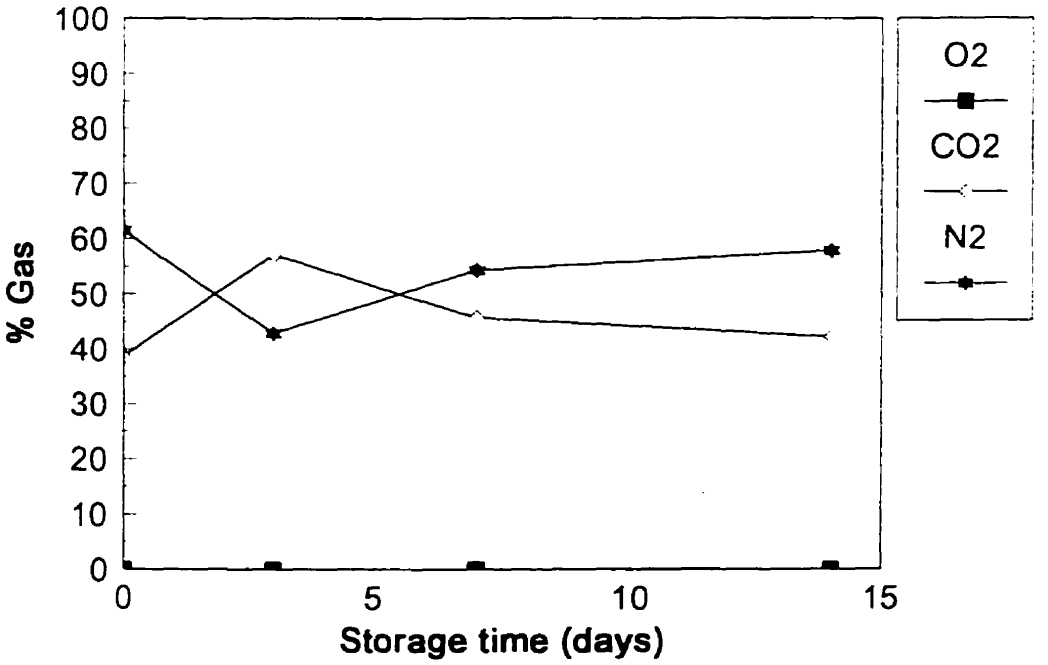


Fig.25a. Changes in headspace gas composition of chicken thighs inoculated with S. enteritidis stored with under vacuum at 4°C.

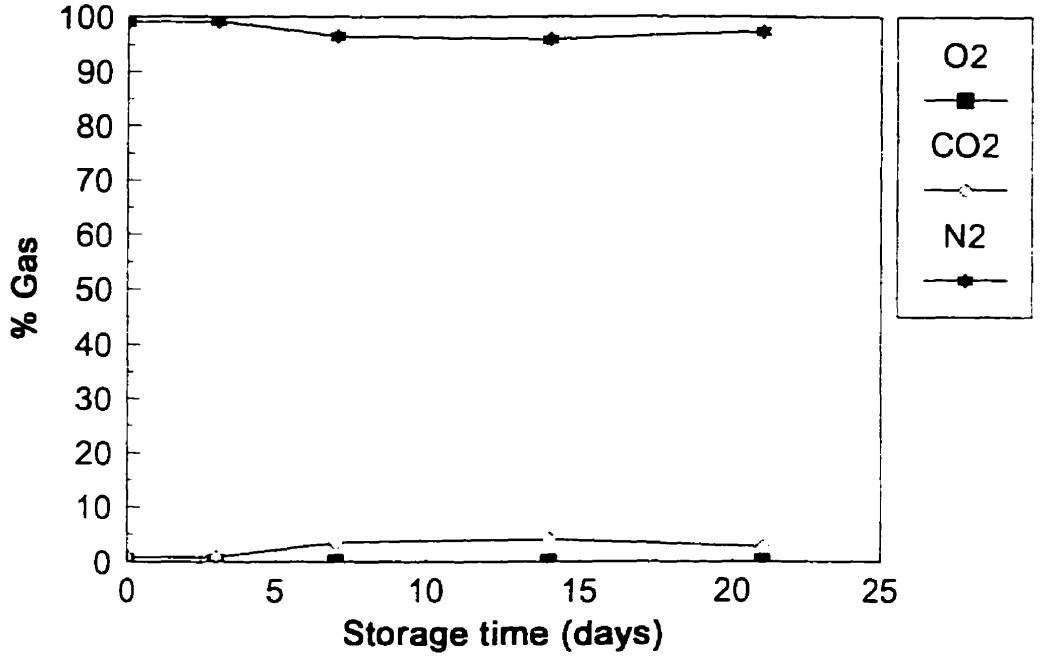


Fig.25b. Changes in headspace gas composition of chicken thighs inoculated with S. enteritidis stored under vacuum at 12°C.

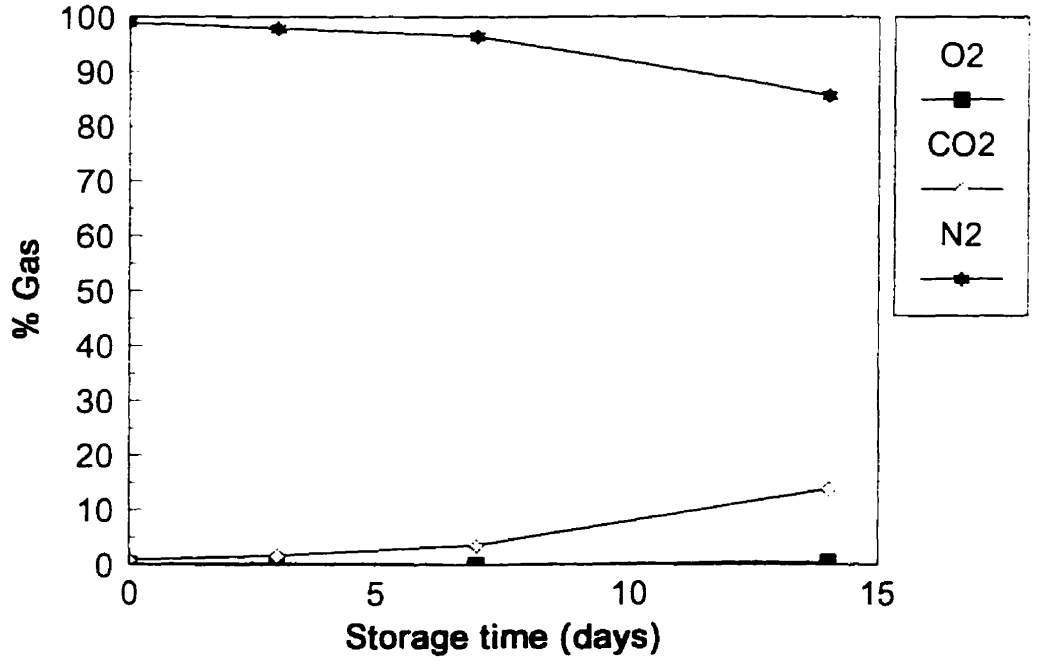


Fig.26a. Changes in headspace gas composition of chicken thighs inoculated with S. enteritidis stored with oxygen absorbent at 4°C.

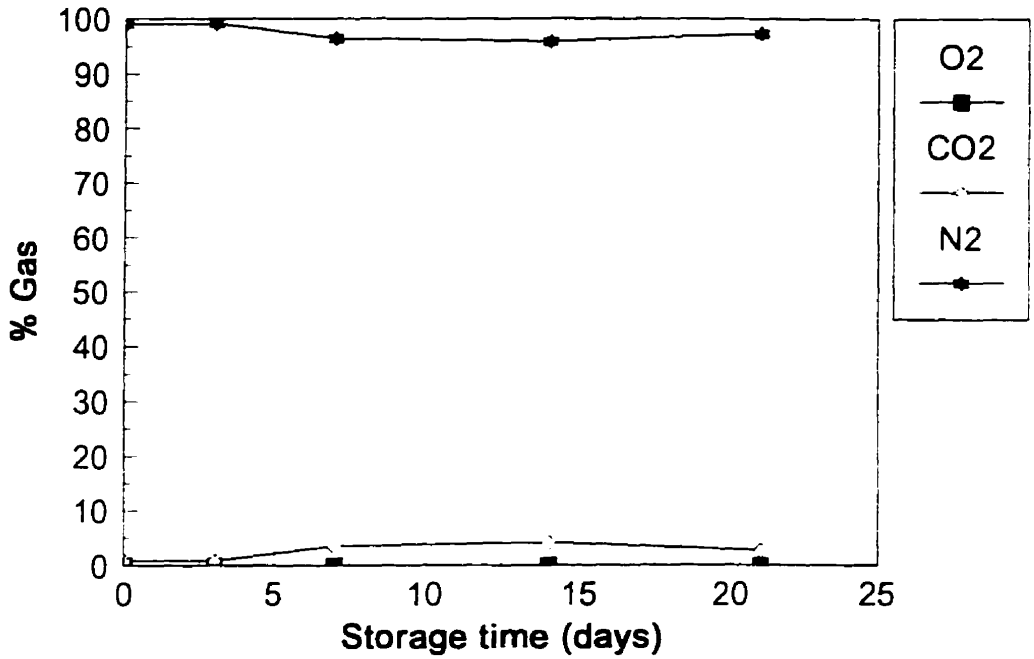
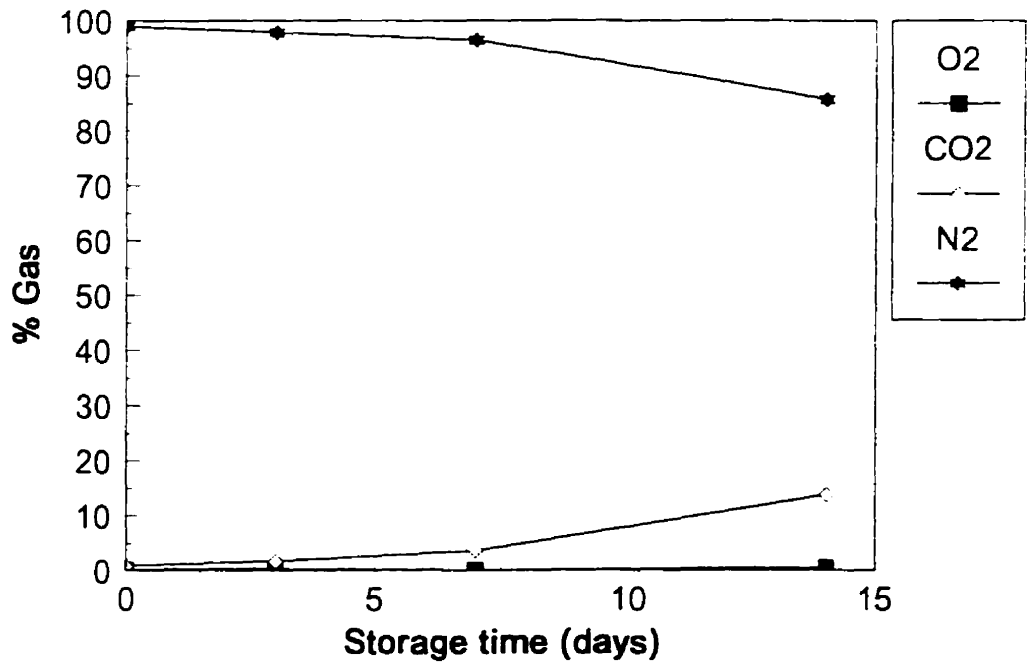


Fig.26b. Changes in headspace gas composition of chicken thighs inoculated with S. enteritidis stored with oxygen absorbent at 12°C.



3.3.3. Color analyses

Changes in the L* (lightness), a* (redness) and b* (yellowness) color values for the different packaging treatments of chicken thighs are shown in Figures 27-29 a,b respectively.

For products stored at 4°C, L* values increased significantly ($P<0.05$) with storage time (Figure 27a). However, L* values did not differ significantly ($P<0.05$) among treatments. This may be attributed to less oxidation of the myoglobin pigment to darker metmyoglobin or less drip loss and hence less solubilization of the myoglobin pigment in all packaged products. Similar changes in L* values were observed for treatments stored at 12°C. However, in this case, L* values were significantly greater ($P<0.05$) at the higher storage temperature (12°C) than at 4°C. These results were somewhat contradictory from L* changes during storage trials and can only be explained by intrinsic variations in the muscle proteins.

Changes in both a* and b* values fluctuated throughout storage (Figures 28-29 a,b respectively). Generally, chicken thighs had a consistently higher a* values than b* values indicating that samples were more red than yellow. At 4°C, a* values tended to increase throughout storage (Figure 28a) while b* values decreased (Figure 29a) i.e., chicken thighs became more red and less yellow. This trend could again be attributed to less oxidation of myoglobin to metmyoglobin in packaged products. At 12°C, a* values increased in all treatments, then rapidly decreased for the air and the vacuum packaged samples at the end of the storage trial (Figure 28b). Similar trends were also observed for b* values at higher storage temperature (Figure 29b).

Although variation in skin color was observed between treatments by panellists during sensory analysis, the L*, a* and b* values did not confirm any significant color differences. Thus, changes in color when measured objectively may not be a reliable indicator of color stability and hence safety of MAP products compared to subjective methods.

Fig.27a. Changes in L* coordinates of chicken thighs inoculated with S. enteritidis at 4°C.

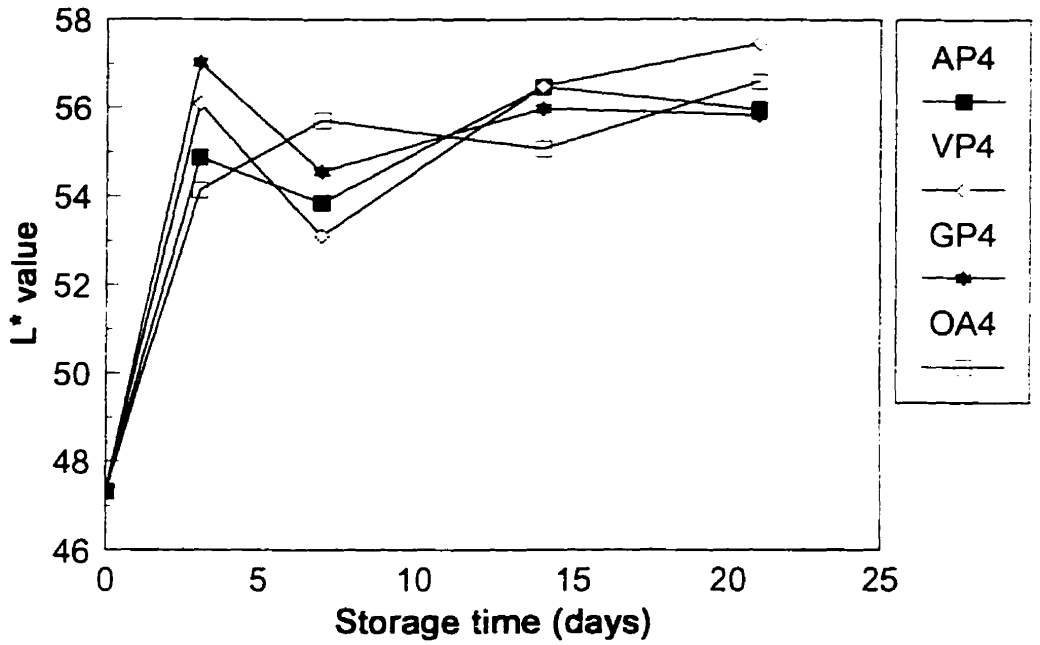
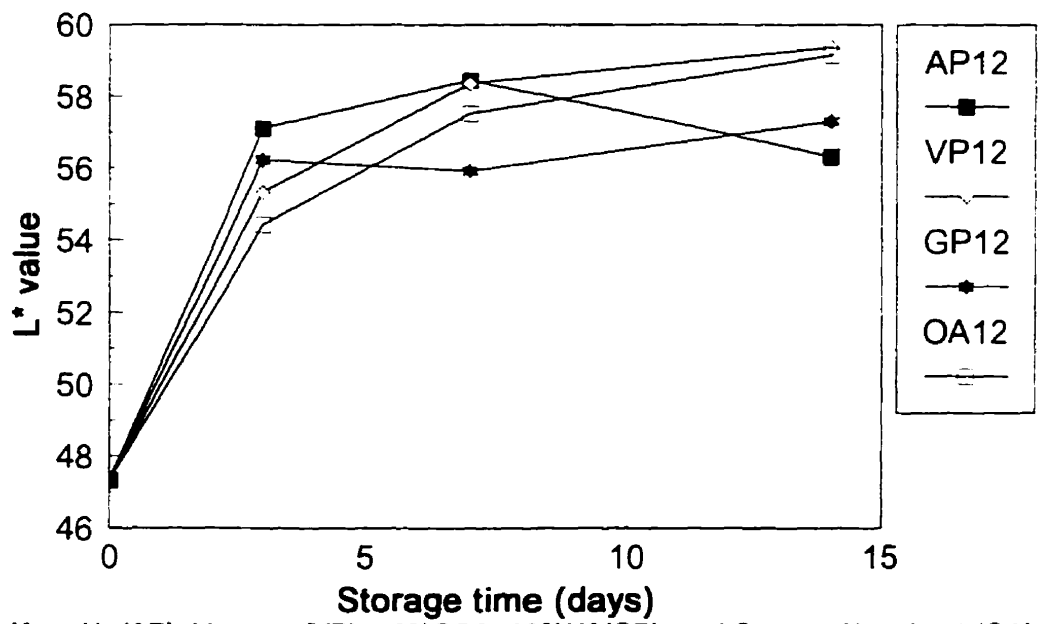


Fig.27b. Changes in L* coordinates of chicken thighs inoculated with S. enteritidis at 12°C.



Key: Air (AP), Vacuum (VP), 60%CO2+40%N2(GP), and Oxygen Absorbent (OA)

Fig.28a. Changes in a* coordinates of chicken thighs inoculated with S. enteritidis at 4°C.

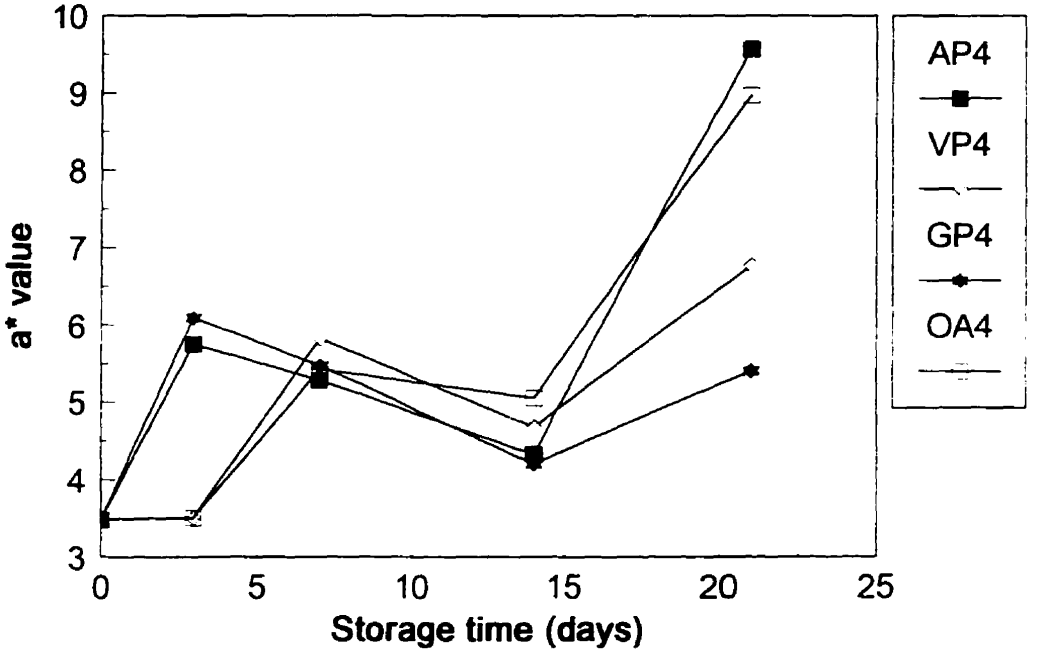
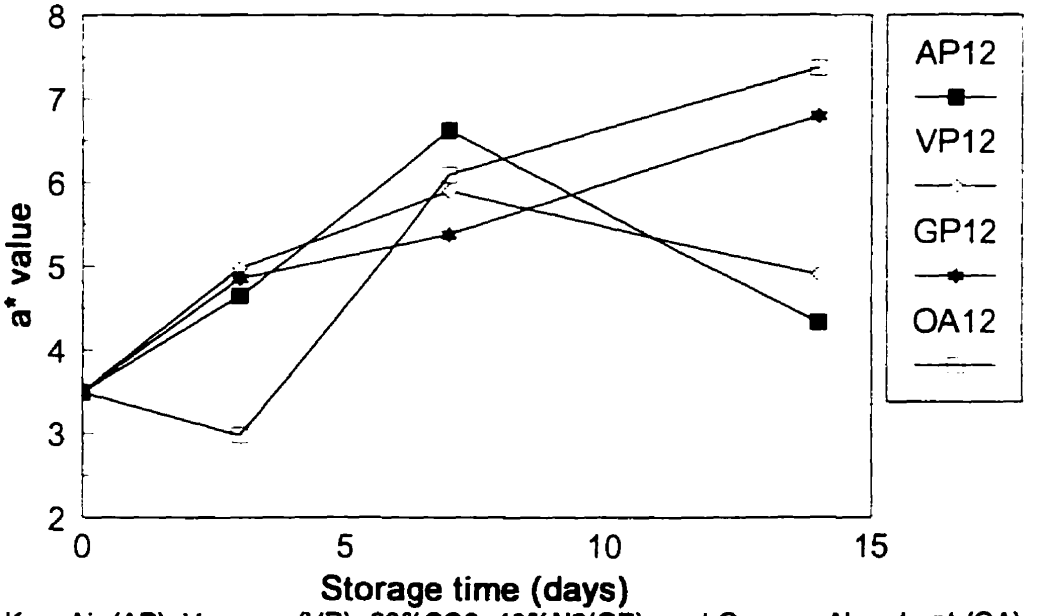


Fig.28b. Changes in a* coordinates of chicken thighs inoculated with S. enteritidis at 12°C.



Key: Air (AP), Vacuum (VP), 60%CO2+40%N2(GP), and Oxygen Absorbent (OA)

Fig.29a. Changes in b* coordinates of chicken thighs inoculated with S. enteritidis at 4°C.

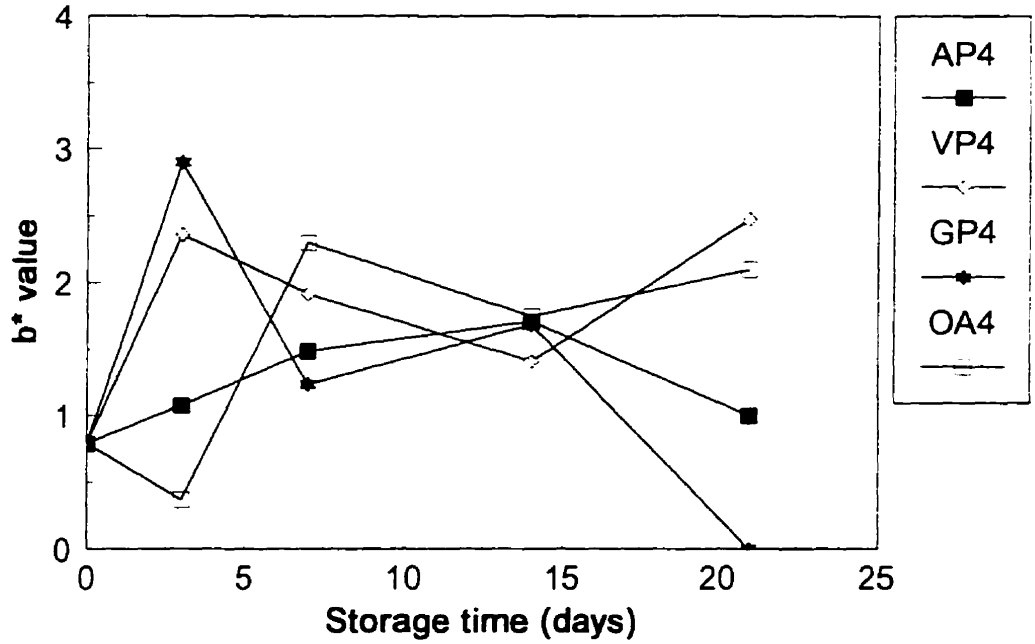
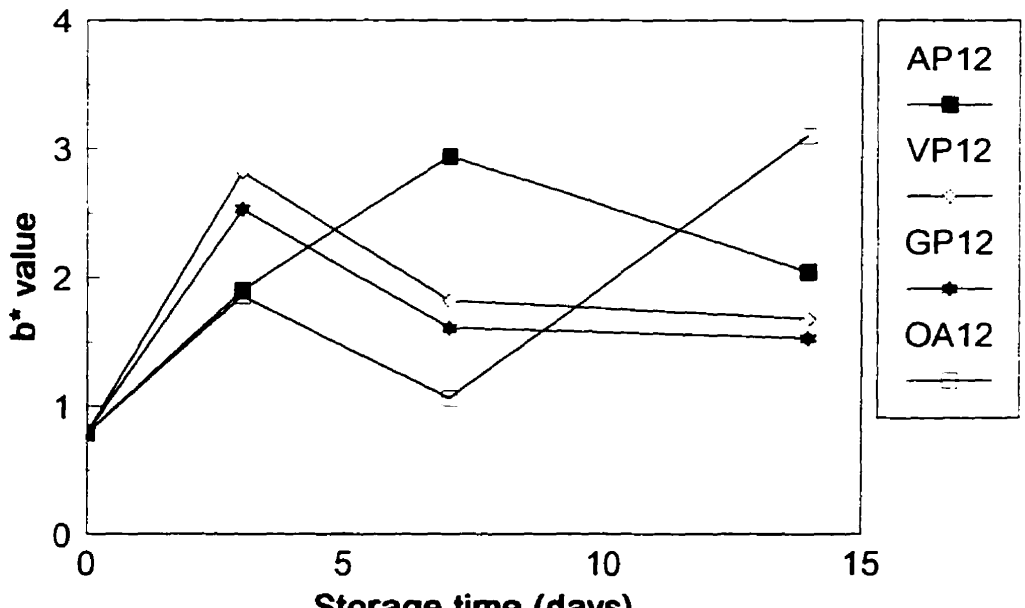


Fig.29b. Changes in b* coordinates of chicken thighs inoculated with S. enteritidis at 12°C.



Key: Air (AP), Vacuum (VP), 60%CO2+40%N2(GP), and Oxygen Absorbent (OA)

3.3.4. Drip loss

The drip loss of chicken thighs (% w/w) inoculated with *S. enteritidis* and packaged in air, vacuum, 60%CO_2+40%N_2 and with Ageless SS oxygen absorbent is shown in Figures 30 a,b respectively. Storage temperature, packaging treatment and storage time all had a significant ($P<0.05$) effect on drip loss. Generally, drip loss increased with storage time and temperature. Drip loss was greater in vacuum packaged samples than all other packaging treatments.This may be due to the effect of the strong vacuum on soft muscle resulting in greater drip (Figures 30 a,b). Drip loss from chicken packaged in 60%CO_2+40%N_2 and with an oxygen absorbent did not differ significantly ($P<0.05$) and showed significantly less drip compared to vacuum packaged samples (Figure 30a). For example, drip loss in samples stored in 60%CO_2+40%N_2 or with an oxygen absorbent were 3.97% and 4.5% respectively compared to 8.68% for the vacuum packaged samples after 14d at 4°C (Figure 30a). O'Keefe and Hood (1981) reported that packages containing high levels of N_2 displayed less drip loss. Therefore, the decrease in drip loss in samples packaged in 60%CO_2+40%N_2 or with an oxygen absorbent could be attributed to less microbial growth and/or to high concentration of nitrogen in the package headspace. Another reason may be that absence of a strong vacuum in these treatments resulting in less drip loss.

Fig.30a. Changes in drip loss (%w/w) of chicken thighs inoculated with S. enteritidis at 4°C.

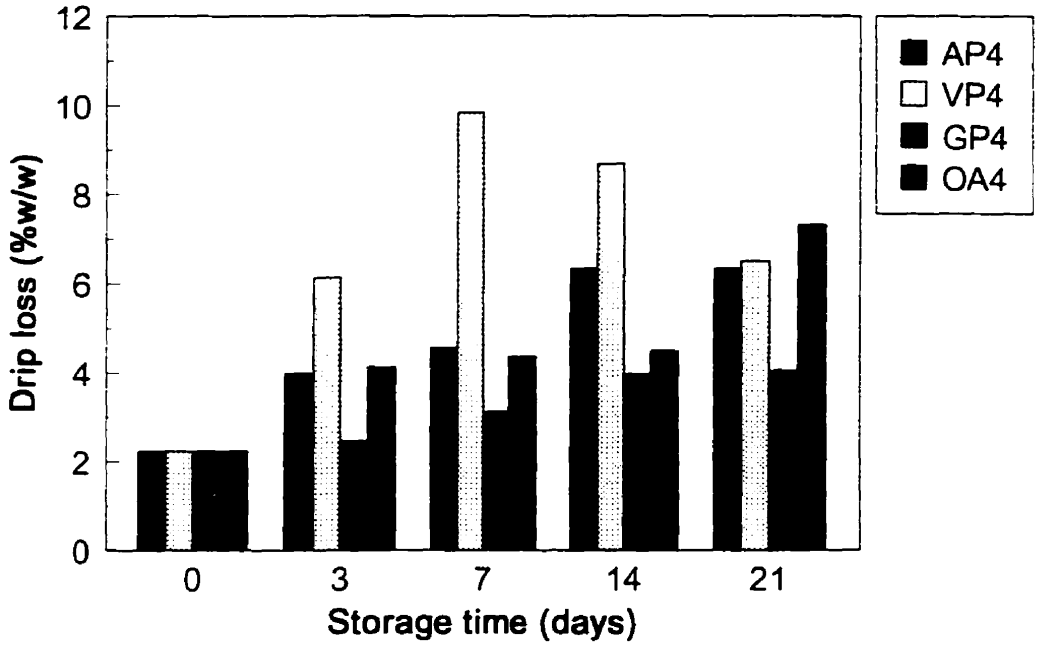
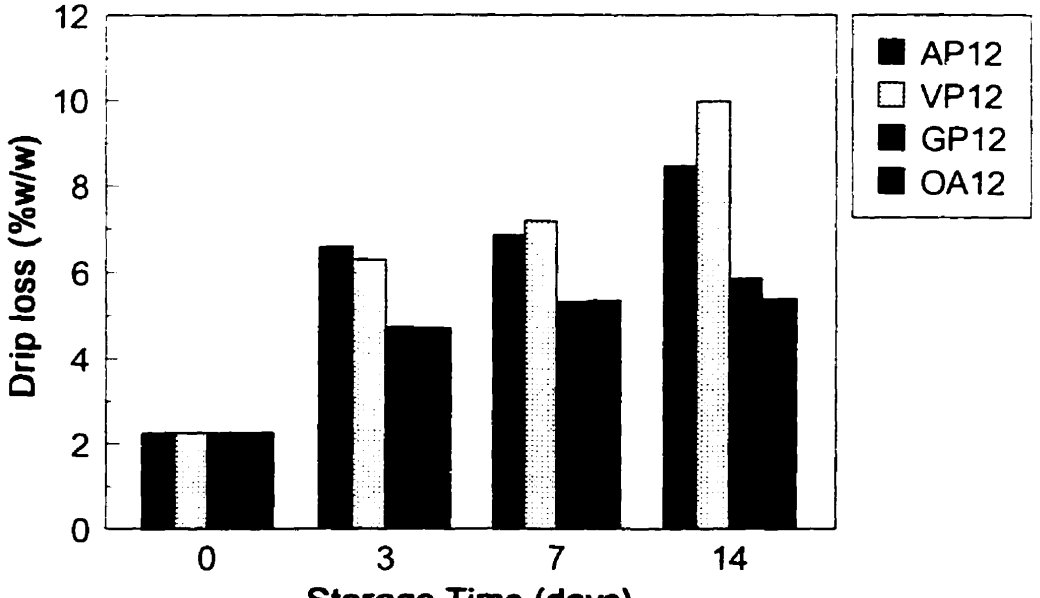


Fig.30b. Changes in drip loss (%w/w) of chicken thighs inoculated with S. enteritidis at 12°C.



Key: Air (AP), Vacuum (VP), 60%CO2+40%N2(GP), and Oxygen Absorbent (OA)

3.3.5. Sensory analyses

The sensory quality scores (color&odor) of chicken thighs are shown in Figures 31-32 a,b respectively. Surface discoloration was greatest ($P<0.05$) in samples packaged in air or with an oxygen absorbent reaching an unacceptable score of six after 10-12d at both 4 and 12°C (Figure 31 a,b). Vacuum packaged samples and samples packaged in 60%CO_2+ 40%N_2 were the most stable with respect to color at both storage temperatures (Figure 31 a,b). In addition, the rate of discoloration increased with increasing temperature. Oxidation of myoglobin to metmyoglobin, which is responsible for the discolouration of muscle, usually occurs at low partial pressures of O_2. O'Keefe and Hood (1981) suggested that an O_2 level of 0.1% or less was required to prevent discoloration of meat. The observation that minimal discoloration occurred in samples packaged in 60%CO_2+40%N_2 after 21d at 4°C supports these findings as the oxygen levels were consistently below the critical limits for discoloration to occur. By comparison, surface discoloration occurred in samples packaged in an oxygen absorbent after 14d at 4°C, even though the O_2 concentration decreased below the critical limits (<0.1%) in the early stages of storage. Surface discoloration in these packages may be due to microbial deterioration by microaerophilic/facultative anaerobes, rather than the development of metmyoglobin.

Similiar results were obtained by panellists for off odor (Figures 32 a,b). Samples packaged in air had significantly higher odor scores than all other packaging treatments at both 4°C and 12°C (Figure 32 a,b). Odor scores of chicken thighs packaged with an oxygen absorbent usually were similiar to those of the air packaged samples stored at 12°C (Figure 32 b). Samples packaged in 60%CO_2+ 40%N_2 had significantly ($P<0.05$) lower odor scores than all other packaging treatments at both 4 and 12°C. The sensory odor of samples packaged in 60%CO_2+ 40%N_2 remained acceptable for 20d at 4°C compared to 16d at 12°C. These results confirm our previous observations that gas packaging inhibited the aerobic spoilage bacteria resulting in a lower rate of proteolysis and off-odors.

Fig.31a. Changes in sensory color of chicken thighs inoculated with S. enteritidis at 4°C.

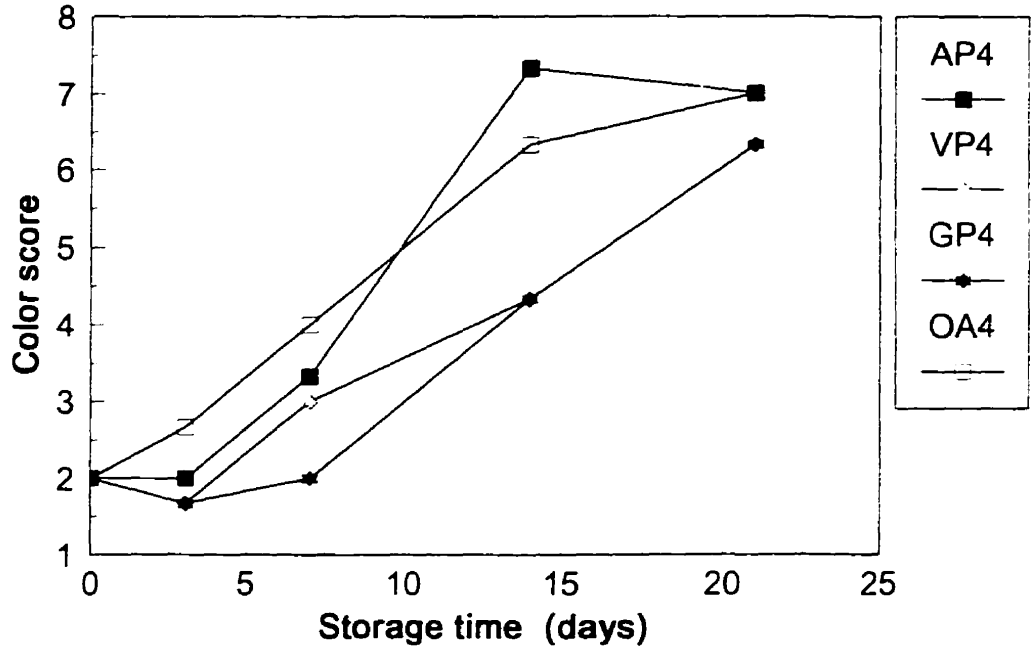
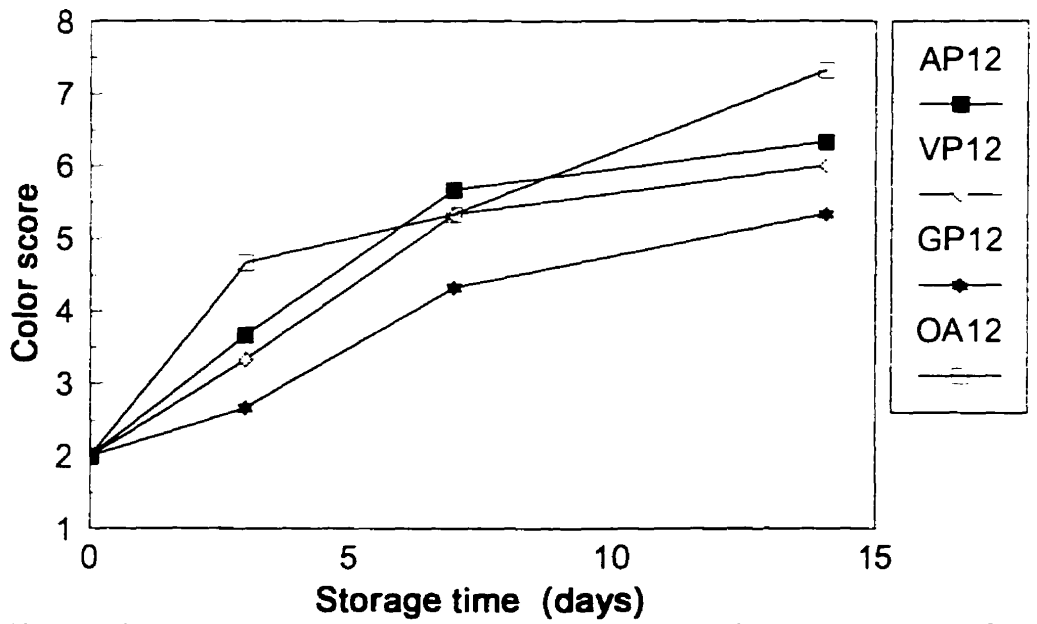


Fig.31b. Changes in sensory color of chicken thighs inoculated with S. enteritidis at 12°C.



Key: Air (AP), Vacuum (VP), 60%CO2+40%N2(GP), and Oxygen Absorbent (OA)

Fig.32a. Changes in sensory odor of chicken thighs inoculated with S. enteritidis at 4°C.

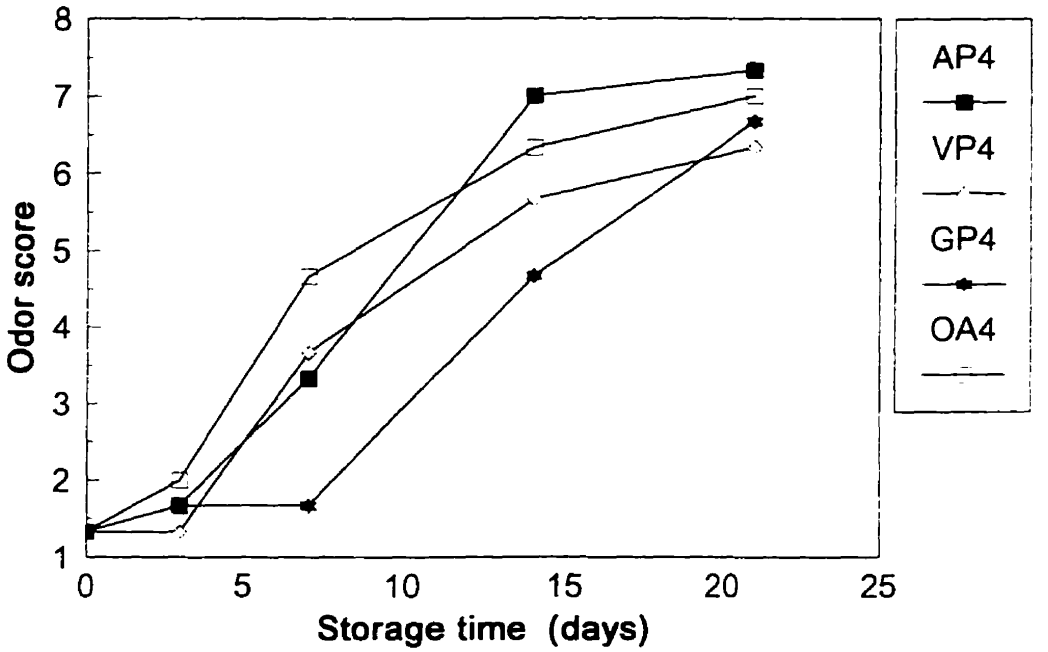
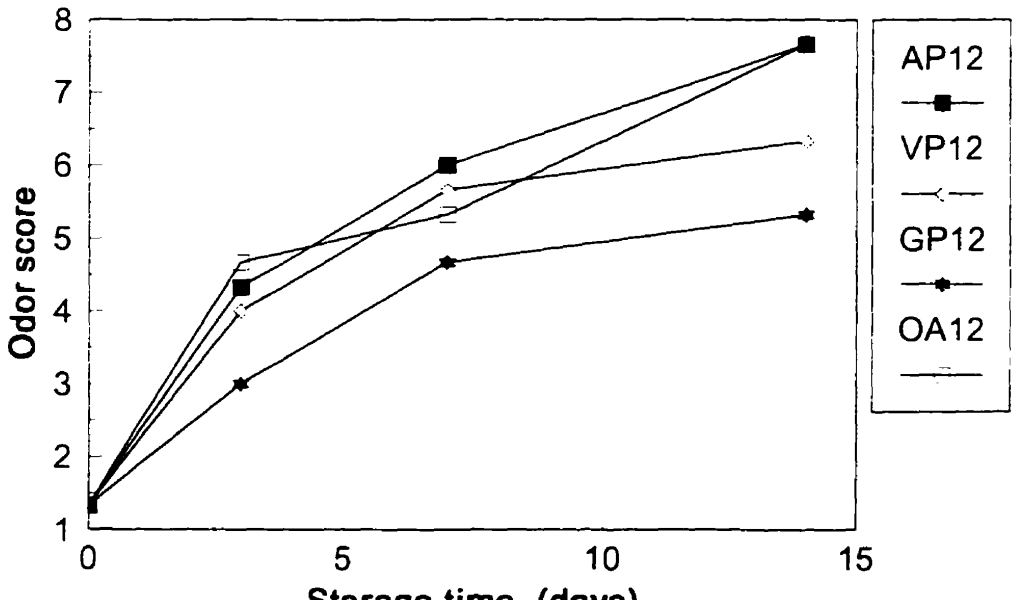


Fig.32b. Changes in sensory odor of chicken thighs inoculated with S. enteritidis at 12°C.



Key: Air (AP), Vacuum (VP), 60%CO2+40%N2(GP), and Oxygen Absorbent (OA)

3.3.6. PH

The surface pH of chicken thighs inoculated with *S. enteritidis* packaged in various atmosphere conditions and stored at 4 and 12°C are shown in Figure 33 a,b. The pH of chicken thighs did not change significantly ($P<0.05$) in any of the different packaging treatments throughout storage and remained within 0.3 pH units of the initial pH of 6.3. It has been previously reported that the growth of *Salmonella* occurs between pH 4 and 9.6 with optimum growth near pH 7 (Farber,1989). Thus, these results indicate that chicken thighs may be conducive to the growth of *S. enteritidis* in the packaged samples. The lack of significant pH change confirmed previous observations in storage trials and was attributed to the buffering effect of proteins.

3.3.7. Microbial analyses

The growth of *S. enteritidis* on chicken thighs packaged in air, vacuum, 60%CO_2+ 40%N_2 and with an Ageless SS oxygen absorbent and stored at 4°C and 12°C are shown in Figure 34 a,b. The initial inoculum level at day 0 averaged 5.4×10^3 CFU/g i.e., a level considerably higher than found naturally in contaminated chicken. It is generally accepted that high numbers of *Salmonella* (10^5-10^6CFU/g) are required to cause food poisoning. However, the FDA have determined a zero tolerance limit (< 1 CFU per 25g of sample) for *Salmonella* spp. in foods.

For most packaging treatments, growth of *S. enteritidis* was more rapid at 12°C than at 4°C. For products stored at 4° C, growth of *S. enteritidis* increased gradually and then decreased throughout storage (Figure 34a). However at 12°C, growth of *S. enteritidis* continued to increase steadily throughout storage (Figure 34b). In air packaged samples, growth of *S. enteritidis* was ($P<0.05$) higher than all other treatments, reaching 10^6 CFU/g after 7d at 12°C and 14d at 4°C. Similar trends were observed for chicken thighs packaged under vacuum or with an oxygen absorbent at both 4 and 12°C (Figure 34 a,b). However,

in the gas packaged samples, growth of *S. enteritidis* was significantly less rapid ($P<0.05$) compared to all other treatments. This decrease was more pronounced at 4 than at 12°C. The inhibitory effect of CO_2 on *Salmonella* spp. at low temperatures has been previously reported (Nychas, 1993; Baker et al., 1986). In inoculation studies with *S. enteritidis* and packaging in vacuum and various modified atmospheres (100%CO_2, 100%N_2 and 20%CO_2 – 80% air), counts of *S. enteritidis* in chicken thighs increased more significantly at 10°C than at 3°C (Nychas, 1993). In addition, results indicated that even elevated levels of CO_2 (100% CO_2) decreased counts by only one log unit after 12d of storage at 10°C, again indicating the importance of temperature control. Similiar decreases in *Salmonella* counts were observed in CO_2 enriched atmospheres by Gray et al. (1984). Our results confirm the observations that elevated levels of CO_2 (60%CO_2 – 40%N_2) and low temperature were the most inhibitory combination to the growth of *S. enteritidis*. The greater inhibitory effect of this packaging atmosphere could be explained by the inhibitory effect of CO_2 on the metabolism of *S. enteritidis*. Another reason may be the growth of psychrotrophic lactic acid bacteria which become the predominant spoilage microflora in MAP chicken and the competitive inhibition of these bacteria on *Salmonella* spp.

Fig.33a. Changes in pH of chicken thighs inoculated with S. enteritidis and stored at 4°C.

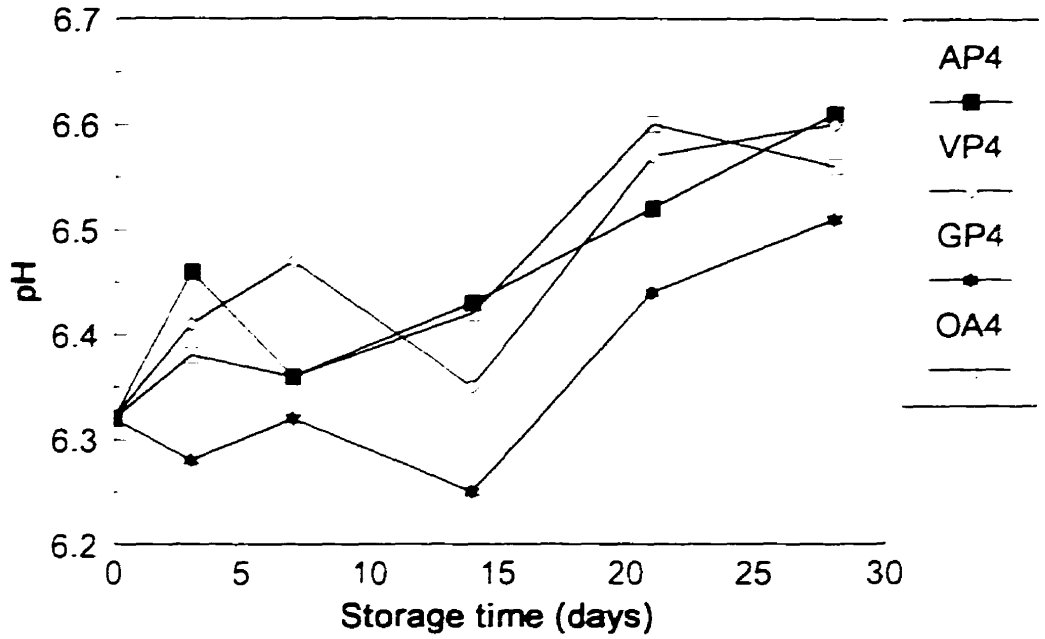
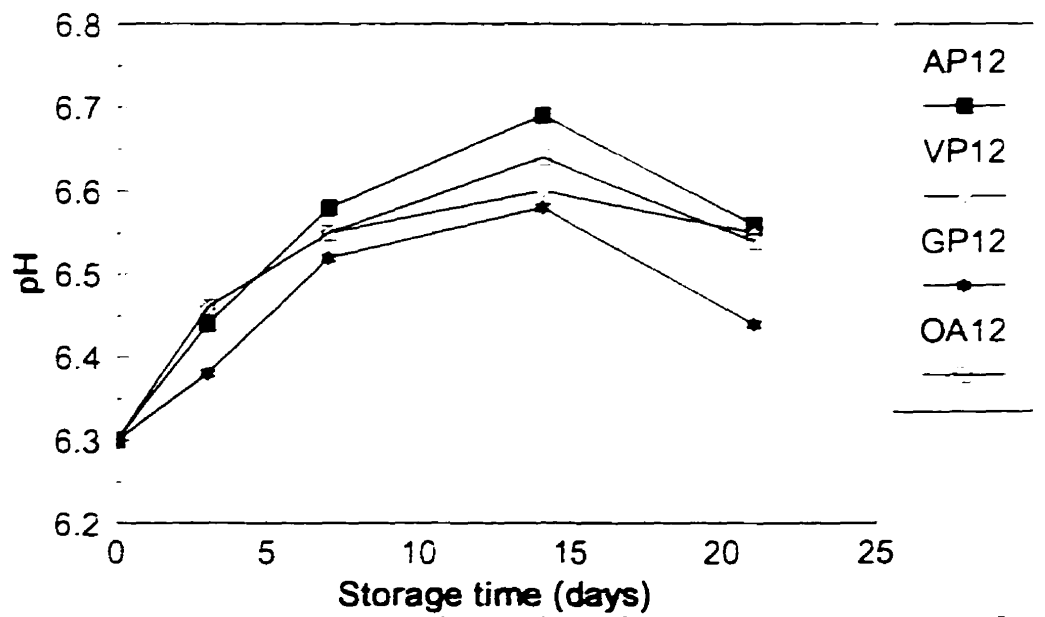


Fig.33b. Changes in pH of chicken thighs inoculated with S. enteritidis and stored at 12°C.



Key: Air (AP), Vacuum (VP), 60% CO2+40%N2 (GP), And Oxygen Absorbent (OA)

Fig.34a. Growth of S. enteritidis in chicken thighs at 4°C.

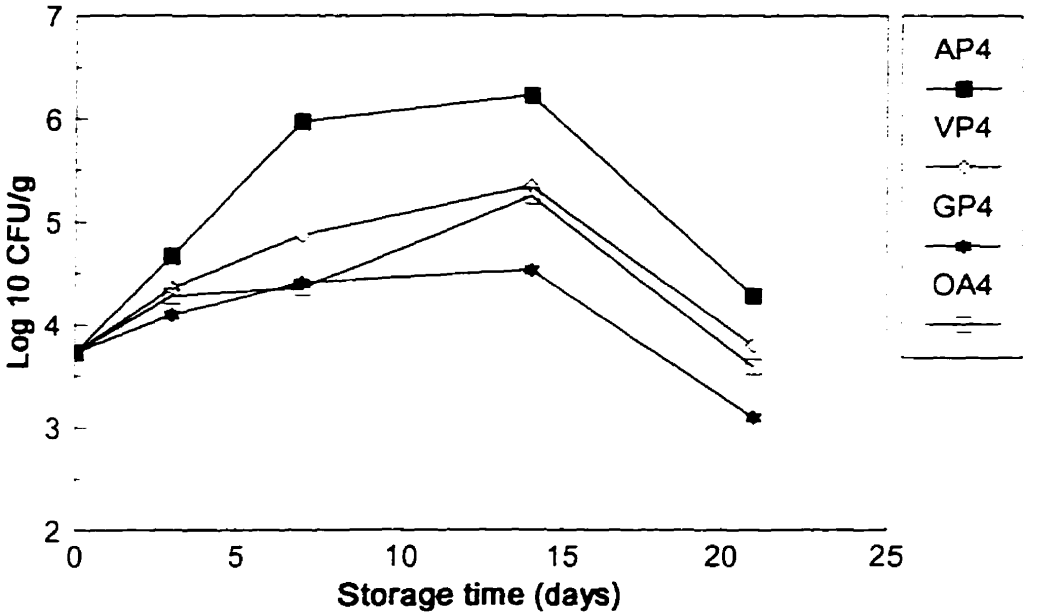
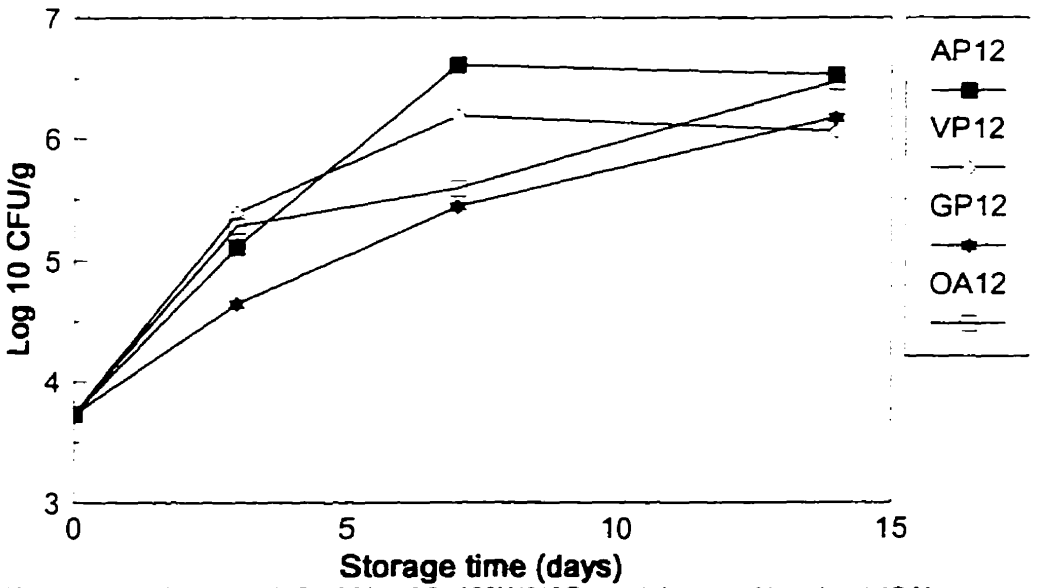


Fig.34b. Growth of S. enteritidis in chicken thighs at 12°C.



Key: Air (AP), Vacuum (VP), 60%CO2+40%N2(GP), and Oxygen Absorbent (OA)

3.3.8. Shelf-life

The sensory shelf-life of chicken thighs inoculated with *S. enteritidis* stored in air, vacuum, 60%CO_2+ 40%N_2 and with an Ageless SS oxygen absorbent are shown in Table 14. The storage shelf-life of chicken was based on the time to reach a sensory score (odor/color) of six or a *Salmonella* count of 10^6 CFU/g. Generally, results show that surface discoloration and off odor development occurred at the same time at both temperatures for all modified atmosphere conditions. However, in the air packaged samples at 12°C, off odor usually preceded color deterioration. These results reflect the high *Salmonella* counts found in air packaged samples throughout the storage trial. Most treatments were considered organoleptically spoiled at or before the time *S. enteritidis* reached maximum numbers (10^6 CFU/g) at 4°C. However, at 12°C, the growth of *S. enteritidis* limited the overall shelf-life of chicken thighs. Thus, samples containing high levels of *S. enteritidis* counts were judged organoleptically acceptable by the sensory panellists - a potentially hazardous scenario.

Conclusion

Results from this study indicate that gas packaging (60%CO_2+ 40%N_2), in combination with low temperature (4°C), could retard the growth of *S. enteritidis* for up to 20d with spoilage being evident at approximately the same time. Results also clearly show that while gas packaging extends the shelf-life of chicken thighs at 4°C, the growth rate of *S. enteritidis* was less affected by the inhibitory effect of CO_2 at abusive temperature (12°C). Thus, *S. enteritidis* may reach high numbers before sensory rejection. In this respect, greater emphasis on proper temperature control or additional barriers is considered critical to ensure the micrbiological safety and quality of poultry products, particularly at temperature abuse conditions.

Table 14. Estimated shelf-life of chicken thighs inoculated with *S. enteritidis* in different packaging treatment and stored at 4 and 12°C.

Packaging Treatment[a]	Rejection point/color[b]	Rejection point/odor[b]	Microbial shelf life[c]	Shelf life[d]
Storage at 4°C				
A	10	11	7	7
B	21	20	>21	20
C	21	20	>21	20
D	13	14	>21	13
Storage at 12°C				
A	13	7	5	5
B	14	14	6	6
C	16	16	12	12
D	10	9	10	10

[a] A, Air B, Vacuum C, 60%CO_2:40%N_2 D, Ageless SS.

[b] Time to reach a score of six

[c] Time to reach a *Salmonella* count of 10^6 CFU/g.

[d] Earliest rejection point on terms of odor,color and microbial load

CHAPTER 4

"HURDLE" APPROACH TO FOOD SAFETY

4.1. INTRODUCTION

It is well established that the combined effect of one or more "hurdles" or "barriers" will have a far greater effect on microbial growth and survival than when either barrier is used alone. These hurdles may be temperature, redox potential, preservatives, MAP, irradiation, dipping solutions etc. Microorganisms must make a specific effort to overcome each hurdle, the "higher" the hurdle, the greater the effort each microorganism must make. The advantage of this combined approach is that due to synergistic effect of each hurdle, individual hurdles may be used at lower levels/concentrations than would be required if only a single hurdle was used as a preservative technique. In light of the above discussion, this study addresses the potential for extended shelf life/safety of poultry through a "hurdle approach".

4.2. OBJECTIVES

The specific objectives of the research were:

i) To determine the use of MAP and (a) dipping solutions (chitosan and potassium sorbate) and (b) low dose irradiation to extend the shelf-life and safety of chicken thighs;

ii) To monitor the physical, chemical, microbiological and sensorial changes occurring in the treated/packaged products; and

iii) To determine any significant statistical difference between treatments and based on this difference, recommend a combination treatment in conjunction with MAP to ensure the safety of packaged poultry.

4.3. MATERIAL & METHODS

4.3.1. Culture

Storage, subculturing and preparation of *S. enteritidis*^{NAST} inoculum was as described previously **(See Section 3.2.3.)**.

4.3.2. Preparation of chitosan dipping solution

A 1% stock solution (w/v) of chitosan was prepared by dissolving 10 gram of chitosan into 1000 ml of sterile deionized water. Two hundred ml of this stock solution was added to 800 ml of sterile distilled water to give a final concentration of 0.2 % chitosan (vol/vol). Since the addition of chitosan ((initial pH 4.9) may lower meat pH, the pH of the solution was adjusted to pH 6 using sterile food grade lactic acid and autoclaved for 15 minutes at 121°C. The chitosan solution was then stored at 4°C until required.

4.3.3. Dipping treatment and packaging of chicken thighs

Frozen chicken thighs were obtained from Club Price, Montreal. After thawing at 4°C overnight, thighs were dipped in a sterile beaker containing 0.2% chitosan for 1 minute. The thighs were then allowed to drain in a sterile beaker and placed separately (~50g) in 210 · 210 mm high gas barrier bags (Cryovac, Canada). A fresh chitosan solution was used per 25 chicken thighs. The chicken thighs were then inoculated with 0.5 ml of *S. enteritidis*^{NAST} as prepared previously to give a final inoculum level of 10^3 CFU/g **(See Section 2.2.2.)**. Control samples were dipped in sterile water, the excess water drained and then inoculated with *S. enteritidis*^{NAST} as outlined above. All samples were packaged as described previously **(See Section 2.2.3.)** and stored at 4°C and 12°C for up to 21d.

4.3.4. Headspace gas composition

Headspace gas composition were determined as previously described **(See Section 2.2.6.)**

4.3.5. Color analyses

Color analysis was measured as previously described **(See Section 2.2.2.2.)**.

4.3.6. Drip loss

Drip loss were measured as previously described **(See Section 1.1.2.2.6.)**.

4.3.7. Sensory, pH and microbiological analyses

Sensory, pH and microbiological analyses were carried out as previously described **(See Section 3.2.8.).**

4.3.8. Statistical analyses

Data were analyzed using a split plot design in which storage time was the main plot effect and storage temperature and packaging treatment the subplot effect. Bacterial counts were expressed as $\log_{10}$ CFU/g and the reported means were the average of three replicates per treatment. Differences between means were analysed using the least significant difference (LSD) and the Duncan multiple range test. A probability (P) of < 0.05 was considered to be significantly different.

4.4. RESULTS & DISCUSSION

4.4.1. Changes in headspace gas composition

Changes in headspace gas composition of chicken thighs predipped in sterile deionized water or in 0.2% chitosan (v/v) prior to packaging and storage at 4 and 12°C are shown in Figures 35-42 a,b respectively. Generally, no noticeable difference in the gaseous concentrations (O_2, CO_2 & N_2) were observed between the chitosan and non-chitosan dipped (control) samples. However, the headspace gas composition changed significantly as storage temperature increased.

In air packaged, water dipped samples (control), O_2 was depleted to less than 1% after 7d and CO_2 increased to 24% after 21d at 4°C (Figure 35a). Similar results were also observed for the air packaged samples dipped in chitosan i.e., O_2 was depleted to less than 1% after 7d and CO_2 increased to 22% after 21d (Figure 35b). Similar but more dramatic changes were observed for the air packaged samples (control & chitosan) at 12°C. Here, O_2 was rapidly depleted to less than 1% after only 3d and CO_2 increased to >20% after 14d (Figure 36 a,b). These changes in the headspace gas composition are a result of aerobic metabolism of the meat tissue and microbial activity.

In samples packaged with 60% CO_2+40% N_2, the CO_2 concentration decreased to 57% in both water dipped and chitosan dipped samples after 21d at 4°C (Figure 37 a,b). This decrease could be attributed to the dissolution of CO_2 into the meat tissue. The headspace CO_2 concentration of the gas packaged samples (water & chitosan dipped) were both higher at 12°C, reaching 64 % after 14d (Figure 38 a,b). At both 4 and 12°C, the CO_2 concentration remained fairly constant in the early stages of the storage period. These results confirm our previous observations where microbial composition of modified atmosphere packaged chicken thighs becomes dominated by CO_2 resistant lactic acid bacteria. Gariepy et al. (1986) reported a latency period of 10-15d for the growth of lactic acid bacteria in 100% CO_2. Furthermore, Seideman et al. (1979) reported that lactic acid bacteria increased from 1.00

$\log_{10}/cm^2$ to 5.19 $\log_{10}/cm^2$ in pork samples packaged in 40% CO_2 + 60% N_2 after 15d at 3°C These results indicate that changes in CO_2 concentration in the modified atmosphere treatments usually occur at the late stages of the storage period because of the inhibitory effect of CO_2 on the aerobic spoilage bacteria and its stimulatory effect on the growth of lactic acid bacteria.

In samples packaged under vacuum or with an oxygen absorbent, the concentration of CO_2 and O_2 did not change significantly between the water or chitosan dipped samples at 4 and 12°C (Figures 39-42 a,b). However, the CO_2 concentration increased more rapidly at the end of the storage period at 12°C, again indicating the predominance of the lactic acid bacteria. The use of an oxygen absorbent Ageless SS is capable of reducing the O_2 partial pressure to less than 0.01%. The residual O_2 in the package immediately after packaging was 0.25% and decreased to negligible levels towards the end of the storage period.

Fig.35a. Changes in headspace gas composition of water dipped (control) chicken thighs inoculated with S. enteritidis stored in air at 4°C

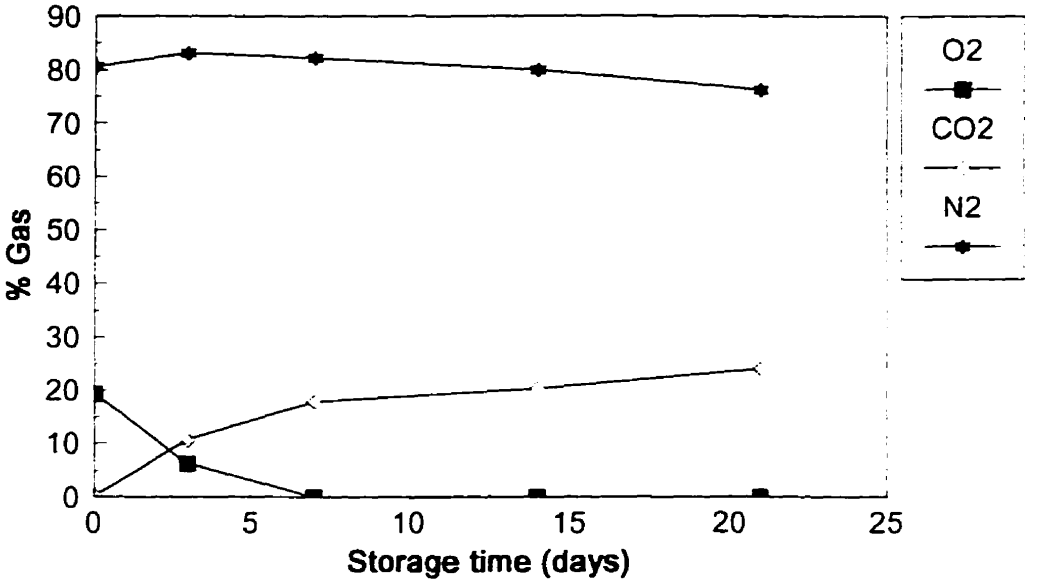


Fig.35b. Changes in headspace gas composition of chitosan dipped chicken thighs inoculated with S. enteritidis stored in air at 4°C.

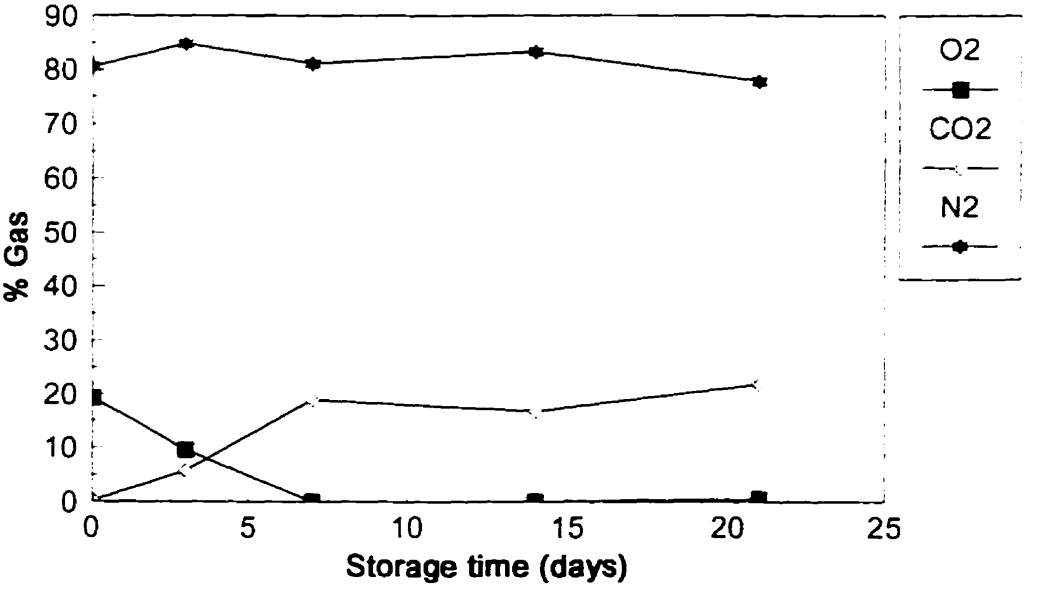


Fig.36a.Changes in headspace gas composition of water dipped (control) chicken thighs inoculated with S. enteritidis stored in air at 12°C.

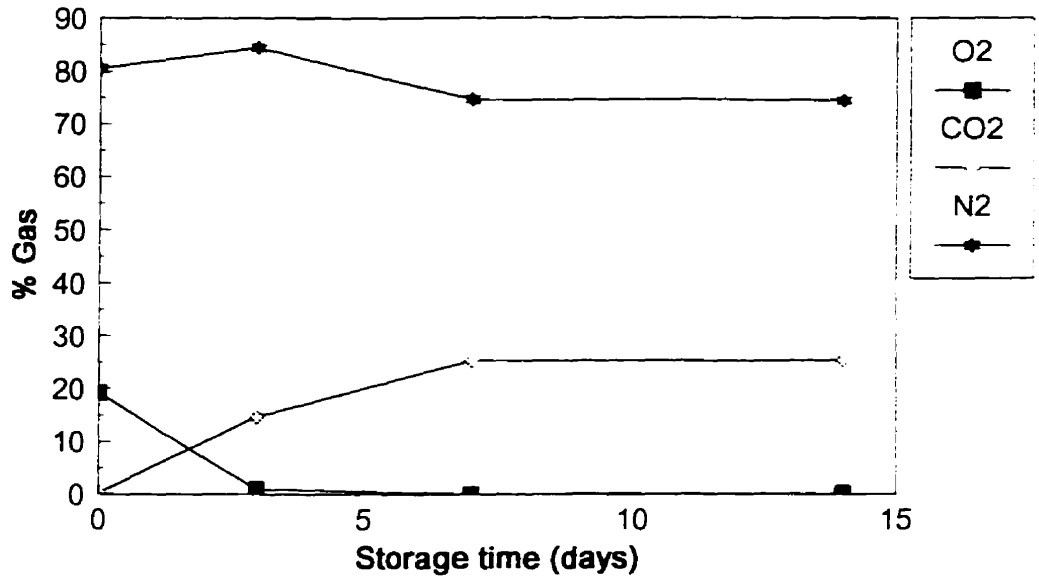


Fig.36b.Changes in headspace gas composition of chitosan dipped chicken thighs inoculated with S. enteritidis stored in air at 12°C.

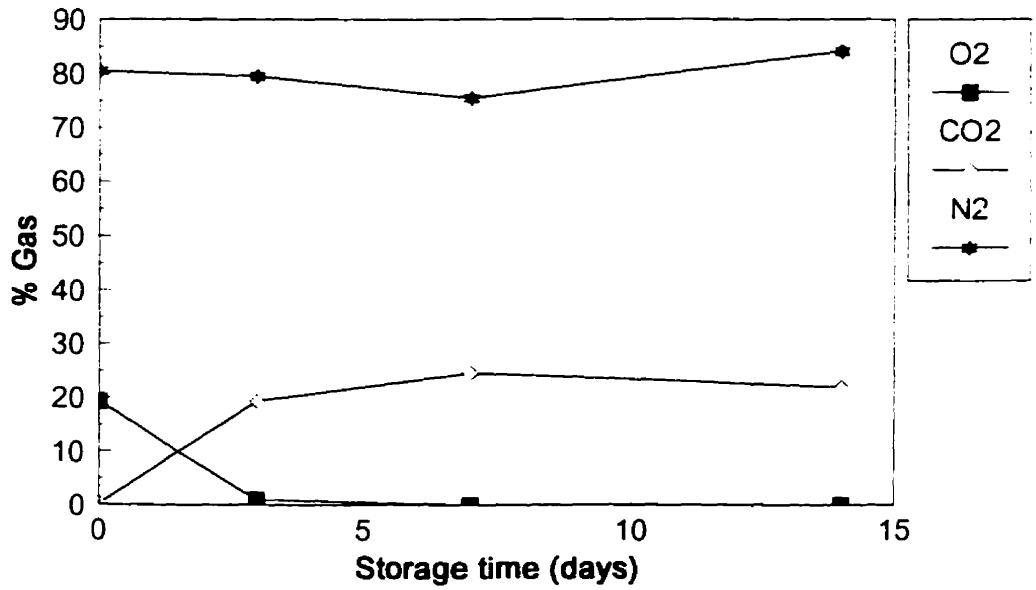


Fig.37a. Changes in headspace gas composition of water dipped (control) chicken thighs inoculated with S. enteritidis stored in 60%CO2+40% N2 at 4°C.

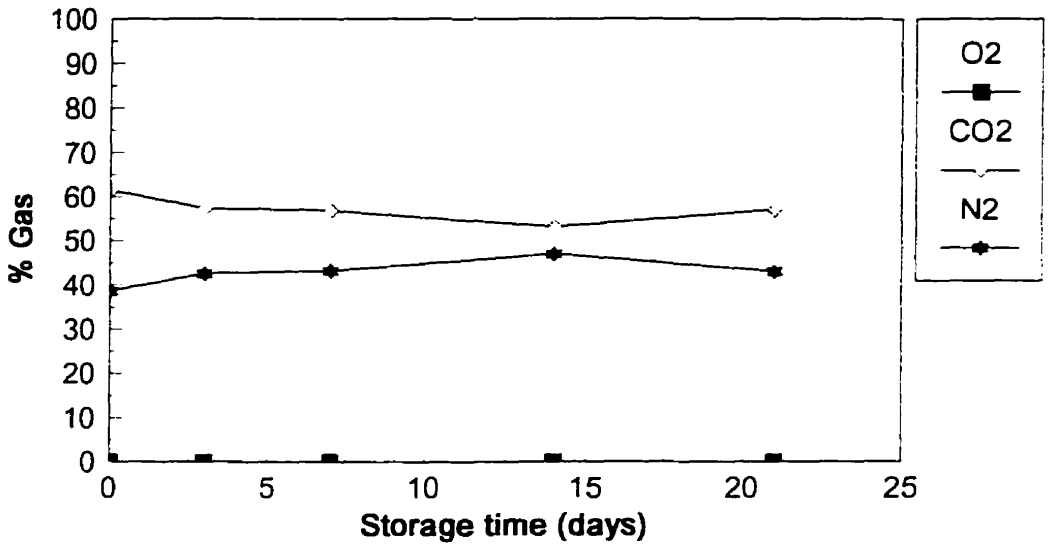


Fig.37b. Changes in headspace gas composition of chitosan dipped chicken thighs inoculated with S. enteritidis stored in 60%CO2+40% N2 at 4°C.

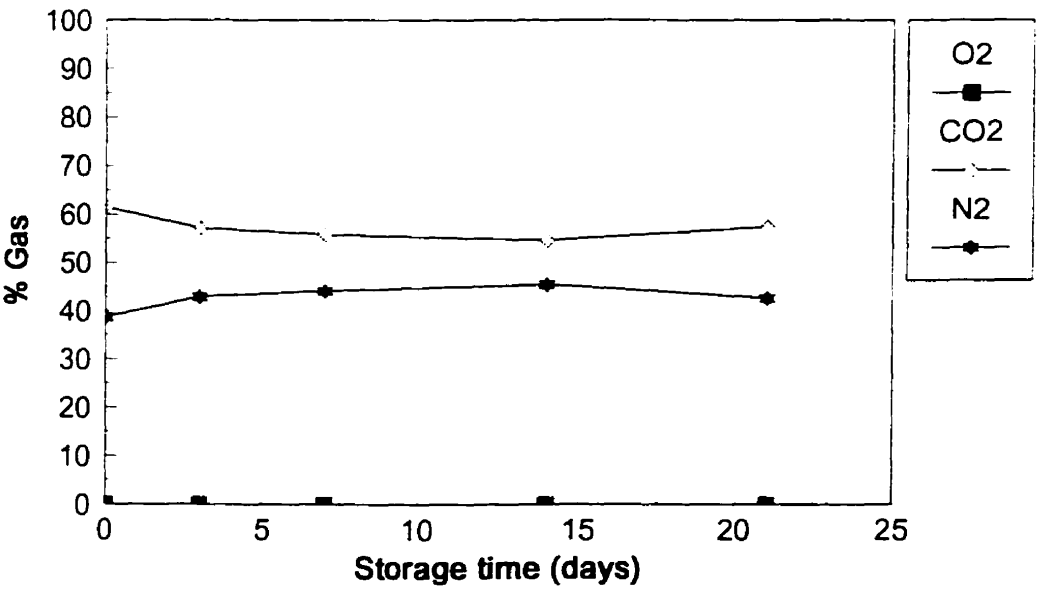


Fig.38a. Changes in headspace gas composition of water dipped (control) chicken thighs inoculated with S. enteritidis stored in 60% CO2+40% N2 at 12°C.

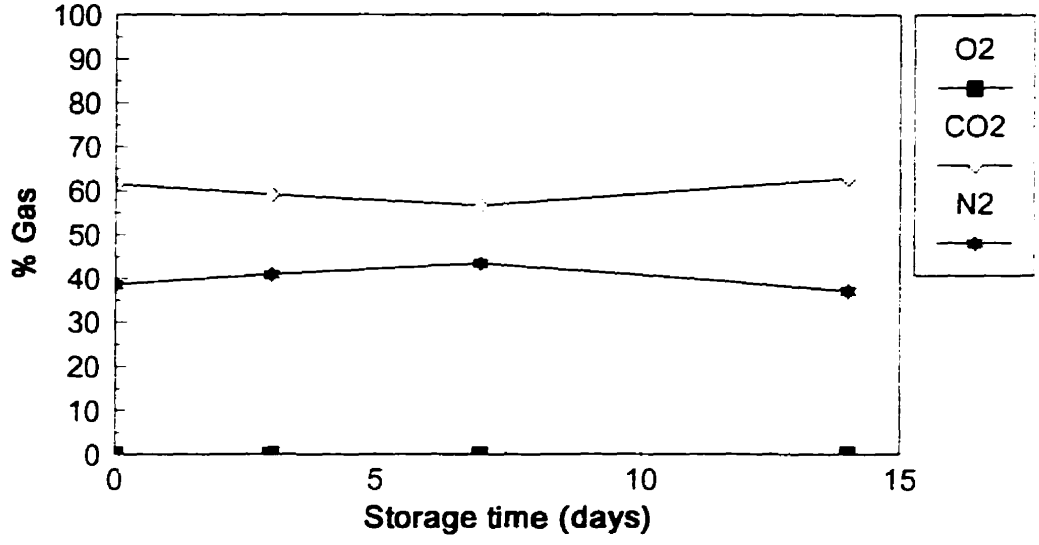


Fig.38b. Changes in headspace gas composition of chitosan dipped chicken thighs inoculated with S. enteritidis stored in 60%CO2+40% N2 at 12°C.

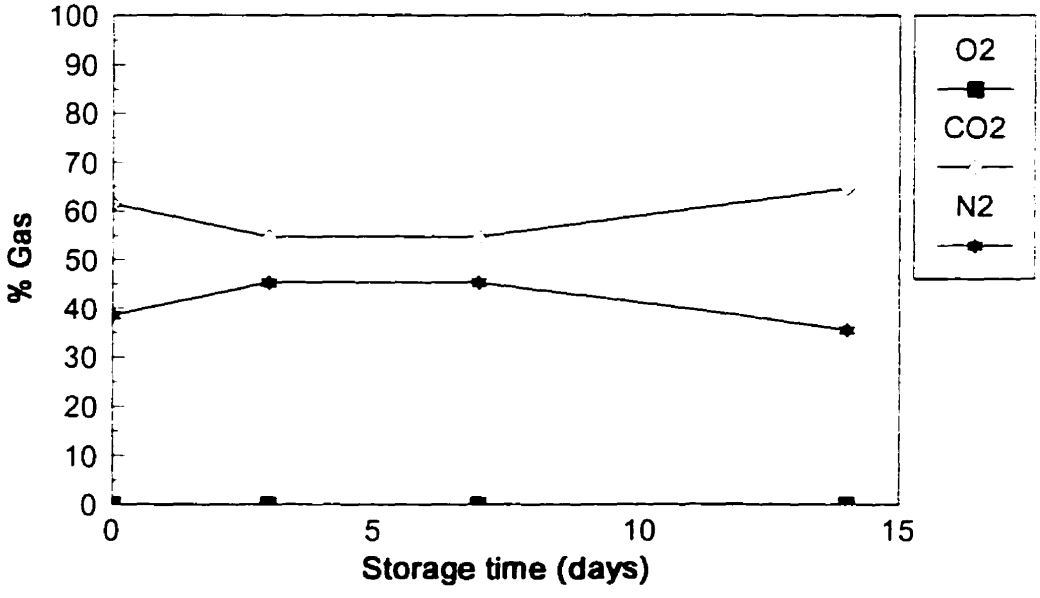


Fig.39a. Changes in headspace gas composition of water dipped (control) chicken thighs inoculated with S. enteritidis stored under vacuum at 4°C.

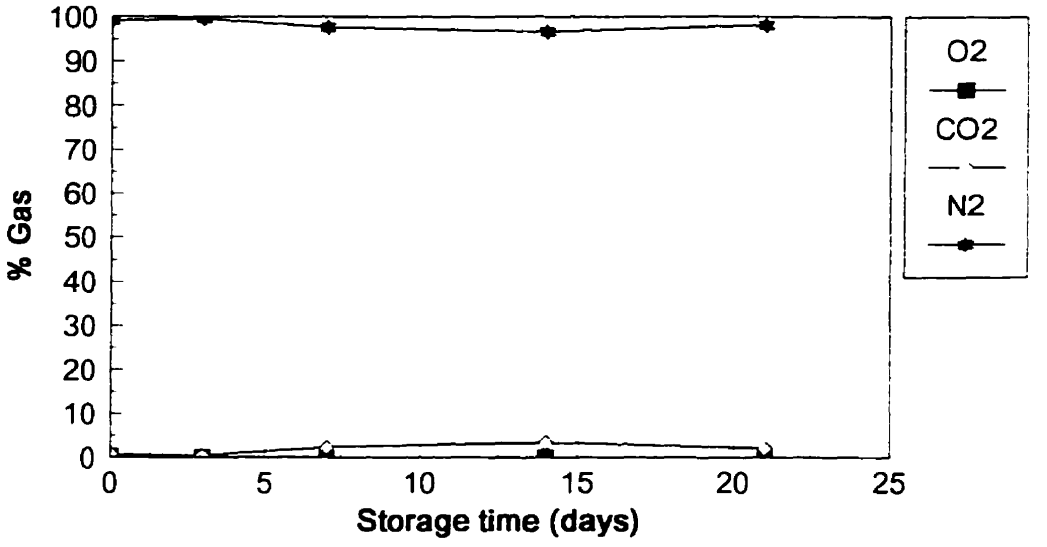


Fig.39b. Changes in headspace gas composition of chitosan dipped chicken thighs inoculated with S. enteritidis stored under vacuum at 4°C.

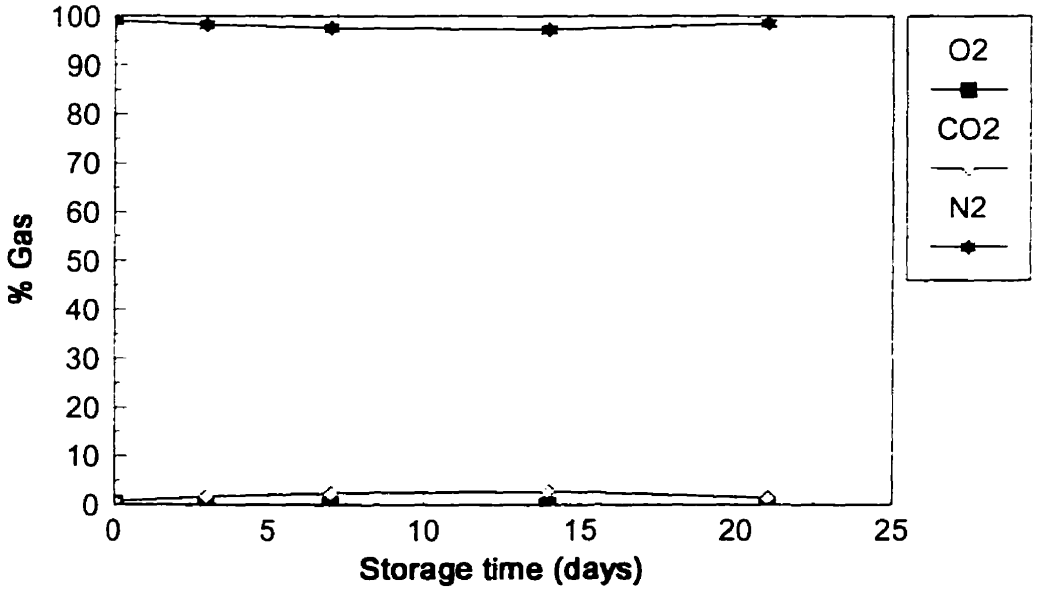


Fig.40a. Changes in headspace gas composition of water dipped (control) chicken thighs inoculated with S. enteritidis stored under vacuum at 12°C.

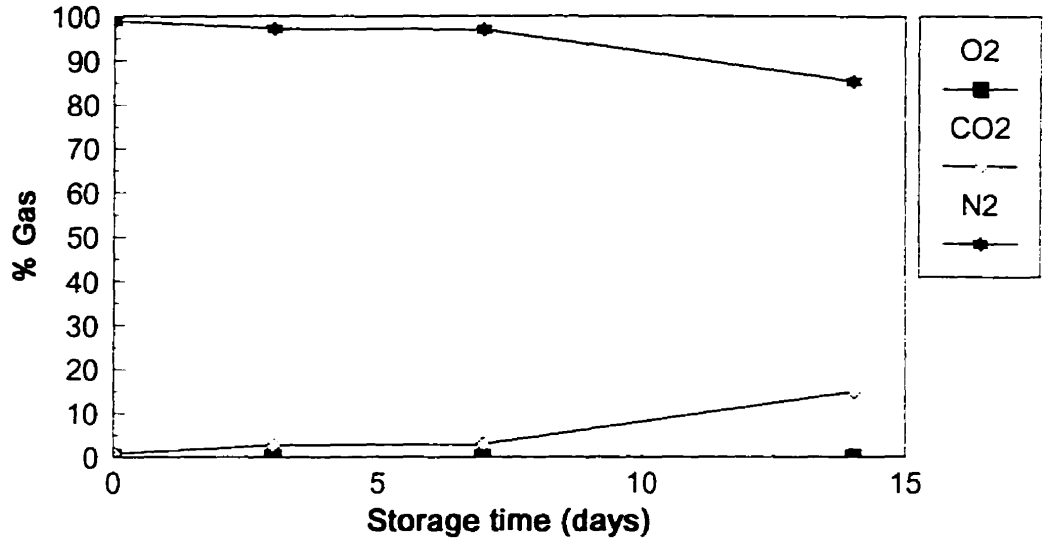


Fig.40b. Changes in headspace gas composition of chitosan dipped chicken thighs inoculated with S. enteritidis stored under vacuum at 12°C.

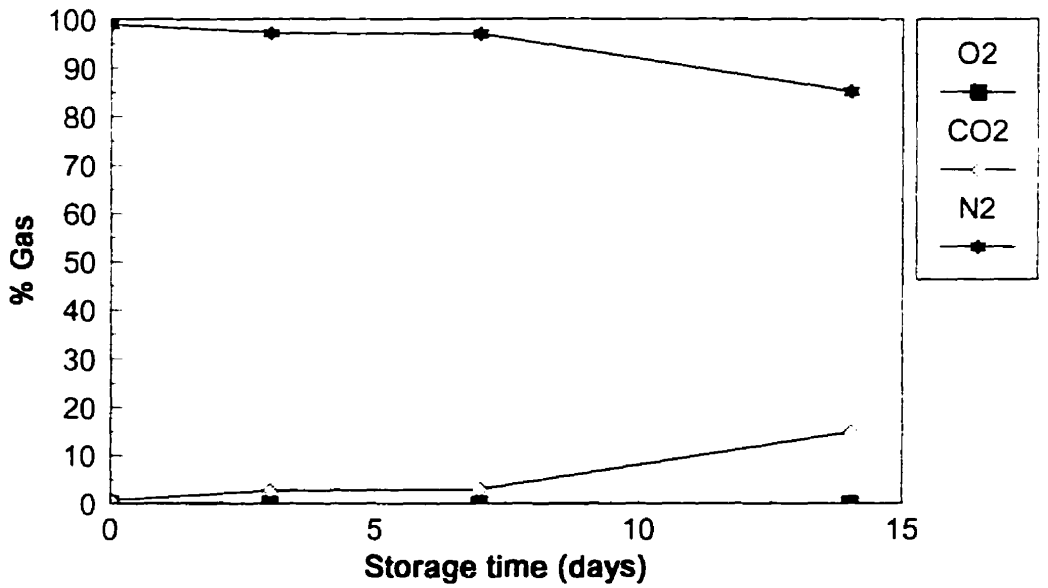


Fig.41a. Changes in headspace gas composition of water dipped (control) chicken thighs inoculated with S. enteritidis stored with oxygen absorbent at 4°C.

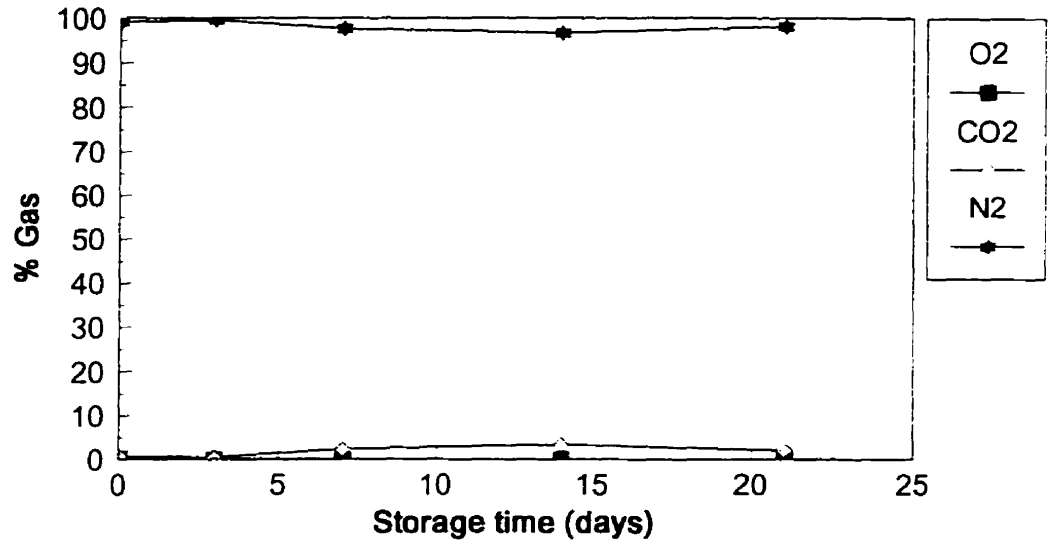


Fig.41b. Changes in headspace gas composition of chitosan dipped chicken thighs inoculated with S. enteritidis stored with oxygen absorbent at 4°C.

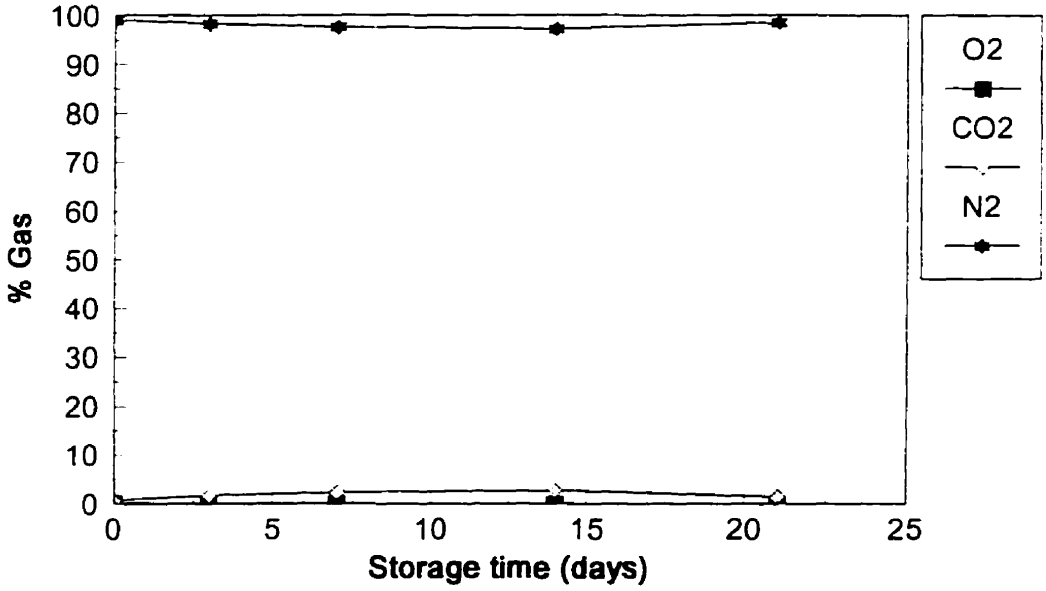


Fig.42a. Changes in headspace gas composition of water dipped (control) chicken thighs inoculated with S. enteritidis stored with oxygen absorbent at 12°C.

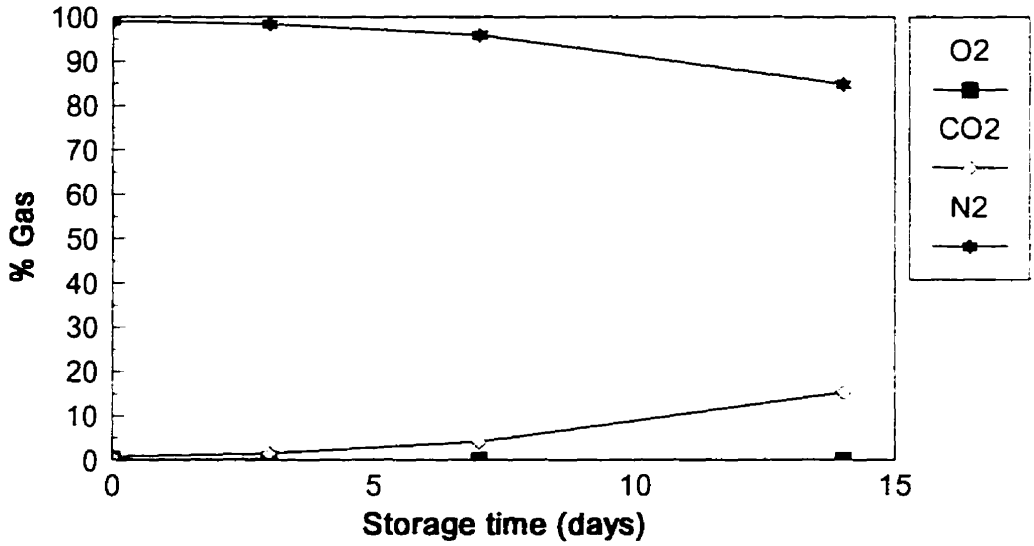
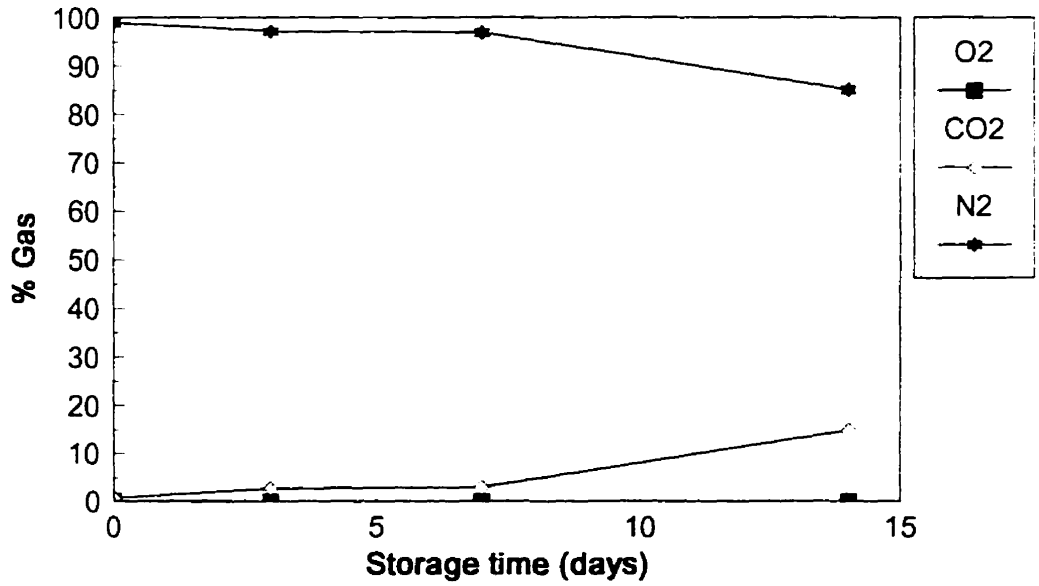


Fig.42b. Changes in headspace gas composition of chitosan dipped chicken thighs inoculated with S. enteritidis stored with oxygen absorbent at 12°C.



4.4.2. Color analyses

Color values (L*, a* and b*) of chicken thighs inoculated with *S. enteritidis*, and pre-dipped in water or 0.2% (v/v) chitosan prior to packaging and storage at 4 and 12°C are shown in Figures 43-48 a,b respectively. In general, the Hunterlab values L* (lightness), a* (redness) and b* (yellowness) increased significantly ($P>0.05$) with increasing temperature and storage time.

Changes in L* values are shown in Figures 43-44 a,b respectively. Generally, the L* values increased throughout the storage period irrespective of the dipping/packaging treatment, indicating that the meat was becoming paler. There was no significant difference ($P>0.05$) between the L* values of non-chitosan and chitosan dipped samples at either 4 or 12°C.

Changes in a* values are shown in Figures 45-46 a,b respectively. Generally, a* values increased throughout storage in both the non-chitosan and chitosan dipped samples. In the air packaged samples (control) at 4°C, a* values increased in the early stages of the storage period but decreased on day 14 and then increased again. This decrease was not observed for the air packaged samples dipped in chitosan and stored at 4°C, indicating that chitosan pre-treatment not only inhibited microbial growth but also enhanced the color stability of chicken thighs (Figure 45b). An initial decrease in a* values was observed for the gas packaged treatments (control & chitosan) after which the a* values increased steadily at both 4 and 12°C. A decrease in a* values were observed for the vacuum packaged treatments (chitosan&control) at 4°C (Figure 45 a,b). This initial decrease in a* values observed in the vacuum and gas packaging treatments have been previously reported (Seideman and Durland, 1982). The presence of residual O_2 immediately after packaging will result in the formation of metmyoglobin. As the residual oxygen is consumed by the meat tissue in the early stages of the storage period to a concentration of less than 0.1%, metmyoglobin is reduced back to the purple red myoglobin which then persists during storage (Seideman and Durland, 1982). Storage at higher temperatures (i.e., 12°C) had a significant effect ($P<0.05$) on a* values of chicken thighs. Samples packaged in air, vacuum and with an oxygen absorbent

(water&chitosan dipped) showed an increase in a* values over 7d of storage followed by a sharp decrease towards the end of the storage period (Figure 46 a,b). However, the addition of chitosan was not significantly more effective in maintaining the color stability of chicken thighs. It has been reported that spoilage bacteria cause the discoloration of meat in their growth phase (Butler et al., 1953). This indicates that the decrease in a* values after day 7 of storage is a result of the growth of aerobic/ facultative aerobic spoilage bacteria that utilize the O_2 resulting in increase metmyoglobin formation. Furthermore, at this time, *Salmonella* counts reached 10^6 CFU/g resulting in rapid spoilage. The effect of microbial deterioration on a* values of gas packaged samples (water&chitosan dipped) was less apparent, the a* values increased steadily throughout the storage period (Figure 46 a,b). These results show that high concentrations of CO_2 may have inhibited the proteolytic activity of microorganisms and thus spoilage of chicken thighs have not occurred.

Changes in b* values are shown in Figures 47-48 a,b respectively. In general, the b* values increased in all packaging treatments, irrespective of the dipping treatment. An increase in b* values was observed in the air packaged samples (control) after 14d at 4°C (Figure 47a) which correlated well with changes in a* values in the air packaged samples at this temperature. Cheysdale and Francis (1971) reported that the formation of metmyoglobin involved a decrease in a* values and an increase in b* values. Thus, these changes in a* and b* values could be attributed to the formation of metmyoglobin. An increase in b* values were also observed for the vacuum and gas packaged treatments (water&chitosan dipped) at day 3 at 4°C. Higher b* values indicate either the presence of myoglobin in its deoxygenated form or that metmyoglobin formation has occurred. In this study, vacuum and gas packaged samples showed a higher b*value and a lower a* value implying that the yellowness of the meat were a result of metmyoglobin formation (Figure 47 a,b). Samples packaged with an oxygen absorbent and dipped in chitosan had significancy higher b* values ($P>0.05$) than any of the other treatments after 14d at 4 and 12°C (Figures 47-48 a,b). Similarly, this increase in b* values were accompanied by higher L* values and lower a* values, again indicating the formation of metmyoglobin.

Fig.43a. Changes in L* coordinates of water dipped (control) chicken thighs inoculated with S. enteritidis at 4°C.

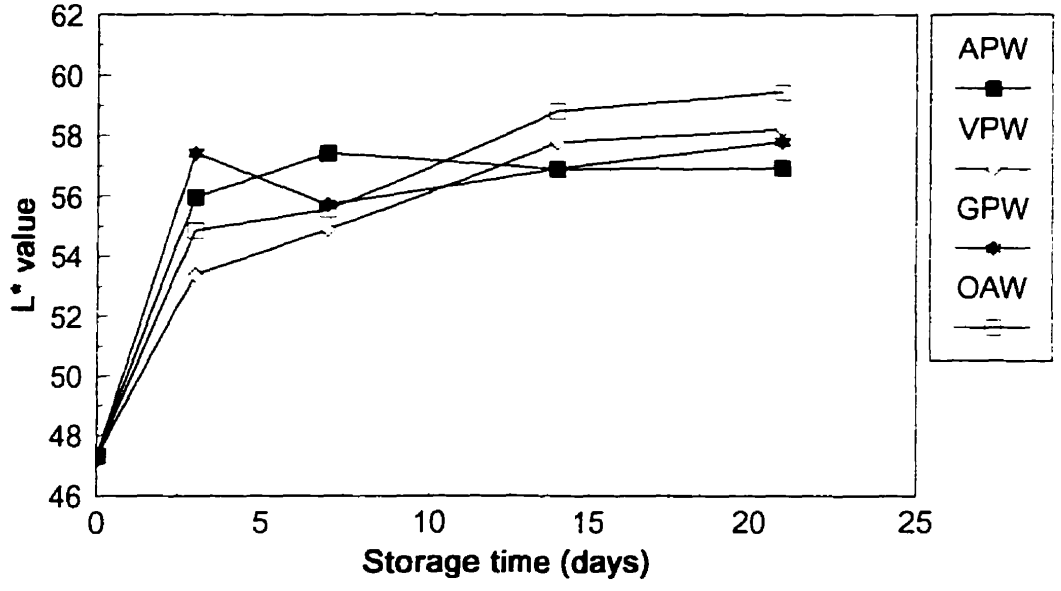
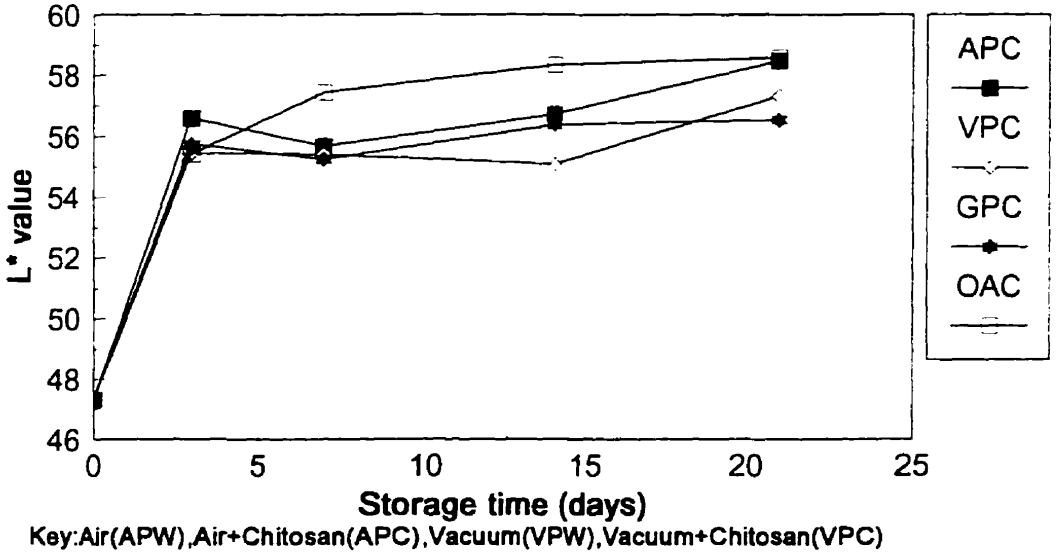


Fig.43b. Changes in L* coordinates of chitosan dipped chicken thighs and inoculated with S. enteritidis at 4°C.



Key:Air(APW),Air+Chitosan(APC),Vacuum(VPW),Vacuum+Chitosan(VPC) Oxygen Absorbent(OAW) And Oxygen Absorbent+Chitosan(OAC)

Fig.44a. Changes in L* coordinates of water dipped (control) chicken thighs inoculated with S. enteritidis at 12°C.

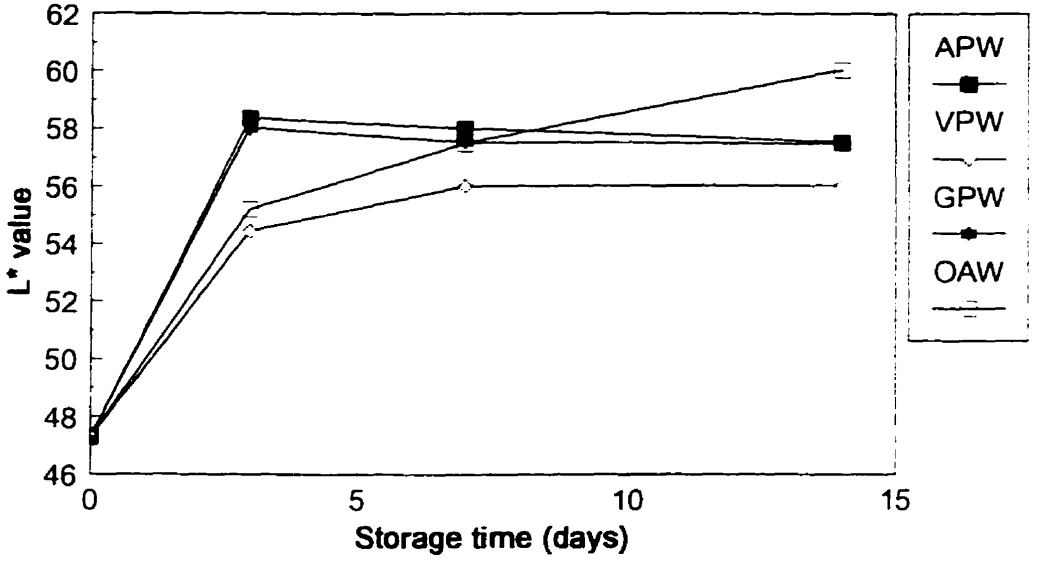
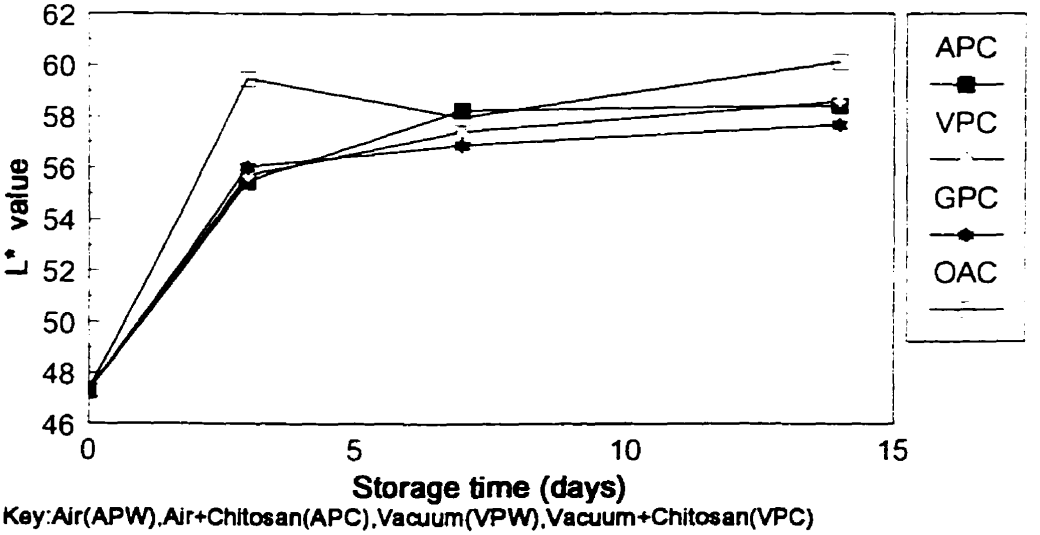


Fig.44b. Changes in L* coordinates of chitosan dipped chicken thighs inoculated with S. enteritidis at 12°C.



Key:Air(APW),Air+Chitosan(APC),Vacuum(VPW),Vacuum+Chitosan(VPC) Oxygen Absorbent(OAW) And Oxygen Absorbent+Chitosan(OAC)

Fig.45a. Changes in a* coordinates of water dipped (control) chicken thighs inoculated with S. enteritidis at 4°C.

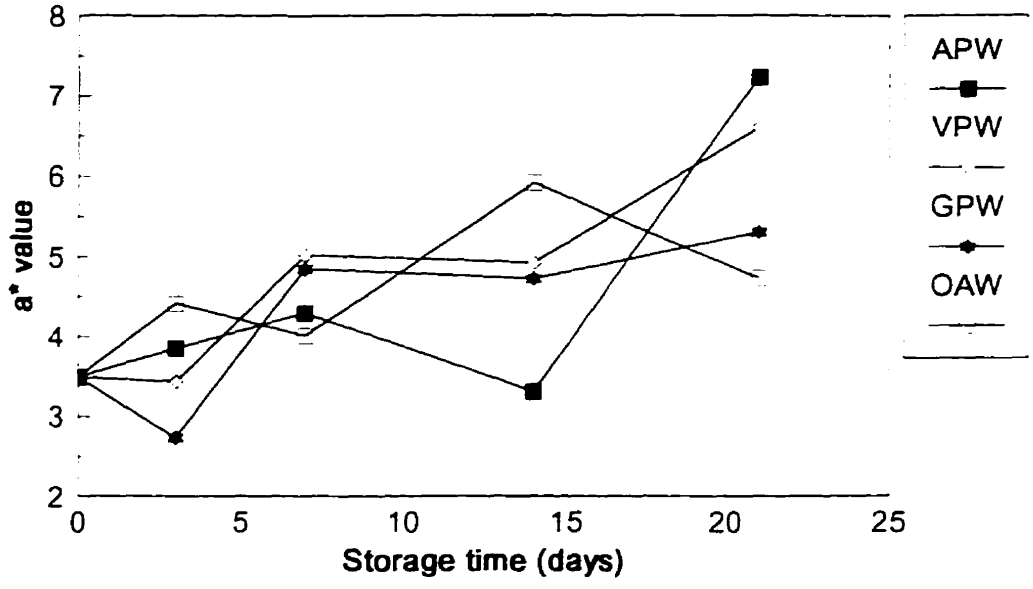
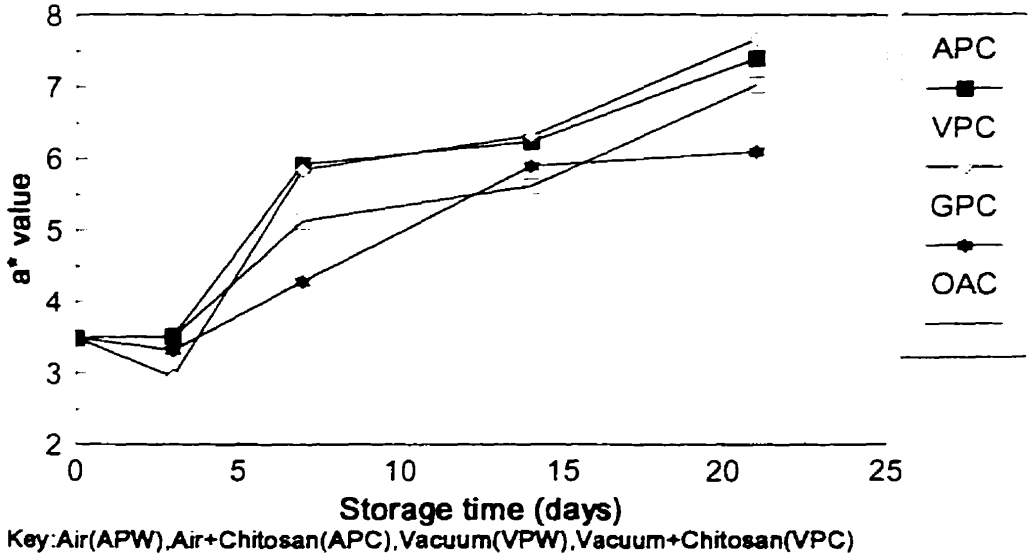


Fig.45b. Changes in a* coordinates of chitosan dipped chicken thighs inoculated with S. enteritidis at 4°C.



Key:Air(APW),Air+Chitosan(APC),Vacuum(VPW),Vacuum+Chitosan(VPC) Oxygen Absorbent(OAW) And Oxygen Absorbent+Chitosan(OAC)

Fig.46a. Changes in a* coordinates of water dipped (control) chicken thighs inoculated with S. enteritidis at 12°C.

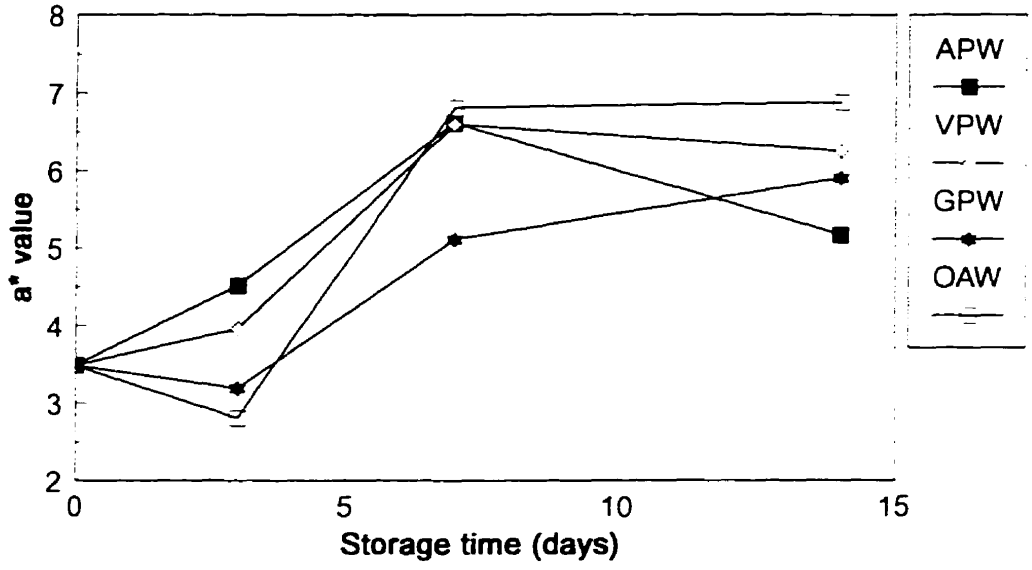
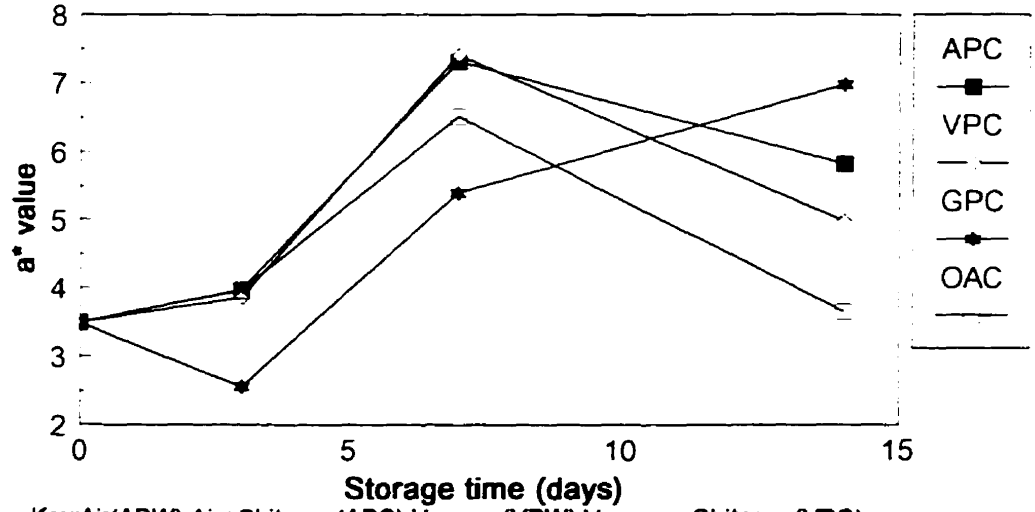


Fig.46b. Changes in a* coordinates of chitosan dipped chicken thighs inoculated with S. enteritidis at 12°C.



Key:Air(APW),Air+Chitosan(APC),Vacuum(VPW),Vacuum+Chitosan(VPC) Oxygen Absorbent(OAW) And Oxygen Absorbent+Chitosan(OAC)

Fig.47a. Changes in b* coordinates of water dipped (control) chicken inoculated with S. enteritidis at 4°C.

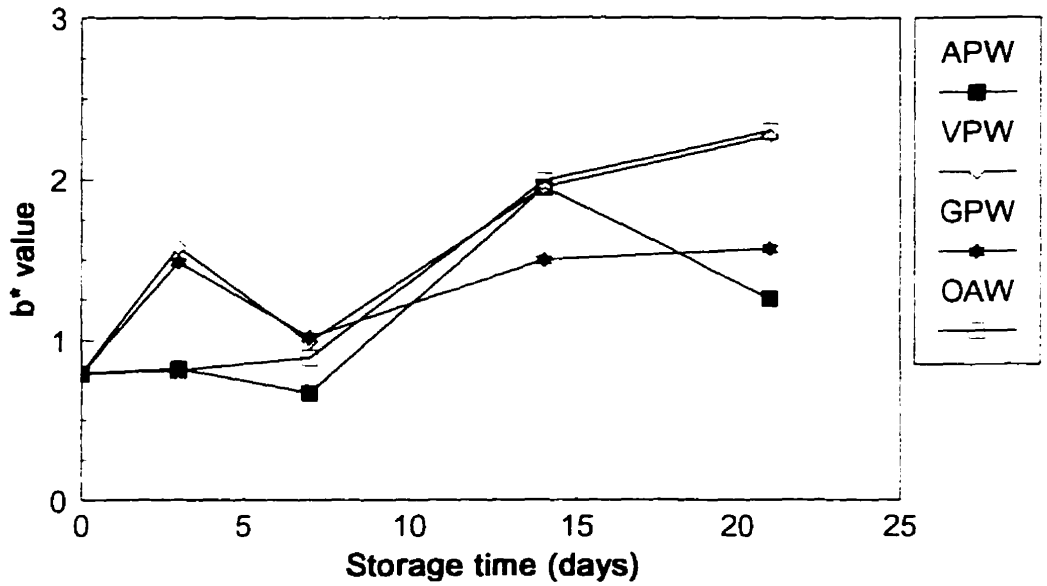
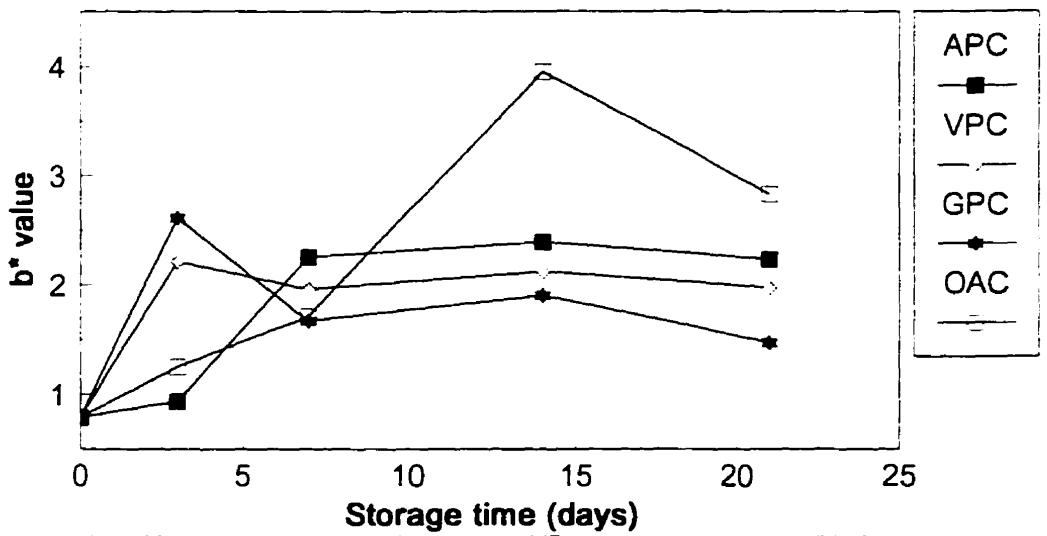


Fig.47b. Changes in b* coordinates of chitosan dipped chicken thighs inoculated with S. enteritidis at 4°C.



Key:Air(APW),Air+Chitosan(APC),Vacuum(VPW),Vacuum+Chitosan(VPC) Oxygen Absorbent(OAW) And Oxygen Absorbent+Chitosan(OAC)

Fig.48a. Changes in b* coordinates of water dipped (control) chicken inoculated with S. enteritidis at 12°C.

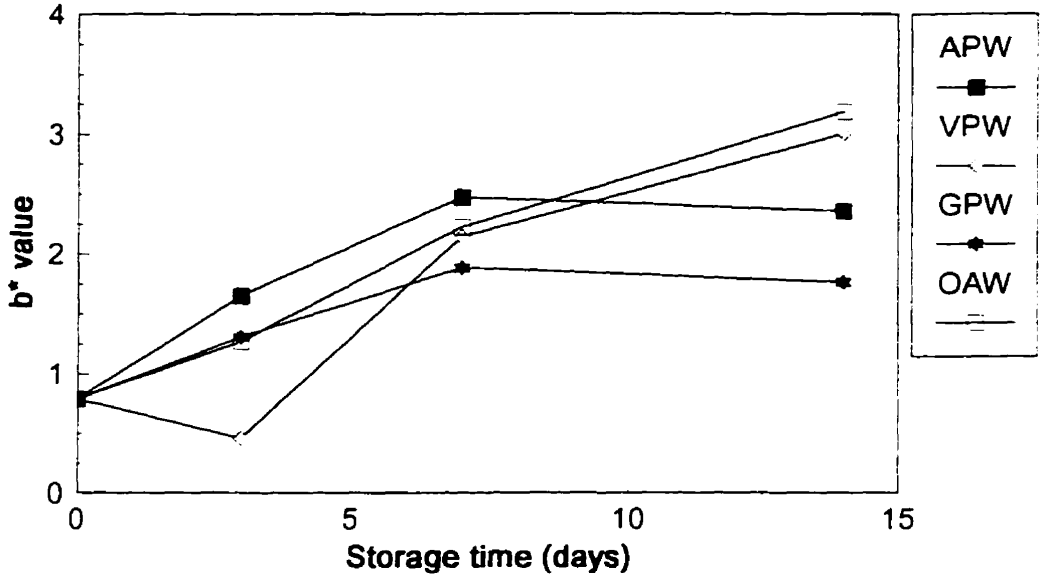
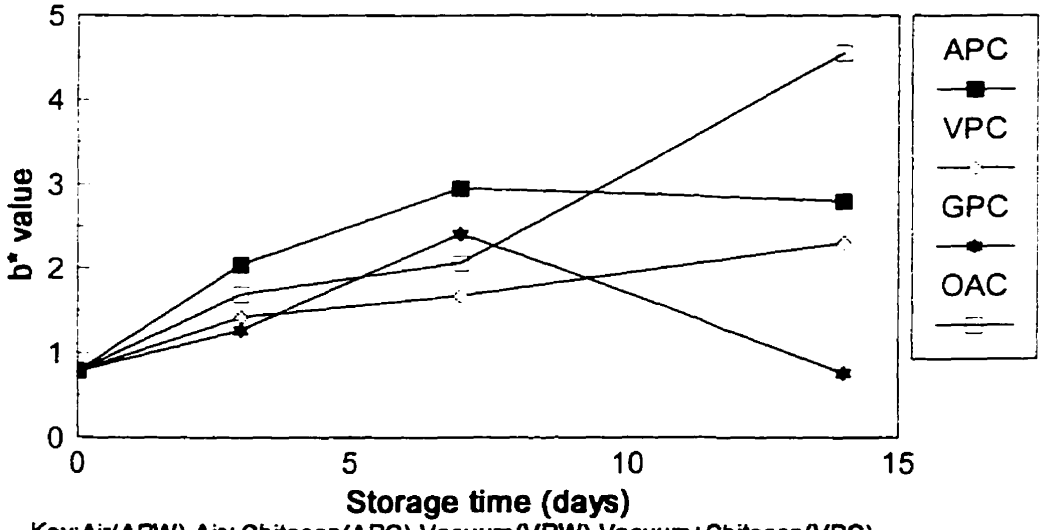


Fig.48b. Changes in b* coordinates of chitosan dipped chicken thighs inoculated with S. enteritidis at 12°C.



Key:Air(APW),Air+Chitosan(APC),Vacuum(VPW),Vacuum+Chitosan(VPC) Oxygen Absorbent(OAW) And Oxygen Absorbent+Chitosan(OAC)

4.4.3. Drip loss

The percentage drip loss (w/w) of pre-dipped chicken thighs inoculated with *S. enteritidis*, and packaged under various atmospheres and stored at 4 and 12°C are shown in Figures 49-50 a,b respectively. Drip loss was significantly affected ($P>0.05$) by packaging treatment, storage time and temperature. Difference in drip loss between treatments was independent of pH as changes in pH were minimal and would not have a direct effect on drip loss. O'Keefe and Hood (1981) concluded that differences in water holding capacity, even when the rate and extent of pH fall is identical, suggest that there were different types of proteins present. Samples packaged under vacuum showed significantly higher drip ($P>0.05$) compared to the other treatments after 7d of storage at 4°C (Figure 49a). Similar results were observed for the chitosan dipped samples packaged under vacuum and stored at the same temperature (Figure 49b). O'Keefe and Hood (1981) reported that high drip loss in vacuum packaged samples is a result of the negative pressure of vacuum forcing the moisture out of the muscle. Pre-dipping in chitosan prior to packaging also had a pronounced effect on drip at both 4 and 12°C (Figure 49-50 b). Samples packaged in air, and with an oxygen absorbent and dipped in chitosan had significantly lower drip ($P<0.05$) than the control samples throughout the storage period. Although samples packaged in 60% CO_2+ 40%N_2 had less drip loss than all other treatments, there were no significant difference ($P>0.05$) in drip loss between the chitosan and the water dipped samples (Figure 50 a,b) . These results are in close agreement with the microbial analysis data, showing that chitosan had little effect on the drip loss in samples packaged in 60% CO_2+40% N_2.

Fig.49a. Changes in drip loss (%w/w) of water dipped (control) chicken thighs inoculated with S. enteritidis at 4°C.

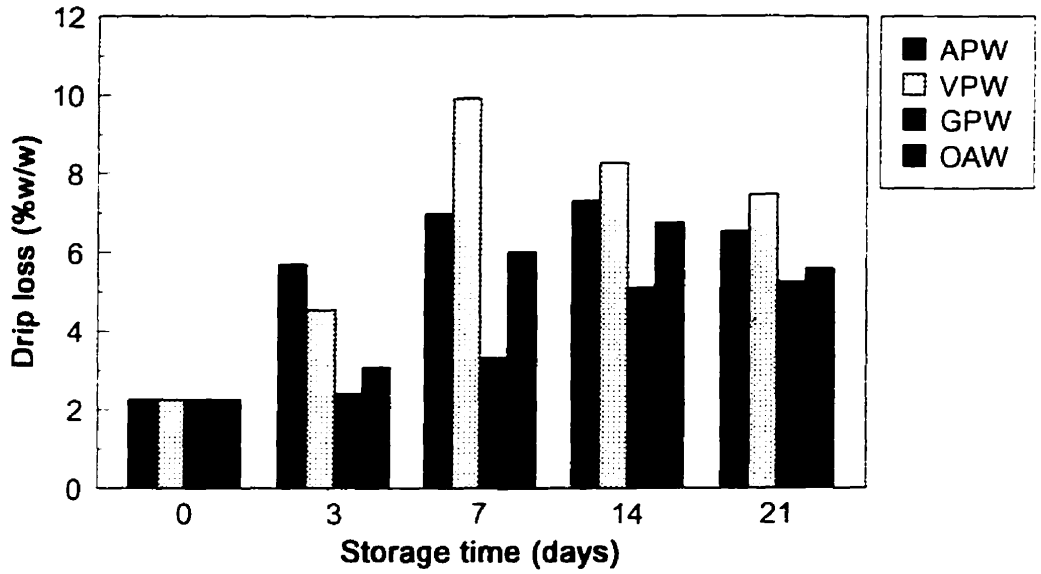
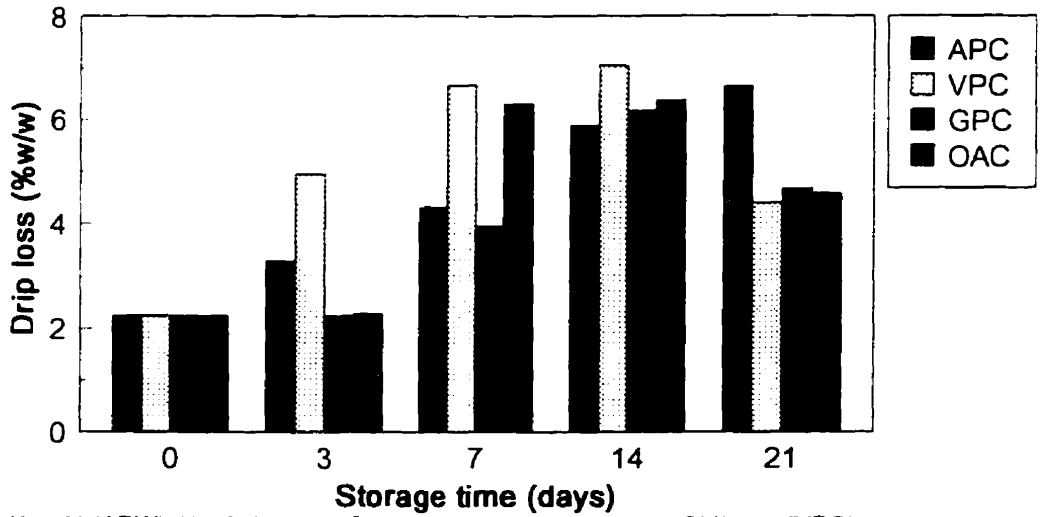


Fig.49b. Changes in drip loss (%w/w) of chitosan dipped chicken thighs inoculated with S. enteritidis at 4°C.



Key:Air(APW),Air+Chitosan(APC),Vacuum(VPW),Vacuum+Chitosan(VPC) Oxygen Absorbent(OAW) And Oxygen Absorbent+Chitosan(OAC)

Fig.50a. Changes in drip loss (%w/w) of water dipped (control) chicken thighs thighs inoculated with S. enteritidis at 12°C.

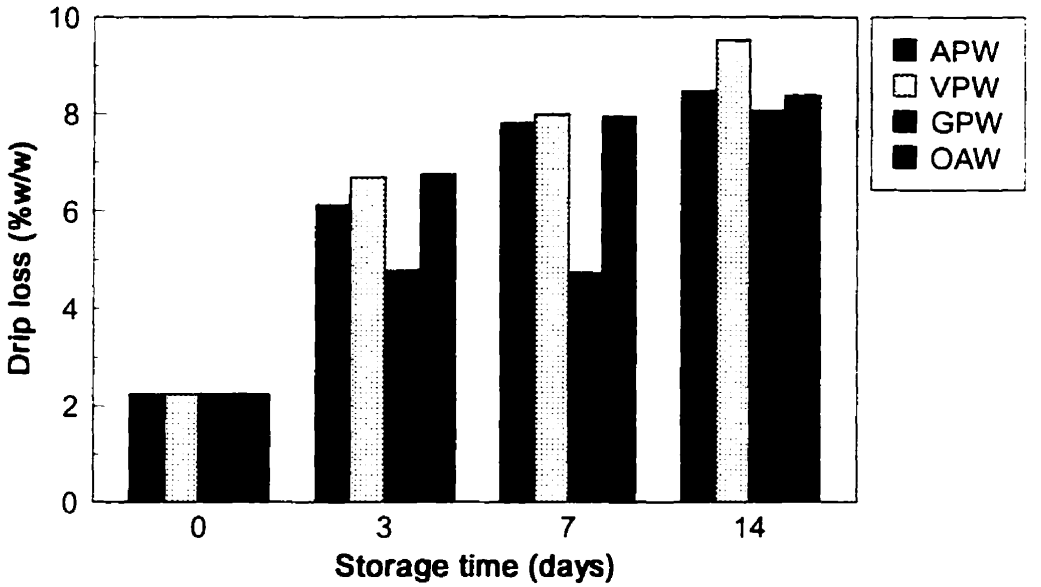
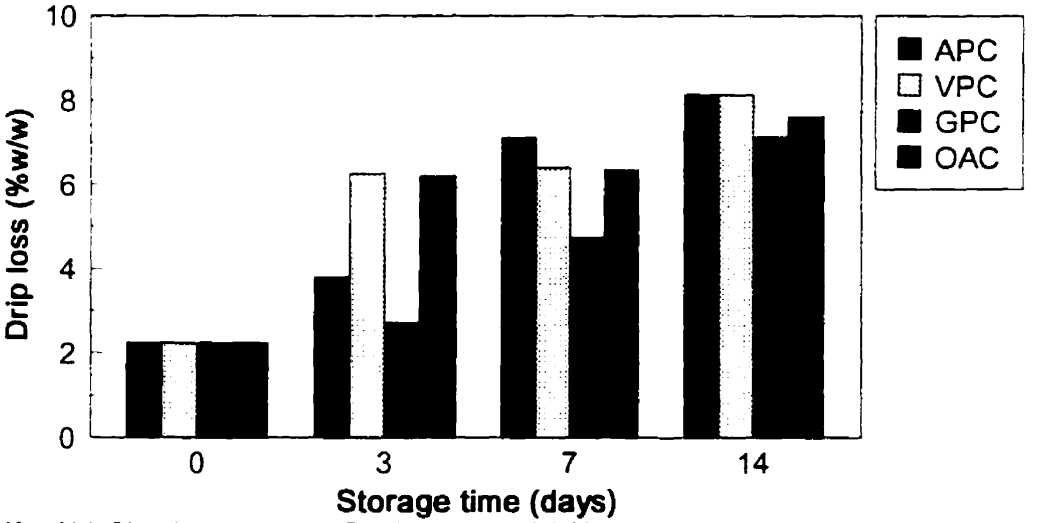


Fig.50b. Changes in drip loss (%w/w) of chitosan dipped chicken thighs inoculated with S. enteritidis at 12°C.



Key:Air(APW),Air+Chitosan(APC),Vacuum(VPW),Vacuum+Chitosan(VPC) Oxygen Absorbent(OAW) And Oxygen Absorbent+Chitosan(OAC)

4.4.4. Sensory analyses

The sensory quality scores (color&odor) of chicken thighs inoculated with *S. enteritidis*, and pre-dipped in deionized water or 0.2 % chitosan and stored in air, vacuum, 60%CO_2+40%N_2 and an ageless SS oxygen absorbent are shown in Figures 51-54 a,b respectively. In this study, there was a significant change ($P<0.05$) in color scores between chitosan treated samples and the water dipped samples packaged in air at 4°C (Figure 51 a,b). However, the panellists did not detect any significant color change ($P>0.05$) in samples packaged under vacuum, oxygen absorbent or gas packaged samples and the respective controls (Figure 51 a,b). Packaging in 60%CO_2+40%N_2 alone or with chitosan showed a lower color score than all treatments (Figure 51 a,b). Similarly, gas packaged samples showed a lower odor score than all treatments, although this decrease was more in chitosan dipped than the water dipped thighs (Figure 53 a,b). Dipping in chitosan prior to gas packaging did not have an appreciable effect on the color of chicken thighs at 4°C. However, a significant effect of chitosan predipping and gas packaging were observed on the odor shelf life of chicken thighs at 4°C. For non-chitosan dipped thighs, samples were rejected after 18 days compared to more than 28d for the chitosan dipped thighs. These results again show the synergistic effect of modified atmosphere and chitosan on the growth of aerobic spoilage bacteria.

Fig.51a. Changes in sensory color of water dipped (control) chicken thighs inoculated with S. enteritidis at 4°C.

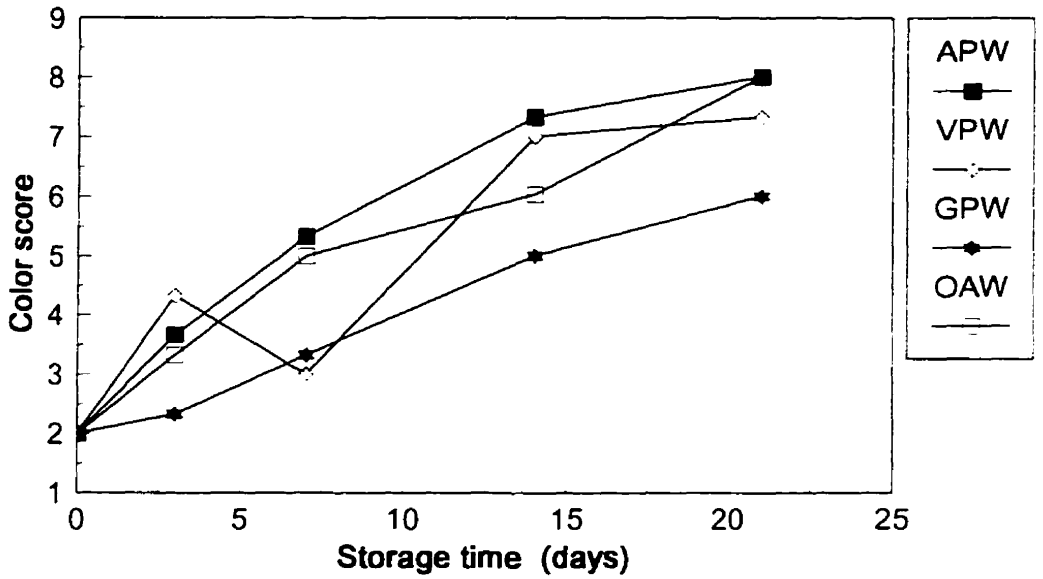
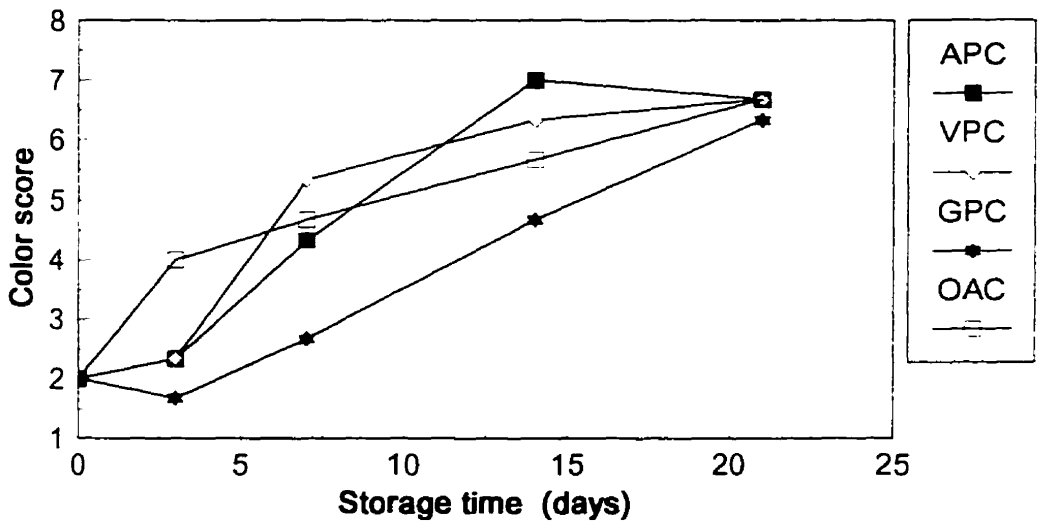


Fig.51b. Changes in sensory color of chitosan dipped chicken thighs inoculated with S. enteritidis at 4°C.



Key:Air(APW),Air+Chitosan(APC),Vacuum(VPW),Vacuum+Chitosan(VPC)
Oxygen Absorbent(OAW) And Oxygen Absorbent+Chitosan(OAC)

Fig.52a. Changes in sensory color of water dipped (control) chicken thighs inoculated with S. enteritidis at 12°C.

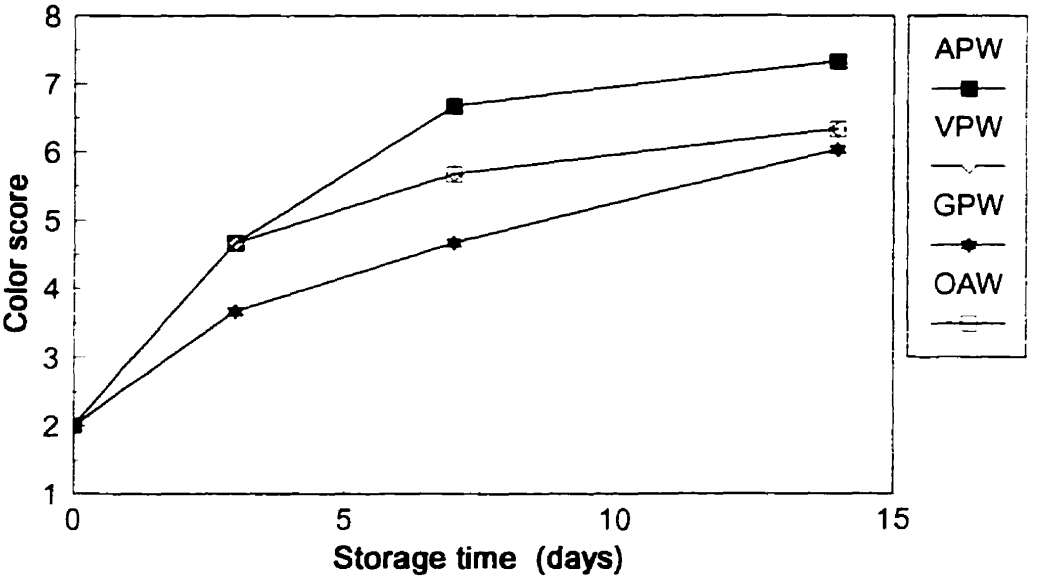
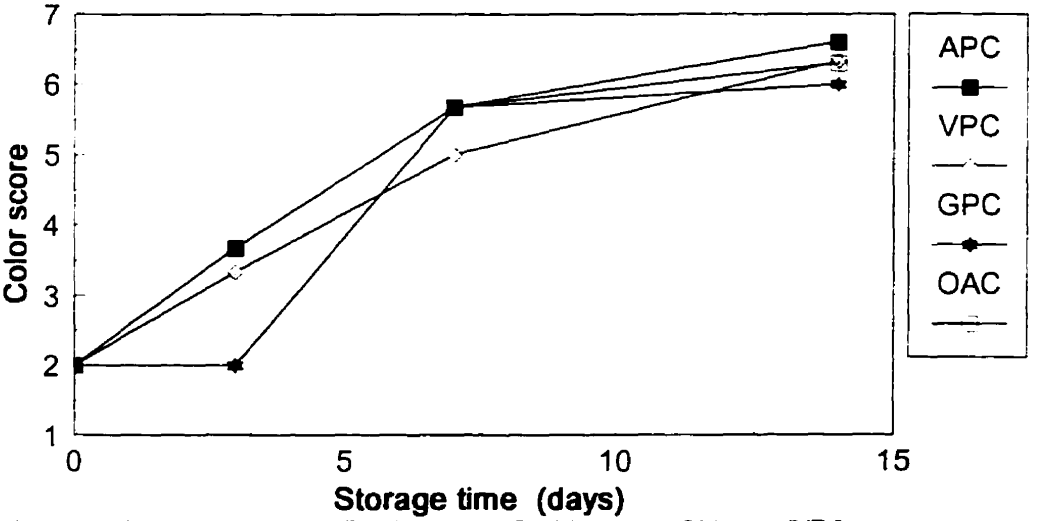


Fig.52b. Changes in sensory color of chitosan dipped chicken thighs inoculated with S. enteritidis at 12°C.



Key:Air(APW),Air+Chitosan(APC),Vacuum(VPW),Vacuum+Chitosan(VPC) Oxygen Absorbent(OAW) And Oxygen Absorbent+Chitosan(OAC)

Fig.53a. Changes in sensory odor of water dipped (control) chicken thighs inoculated with S. enteritidis at 4°C.

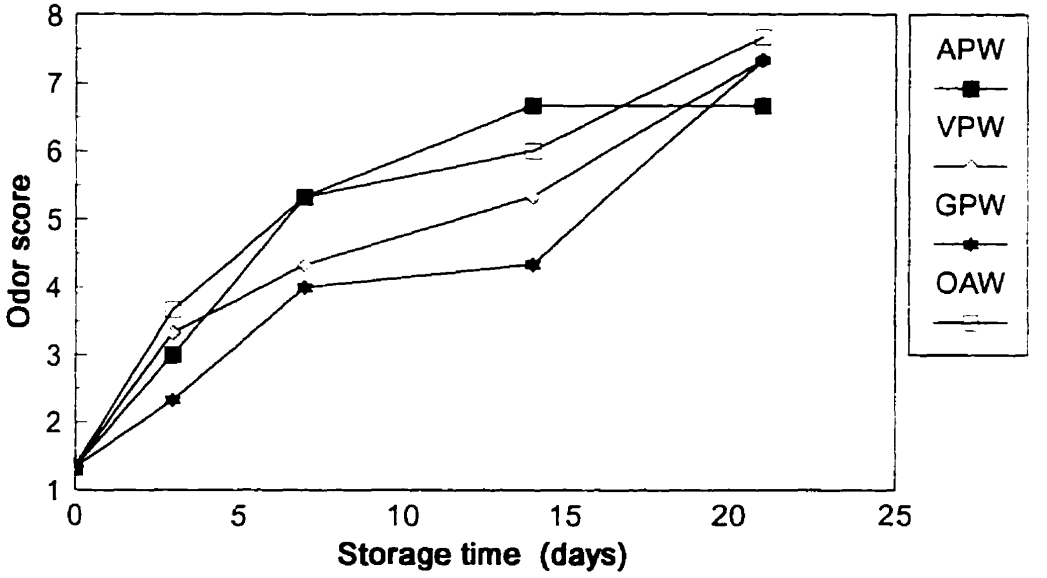
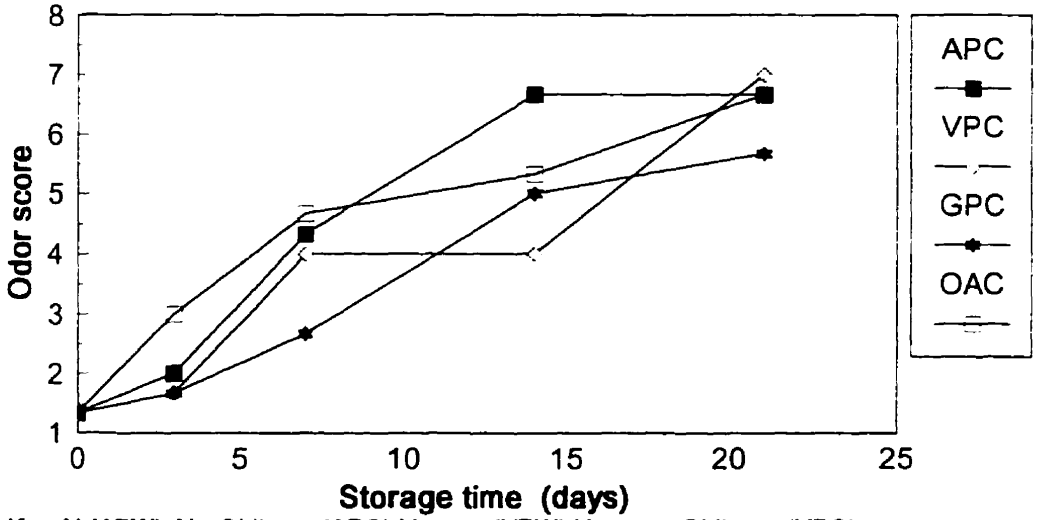


Fig.53b. Changes in sensory odor of chitosan dipped chicken thighs inoculated with S. enteritidis at 4°C.



Key:Air(APW),Air+Chitosan(APC),Vacuum(VPW),Vacuum+Chitosan(VPC) Oxygen Absorbent(OAW) And Oxygen Absorbent+Chitosan(OAC)

Fig.54a. Changes in sensory odor of water dipped (control) chicken thighs inoculated with S. enteritidis at 12°C.

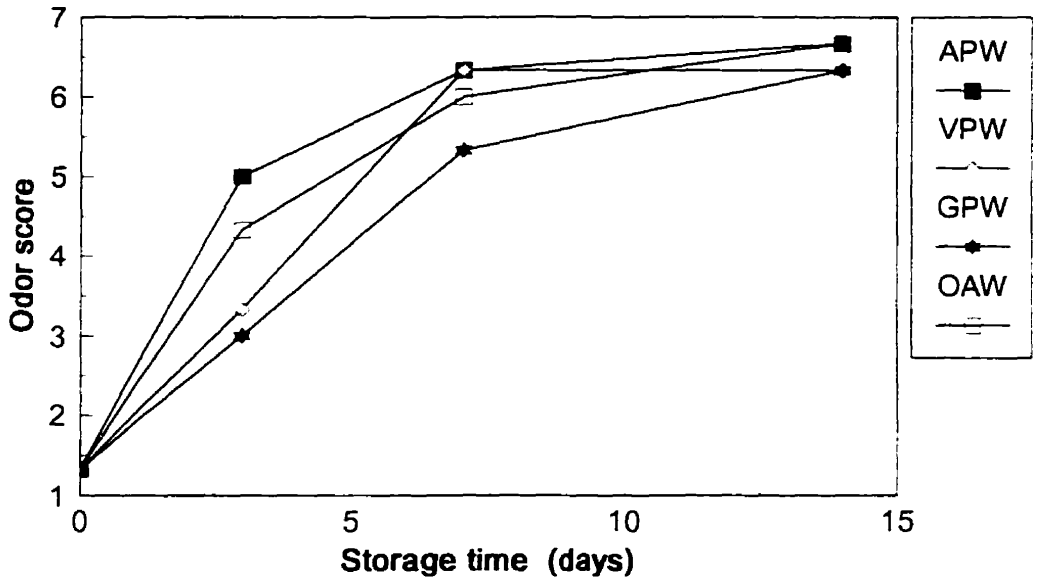
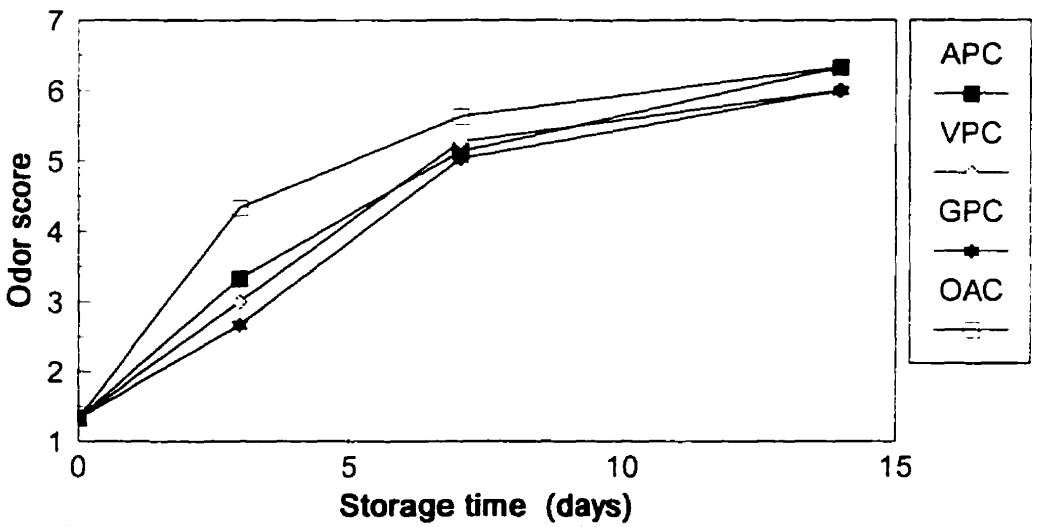


Fig.54b. Changes in sensory odor of chitosan dipped chicken thighs inoculated with S. enteritidis at 12°C.



Key:Air(APW),Air+Chitosan(APC),Vacuum(VPW),Vacuum+Chitosan(VPC) Oxygen Absorbent(OAW) And Oxygen Absorbent+Chitosan(OAC)

4.4.5. pH

The pH of pre-dipped chicken thighs inoculated with *S. enteritidis*, in different atmosphere conditions and stored at 4 and 12°C are shown in Figures 55-56 a,b respectively. At day 0, the average pH of chicken thighs was 6.2 and 6.25 at 4 and 12°C respectively. As storage progressed, there were no significant difference ($P>0.05$) in pH between treatments, although there was a gradual increase in pH in all treatments probably due to microbial activity resulting in the production of volatile amines. The pH of chicken thighs packaged in air increased substantially after 3d and then decreased slightly to the end of the storage period in both the chitosan and non-chitosan dipped samples at 4°C (Figure 55 a,b). Similar results were also observed for the air packaged samples (water& chitosan dipped) at 12°C (Figure 56 a,b). Samples packaged with an oxygen absorbent (water dipped) and stored at 4°C, showed an increase in pH (~0.1-0.2 pH units) (Figure 55a). Similar results were observed for samples packaged with an oxygen absorbent and dipped in chitosan, where an increase in pH were observed in the first 7 d of storage at 4°C (Figure 55b). These changes in pH were not observed in samples packaged with an oxygen absorbent (water&chitosan dipped) at 12°C (Figure 56 a,b). The pH of the vacuum packaged samples (water&chitosan dipped) also increased steadily at both 4 and 12°C, while the pH of chicken thighs packaged in $60\%CO_2+40\%N_2$ (water&chitosan dipped) decreased after day 3 of storage and subsequently increased at all storage temperatures (Figures 55-56 a,b). Furthermore, this decrease was more evident at 4 than at 12°C. This decrease in pH in the gas packaged samples is probably due to the high dissolution of CO_2 into the meat tissue especially at lower temperatures.

4.4.6. Microbial analyses

The growth of *S. enteritidis* in chicken thighs, pre-dipped in deionized water and 0.2% (v/v) chitosan and packaged under various treatments and stored at 4 and 12°C are shown in Figures 57-58 a,b respectively. Initially, levels of *S. enteritidis* increased at 4°C and

then decreased after 14d of storage irrespective of dipping treatment (Figure 57 a,b). However, at 12°C, counts increased after day1 and continued this trend throughout storage (Figure 58 a,b). Packaging of chicken thighs in air, vacuum and with an oxygen absorbent immediately after dipping in 0.2% chitosan (v/v) resulted in significantly lower *Salmonella* counts ($P>0.05$) compared to the water dipped samples (Figure 57 a,b). This decrease in *Salmonella* counts was negligible at higher temperature (Figure 58 a,b). In the air packaged samples stored at 4°C, *Salmonella* grew more rapidly ($P<0.05$) than all other treatments reaching maximum numbers (6.17 $\log_{10}$ CFU/g) after 14d (Figure 57 a). However, thighs packaged in air and pre-dipped in 0.2% chitosan showed significantly lower *Salmonella* counts compared to water dipped samples at 4°C (Figure 57 a,b). Similar results were observed for samples packaged with an oxygen absorbent. Dipping of samples with 0.2% chitosan showed a significant decrease ($P>0.05$) in the growth rate of *S. enteritidis* during the early stages of the storage period after which the rate of growth increased throughout storage. Similarly, vacuum packaged samples pre-dipped in 0.2% chitosan showed delayed growth in the early stages of storage with *S. enteritidis* reaching 4.39 $\log_{10}$ CFU/g after 14d at 4°C. High concentrations of CO_2 have shown to result in an increased lag phase and a decrease in the growth rate of microorganisms (Farber, 1991). This decrease in the logarithmic phase of growth was observed between day 7 and 14 for both the vacuum packaged and gas packaged thighs pre-dipped in chitosan (Figure 57 b). Lambert et al. (1991) reported that CO_2 concentrations released by the tissue in vacuum packaged meat may reach 20%, which explains the similarity between the inhibitory effect of vacuum and gas packaged samples on the growth of *S. enteritidis*. The inhibitory effect of chitosan on the growth of *L. monocytogenes* have been previously reported (Morris et al., 1995). Morris et al. (1995) reported that a combination of 100% N_2 + Ageless FX and dipping in 0.2% chitosan (pH 6) inhibited the growth of *L. monocytogenes* inoculated onto pork at 5°C. The effectiveness of 2.5% chitosan on the inhibition of *S. typhimurium* were also reported by Wang (1992). However in this study, gas packaged samples showed no significant difference ($P>0.05$) from the water dipped samples stored at 4°C (Figure 57 a,b). These results imply that chitosan may be more effective against gram negative bacteria when present in high concentrations. In

addition, it also appears that inhibition of *S. enteritidis* is primarily caused by high concentrations of CO_2 and that chitosan pretreatment has a relatively minimal effect on the growth of this pathogen in the gas packaged samples.

Fig.55a. Changes in pH of water dipped (control) chicken thighs inoculated with S. enteritidis at 4°C.

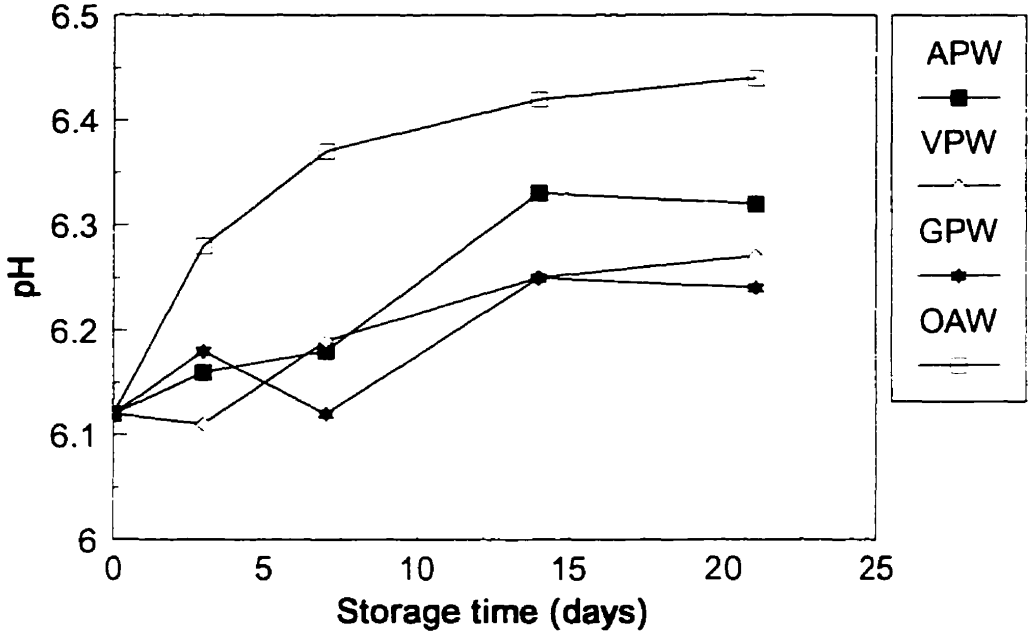


Fig.55b. Changes in pH of chitosan dipped chicken thighs inoculated with S. enteritidis stored at 4°C.

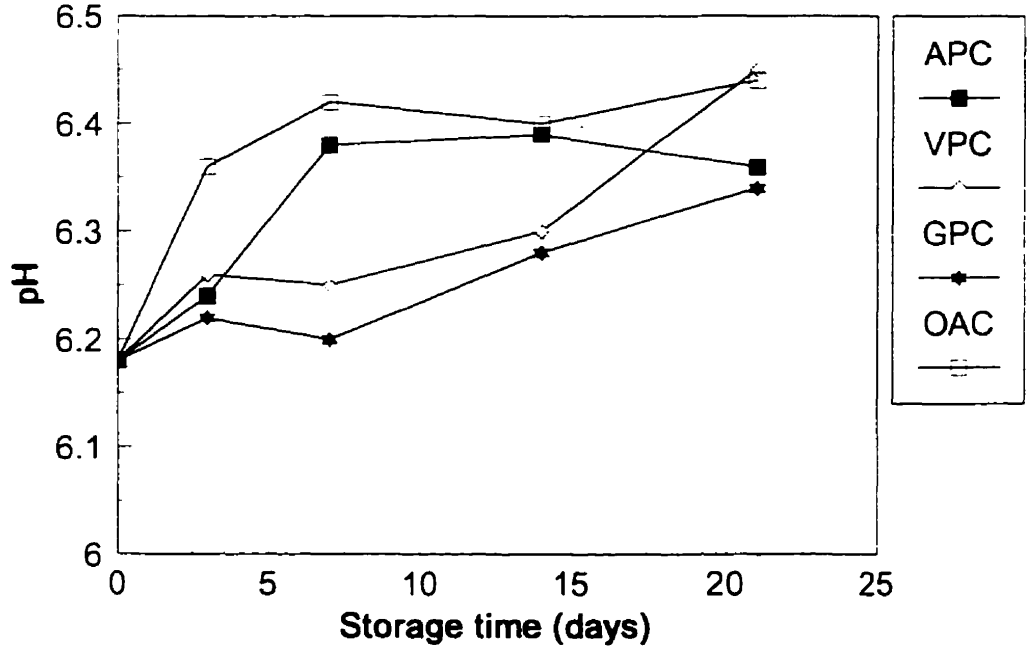


Fig.56a. Changes in pH of water dipped (control) chicken thighs inoculated with S. enteritidis at 12°C.

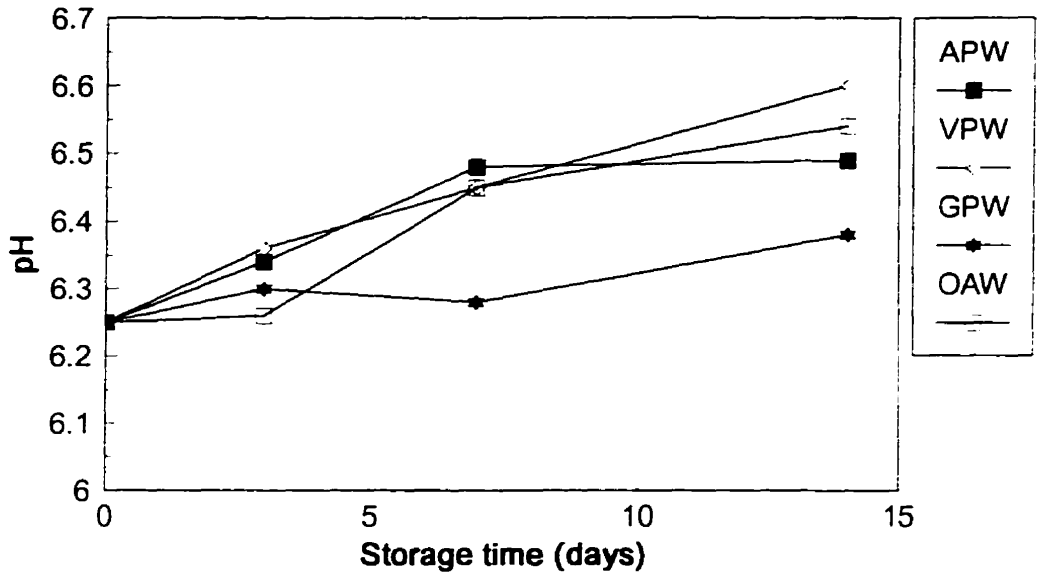


Fig.56b. Changes in pH of chitosan dipped chicken thighs inoculated with S. enteritidis at 12°C.

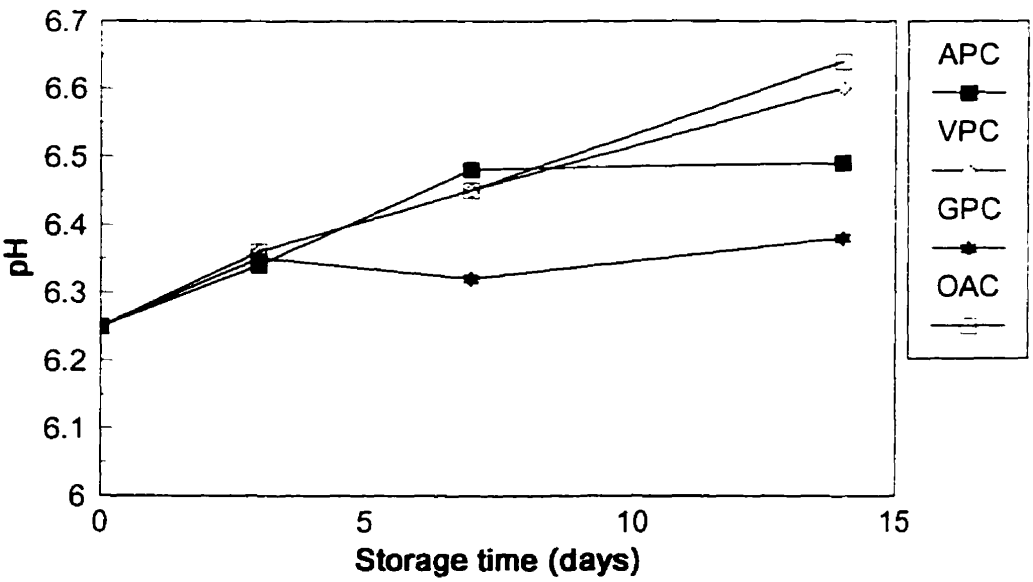


Fig.57a. Effect of packaging atmosphere on the growth of S. enteritidis at 4°C.

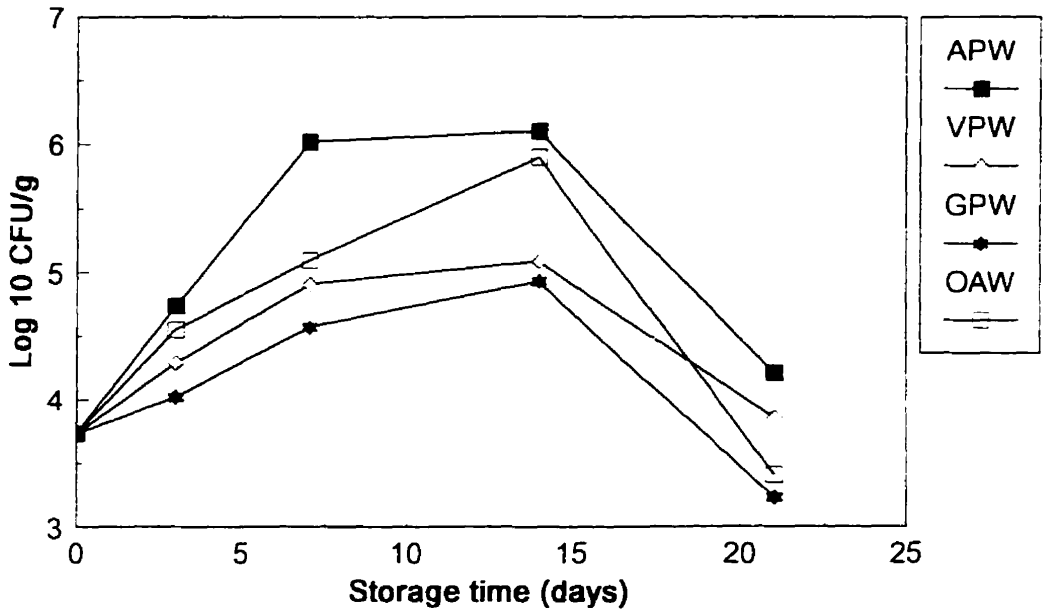
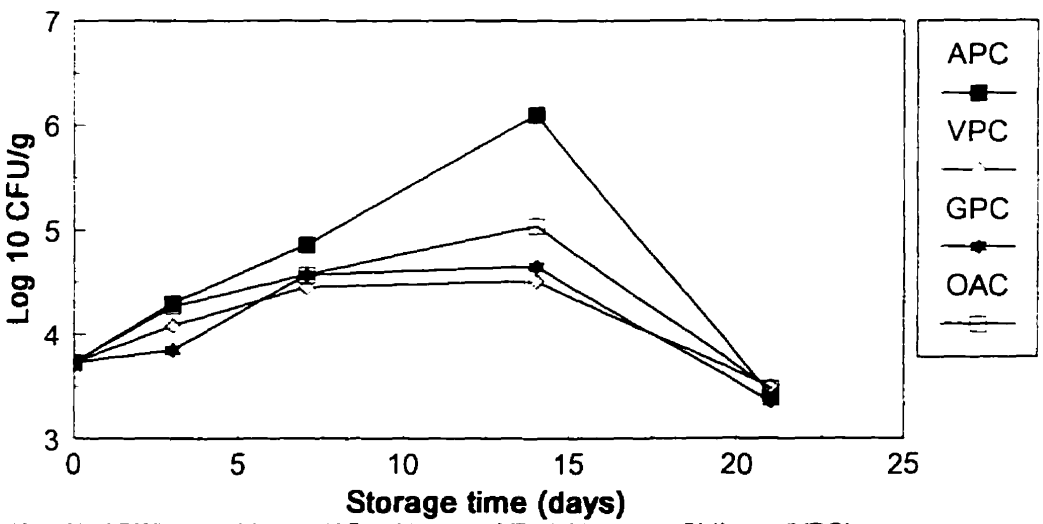


Fig.57b. Effect of chitosan pre-dipping and packaging atmosphere on the growth of S. enteritidis at 4°C.



Key:Air(APW),Air+Chitosan(APC),Vacuum(VPW),Vacuum+Chitosan(VPC)
Oxygen Absorbent(OAW) And Oxygen Absorbent+Chitosan(OAC)

Fig.58a. Effect of packaging atmosphere on the growth of S.enteritidis at 12°C.

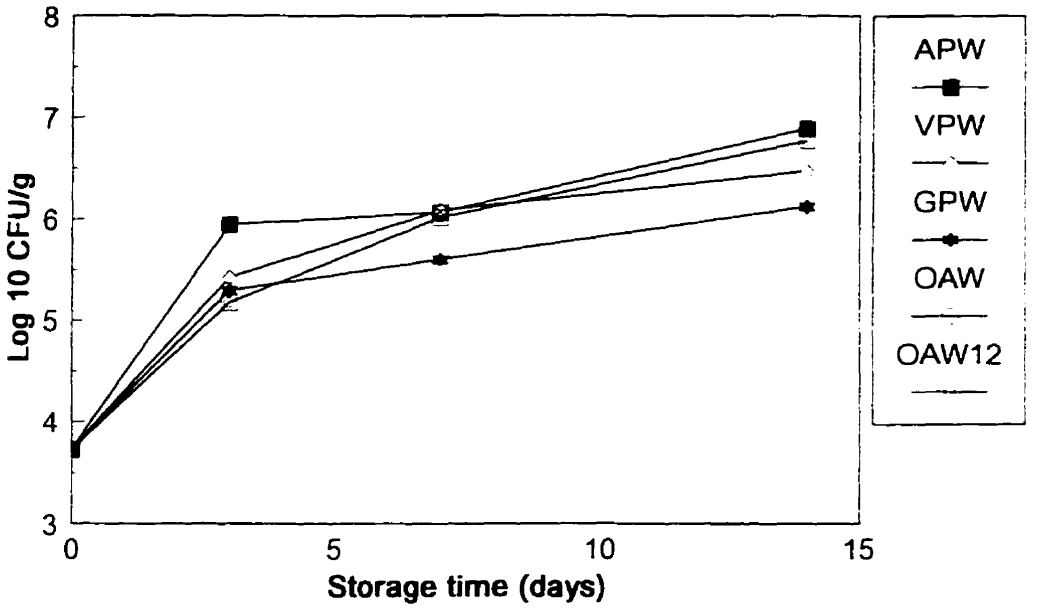
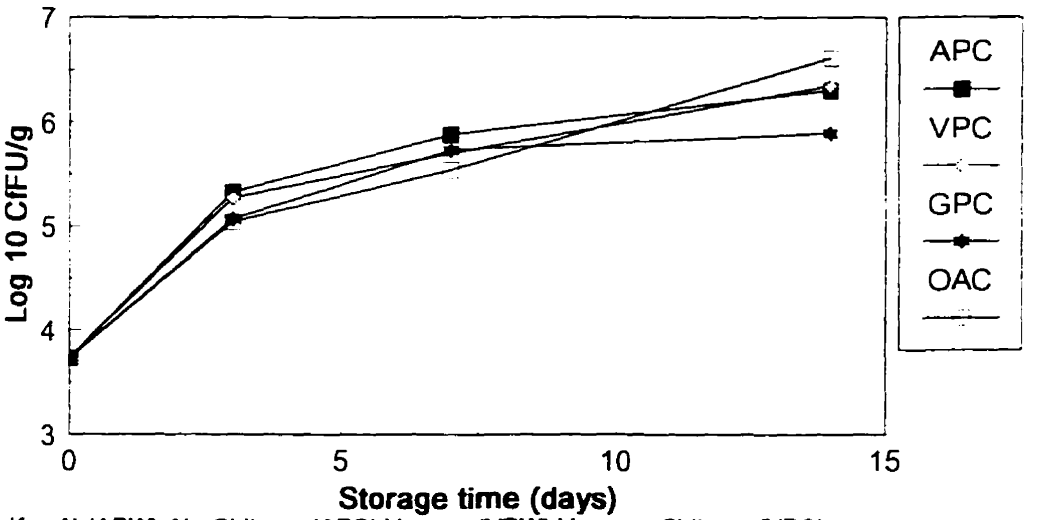


Fig.58b. Effect of chitosan pre-dipping and packaging atmosphere on the growth of S. enteritidis at 12°C.



Key:Air(APW),Air+Chitosan(APC),Vacuum(VPW),Vacuum+Chitosan(VPC)
Oxygen Absorbent(OAW) And Oxygen Absorbent+Chitosan(OAC)

4.4.7. Shelf-life

The sensory shelf-life of chicken thighs inoculated with *S. enteritidis* pre-dipped in deionized water or 0.2%(v/v) chitosan and packaged in various atmospheres and stored at 4°C and 12°C are shown in Table 15. The overall shelf-life extension of chicken thighs was directly proportional to storage temperature, storage time, packaging treatment and the inhibitory effect of chitosan. Treatment with 0.2 % chitosan did not affect the sensory shelf-life of chicken thighs at 4°C. Furthermore, dipping of chicken thighs in 0.2% chitosan resulted in sensory rejection at or before *Salmonella* reached maximum numbers. For example, in air packaged samples, *S. enteritidis* reached maximum numbers before spoilage was detected. However, dipping in chitosan resulted in sensory rejection prior to *S. enteritidis* reaching 10^6 CFU/g. Similarly, an increase in *Salmonella* counts was accompanied by changes in color for samples packaged with an oxygen absorbent. In samples packaged under vacuum, or in 60% CO_2+40% N_2, both the chitosan and the water dipped samples were sensorilly rejected by the panellists before *S. enteritidis* reached high numbers. At higher temperatures (12°C), the growth of *S. enteritidis* limited the overall shelf-life. Chicken thighs packaged in air, vacuum and with an oxygen absorbent were rejected on terms of microbial shelf-life rather than quality deterioration. However, in the gas packaged dipped in chitosan and stored at 12°C, thighs were organoleptically spoiled before *Salmonella* reached high levels indicating that chitosan in conjuction with modified atmosphere can ensure the safety of chicken thighs even under temperature abuse conditions.

Table 15. Estimated shelf-life of water and chitosan pre-dipped chicken thighs inoculated with *S. enteritidis* in various packaging treatments and stored at 4 and 12°C.

Packaging Treatment[a]	Rejection point/color[b] Control	Chitosan	Rejection point/odor[b] Control	Chitosan	Microbial shelf life[c] Control	Chitosan	Shelf life[d] Control	Chitosan
Storage at 4°C								
A	11	12	11	12	7	14	7	12
B	13	12	16	19	>21	>21	13	12
C	>21	20	18	>21	>21	>21	18	20
D	16	15	15	17	14	>21	14	15
Storage at 12°C								
A	6	11	7	12	5	10	5	10
B	14	14	7	13	7	11	7	11
C	14	14	14	>14	14	>14	14	14
D	9	14	10	12	7	11	7	11

[a] A, Air B, Vacuum C, 60%CO_2:40%N_2 D, Ageless SS.

[b] Time to reach a score of six

[c] Time to reach a Salmonella count of 10^6 CFU/g.

[d] Earliest rejection point on terms of odor,color and microbial load

Conclusion

Packaging of chicken thighs after 1 minute pre-dipping in chitosan showed that chitosan substantially retarded the growth of *S. enteritidis* in all treatments and increased the shelf-life of chicken thighs. This effect was negligible at higher temperatures and more pronounced at 4°C. Pre-dipping of thighs in chitosan followed by gas packaging in 60%$C0_2$+40%N_2 could inhibit the growth of *S. enteritidis* to significant levels to 20d compared to 7d for air packaged samples at 4°C. From these results, it appears that gas packaging in conjunction with 0.2 % chitosan could be used to inhibit the growth of *S. enteritidis* and substantially improve the shelf-life and safety of chicken thighs.

CHAPTER 5

COMBINED EFFECT OF MAP AND POTASSIUM SORBATE ON GROWTH OF *SALMONELLA ENTERITIDIS* IN PACKAGED POULTRY

5.1. INTRODUCTION

The use of organic acids and their salts such as sorbates, lactates, propionates and ascorbates have been used successfully to extend the shelf-life of fresh meat and poultry. The antimicrobial activity of these compounds is a result of a decrease in pH and a specific antimicrobial effect of the undissociated acid. However, few studies have examined the combined effect of MAP involving gas packaging and sorbates to inhibit the growth of *S. enteritidis* in poultry. Thus, the objectives of this research were:

1. To determine the optimum pH and concentration of potassium sorbate required to inhibit the growth of *S. enteritidis* in a model broth system;
2. To determine the effectiveness of potassium sorbate in combination with CO_2 enriched atmospheres on the growth of *S. enteritidis* in packaged chicken thighs.

5.2. MATERIAL & METHODS

5.2.1. Preparation of inoculum

S. enteritidis was maintained on TSA ^{NAST} slants at 4°C and transferred every 21d to ensure viability. The inoculum was prepared by transferring a loopful of the isolates from slants and streaking onto TSA^{NAST} agar (Difco) followed by incubation aerobically at 37°C for 48h. Isolate colonies of *S. enteritidis* were then transferred into 10ml of TSBYE^{NAST} broth and incubated at 37 °C for 24h to give a stock solution of ~10^9 CFU/ml. Appropriate

dilutions were made using 0.1 % buffered peptone water (w/v) to give an inoculum level of 2×10^5 CFU/ml for all studies described in this chapter. Preparation of all antibiotic media containing 100 p.p.m. of nalidixic acid and streptomycin sulphate was prepared as previously described **(See Section 3.2.1.)**.

5.2.2. Effect of pH, potassium sorbate (KS) on the growth of *S. enteritidis* in broth system

A 1 % stock solution of potassium sorbate was prepared by adding 10g to 1000ml of sterile, deionized water. Appropriate amounts of potassium sorbate stock solution were then added to $TSBYE^{NAST}$ broth in experimental flasks to give sorbate concentrations of 0, 0.1 & 0.2 % (w/v). The pH of the broths was then adjusted to pH 5 and 6 using food grade lactic acid and 1M sodium hydroxide. All pH measurements of the adjusted broth were carried out using a previously calibrated pH meter. The broths were autoclaved at 121°C (15 PSI for 15 mins) and then cooled. After cooling, 1 ml of a suspension of *S. enteritidis* **(See Section 5.2.1.)** was aseptically transferred into each flask to give an inoculum level of ~10^3 CFU/ml. The flasks were then incubated at 4 and 12°C (two at each temperature). At each sampling time (1-7days), flasks were removed and 0.2 ml of broth spread plated onto TSA^{NAST} agar (in duplicate). All plates were enumerated after aerobic incubation at 37°C for 48h.

5.2.3. Effect of MAP and potassium sorbate (KS) on the growth of *S. enteritidis* in chicken thighs

Based on the previous results, a combination of gas packaging (60% CO_2, 100 % CO_2) and potassium sorbate dip (0.2 % (w/v), pH 5) was used to study their combined effect on the growth of *S. enteritidis* in packaged chicken thighs.

5.2.3.1. Inoculum preparation

Storage, subculturing and preparation of *S. enteritidis*^{NAST} inoculum was as described previously **(See Section 5.2.1.)**.

5.2.3.2. Preparation of potassium sorbate

A 1% stock solution of potassium sorbate was prepared as described previously **(See Section 5.2.2.)** and appropriate dilutions of potassium sorbate stock solution were made to give sorbate concentrations of 0.2 % (w/v) in 3 × 2 litre flasks. The pH of the solution was adjusted to pH 5 using sterile food grade lactic acid and autoclaved for 15 minutes at 121°C. The pH of the sorbate dipping solution was then checked after autoclaving as described previously **(See Section 5.2.2.)** to ensure the final pH of the sorbate solution was identical to its pH prior to autoclaving. The sorbate dipping solutions were then stored at 4°C until used.

5.2.3.3. Preparation of chicken thighs and packaging.

Chicken thighs were again obtained frozen from Club Price, Montreal. After thawing under controlled conditions (4°C), thighs (~50 gram each) were dipped in the 0.2% potassium sorbate dipping solution (pH 5) for 1 minute. After dipping, the chicken thighs were allowed to drain in a sterile beaker and then placed individually in high gas barrier bags (Cryovac, USA). A fresh potassium sorbate dipping solution was used for every 25 chicken thighs. All control samples were dipped in sterile deionized water for the same time and treated as per potassium sorbate dipped chicken thighs.Chicken thighs were then inoculated with 0.5 ml of *S. enteritidis*^{NAST} prepared previously **(See Section 5.2.1.)** to give an inoculum level of ~10^3 CFU/g. All samples were then packaged in the following treatments: air (control), 60% CO_2 + 40% N_2 , 100% CO_2. All packaged products were stored at 4 and 12°C for 28d.

5.2.3.4. Headspace gas composition

Headspace gas composition was determined as previously described **(See Section 3.1.4.)**

5.2.3.5. Color analyses

Color analysis was measured as previously described **(See Section 2.2.2.2.)**.

5.2.3.6. Drip loss

Drip loss were measured as previously described **(See Section 2.2.2.3.)**.

5.2.3.7. Sensory, pH And microbiological analyses

Sensory, pH and microbiological analyses were carried out as previously described **(See Section 3.2.8.)**.

5.2.3.8. Statistical analyses

Data were analyzed using a split plot design in which storage time was the main plot effect and the storage temperature and the packaging treatment the subplot effect. Bacterial counts were expressed as $\log_{10}$ CFU/g and the reported means were the average of three replicates per treatment. Differences between means were analyzed using the least significant difference (LSD) and the Duncan multiple range test. Probability (P) of $P<0.05$ was considered to be significantly different.

5.3. RESULTS & DISCUSSION

5.3.1. Effect of potassium sorbate on *S. enteritidis* in a broth system

The effect of various concentrations (0-0.2 % w/v) of potassium sorbate (KS) on the growth of *S. enteritidis* in TSBYE broth at pH 5 & 6 stored at 4°C and 12°C are shown in Figure 59 a,b . In general, potassium sorbate inhibited the growth of *S. enteritidis* in a model broth system particularly at pH 5. The inhibition was also greater at 4°C and significantly reduced at 12°C. Significant differences ($P<0.05$) between the control (water dipped) samples (pH5&6) and the sorbate treated samples were observed after the first days of storage indicating that inhibitory effect on the growth of *S. enteritidis* was due to potassium sorbate and not pH effect. Counts of *S. enteritidis* in chicken thighs at day0 were ~ 3.5 $\log_{10}$ CFU/g at day0. At day 3, all sorbate treated samples had a 1 log lower *Salmonella* counts than the controls. These results indicate that potassium sorbate had an immediate effect on the initial growth of *S. enteritidis*. Furthermore, this inhibition was greater as sorbate concentration increased and pH decreased. An initial decrease in *S. enteritidis* counts was observed in TSBYE broth containing 0.1% potassium sorbate at pH 5 & 6, followed by increase in growth. Similar results were also observed for higher concentrations of potassium sorbate (0.2 %) in TSBYE broth at pH 6. However, the addition of 0.2 % potassium sorbate in TSBYE broth at pH 5 resulted in an extended lag phase followed by slow growth with counts being 1-2 logs less than all other sorbate treated samples. These results are in agreement with previous studies which show that the inhibitory effect of potassium sorbate is both concentration and pH dependent. Potassium sorbate has a pKa value of 4.72 therefore a low pH will result in a higher concentration of the acid in the undissociated state and hence greater antimicrobial activity. Thus, it can be concluded from this study that a level of 0.2 %(w/v) or higher of potassium sorbate is required to inhibit the growth of *S. enteritidis* in TSBYE broth at pH 5. Based on these results, a dipping solution (0.2% w/v, pH5) was used in subsequent studies.

Fig.59a. The growth of S. enteritidis in various potassium sorbate concentrations (0.1-0.2 %) and at two pH levels (5-6) at 4°C.

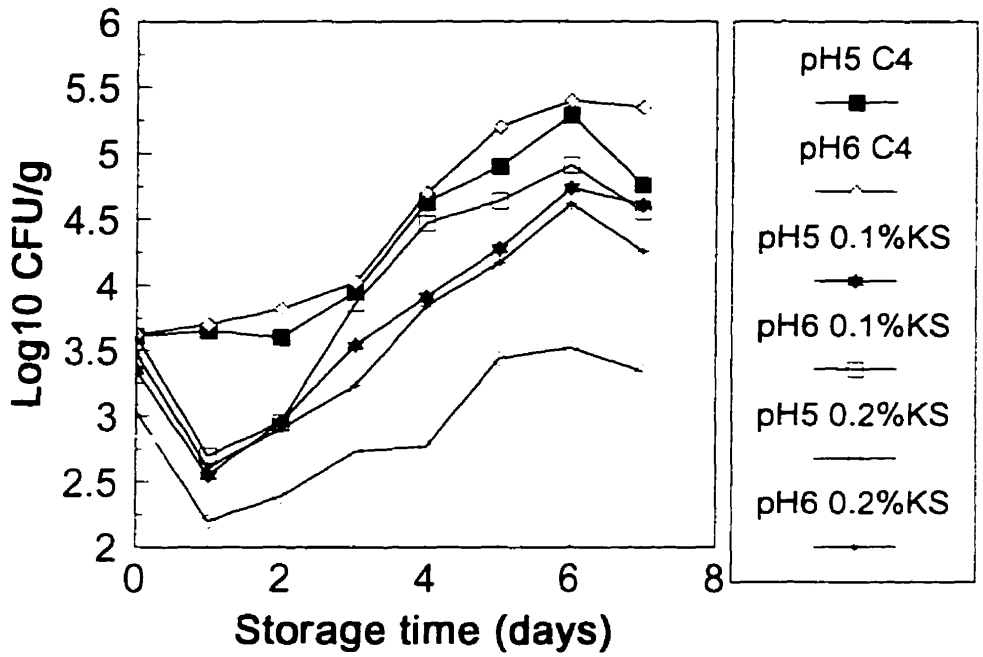
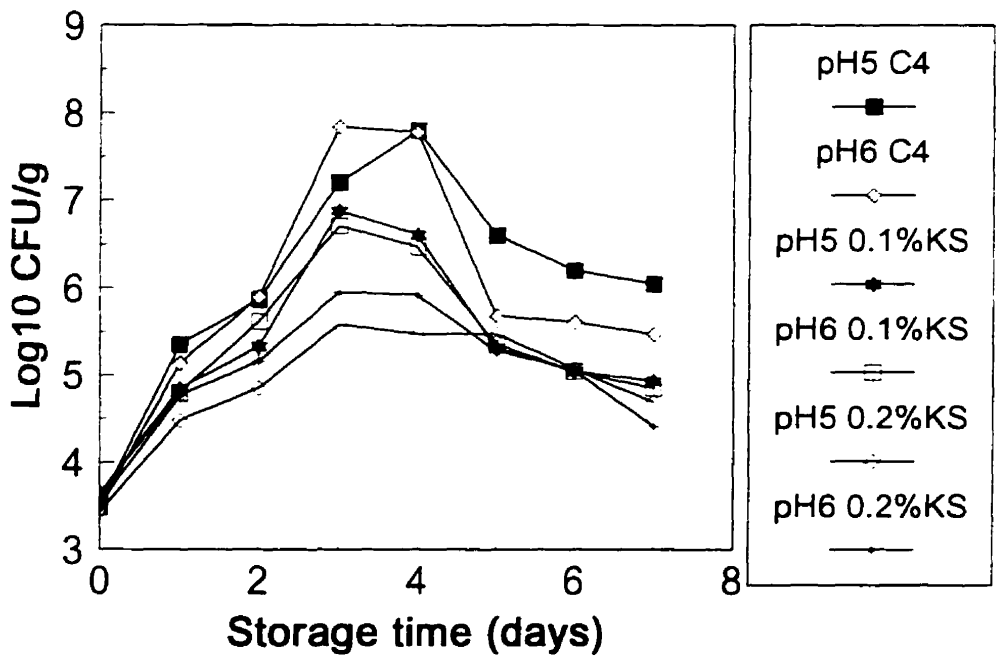


Fig.59b.The growth of S. enteritidis in various potassium sorbate concentrations (0.1-0.2 %) and at two pH levels (5-6) at 12°C.



5.3.2. Changes in headspace gas composition

Changes in the headspace gas composition of chicken thighs dipped in potassium sorbate dipping solution (0.2 %w/v, pH 5), packaged in various atmospheres and stored at 4 and 12°C are shown in Figures 60-65 a,b respectively.

For the air packaged samples (control), O_2 was depleted to less than 2% and CO_2 increased to 12% after 28d at 4°C (Figure 60a). Similar changes were observed for the air packaged samples at 12°C, i.e., O_2 was rapidly depleted to less than 2% and CO_2 increased to 24% after 14d at 12°C (Figure 61a). Similar trends were also observed for the air:sorbate treatment. Headspace O_2 was depleted to less than 4% after 28 and 14d at 4 and 12°C respectively (Figure 60-61 b). Headspace CO_2 in the air:sorbate treatment increased to more than 15% and 24% at 4 and 12°C respectively (Figure 60-61b). These changes in the headspace gas composition can be attributed to the growth and metabolism of aerobic and facultative aerobic spoilage bacteria that utilizes O_2 and produce CO_2.

For samples packaged in 60% CO_2, with or without potassium sorbate, a decrease in CO_2 concentration was observed in the early stages of the storage at 4°C. Following this initial decrease, a steady increase in CO_2 was observed after 7 and 14d followed by a decrease towards the end of the storage period (Figures 62 a,b). Similar, but less dramatic trends, were also observed for all 60% CO_2 treatments at 12°C (Figures 63 a,b). Headspace CO_2 decreased to approximately 58% after day 3 and then increased steadily throughout storage. Such changes in headspace gas composition have been previously observed by other researchers (Laleye et al., 1984; Seideman et al., 1979). They reported an initial decrease in headspace CO_2 in the early stages of the storage period followed by an increase in CO_2 and a relative decrease in headspace O_2. These changes in headspace gas composition have been attributed to post mortem metabolic and microbial activities. Low levels of residual O_2 (0.1-0.2%) were detected only in the 60% CO_2 sorbate treatments between 7 and 14d at both 4 and 12°C. The presence of residual O_2 in the other CO_2 enriched atmospheres (control samples) may be an indication of leakage through small pinholes or inappropriate flushing/sealing.

Chicken thighs packaged in 100% CO_2, with or without potassium sorbate, showed

a steady decrease in CO_2 in the initial stages of the storage period at both 4 and 12°C after which time the concentration of CO_2 remained fairly constant (Figures 64-65 a,b). This initial reduction in CO_2 is due to the dissolution of CO_2 into the meat tissue. Headspace O_2 in all 100%CO_2 treatments (control & sorbate) remained negligible towards the end of the storage period (Figures 64-65 a,b). Laleye et al. (1984) reported the absence of O_2 in all modified atmosphere treatments throughout the 49 days of storage at both 3 and 7°C. Furthermore, Seideman et al. (1979) reported that the majority of gas in 100% CO_2 is carbon dioxide and that any presence of oxygen in the headspace is usually attributed to film permeability or leakage.

From these results, it appears that the headspace gas composition of sorbate treated samples in various atmospheres showed slightly lower concentrations of CO_2 compared to the water dipped controls. Gray et al. (1984) concluded that potassium sorbate were less effective against the spoilage flora of poultry compared to *Salmonella* or *S. aureus*. Thus, these small changes in headspace gas composition suggest that potassium sorbate had no effect against the spoilage flora of chicken thighs and that shelf-life extension was primarily due to high concentrations of CO_2 in the package headspace.

Fig.60a. Changes in headspace gas composition of water dipped (control) chicken thighs inoculated with S. enteritidis and stored in air at 4°C.

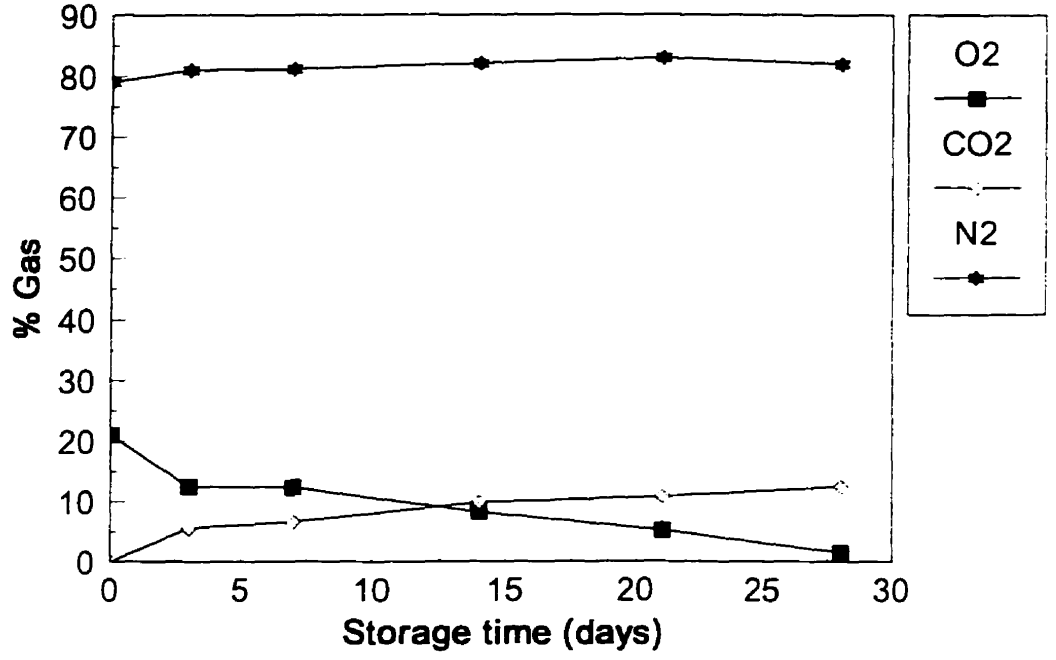


Fig.60b. Changes in headspace gas composition of KS dipped chicken thighs inoculated with S. enteritidis and stored in air at 4°C.

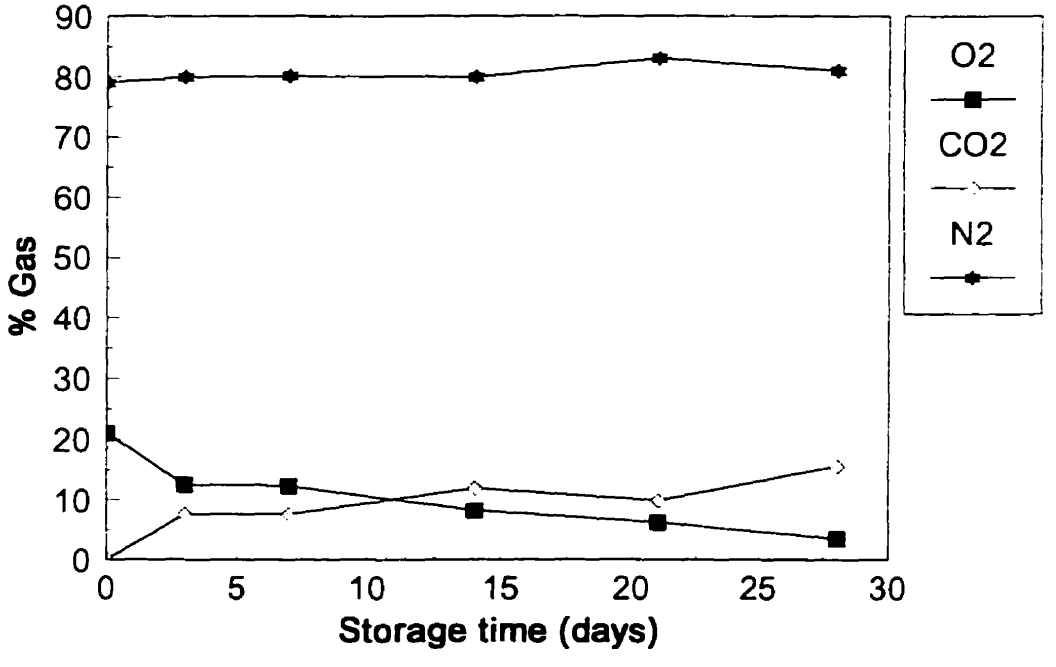


Fig.61a. Changes in headspace gas composition of water dipped (control) chicken thighs inoculated with S. enteritidis and stored in air at 12°C.

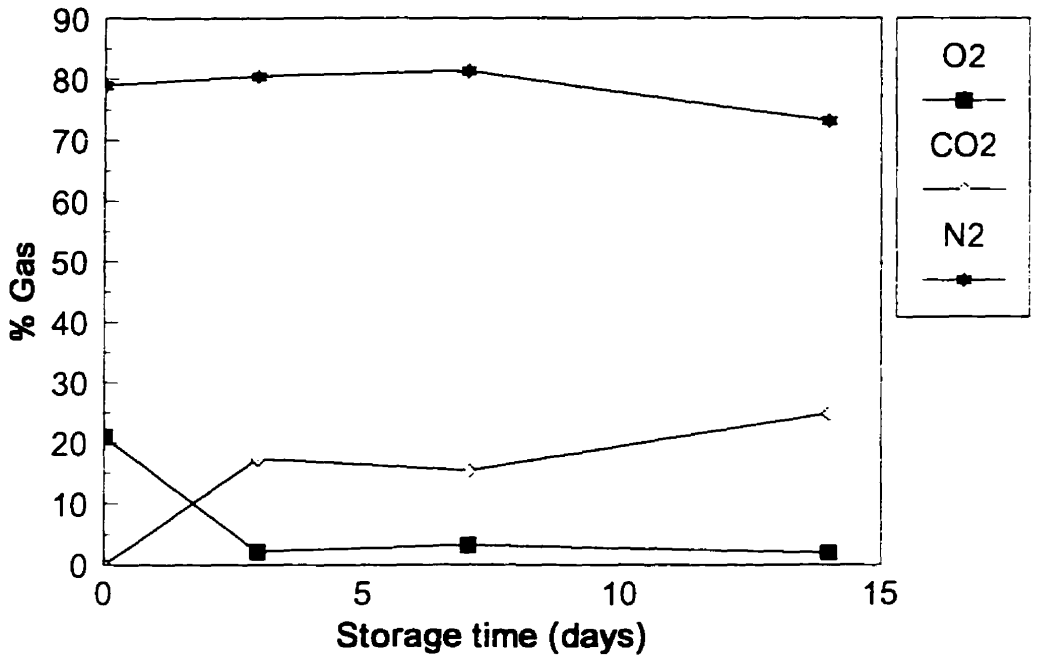


Fig.61b. Changes in headspace gas composition of KS dipped chicken thighs inoculated with S. enteritidis and stored in air at 12°C.

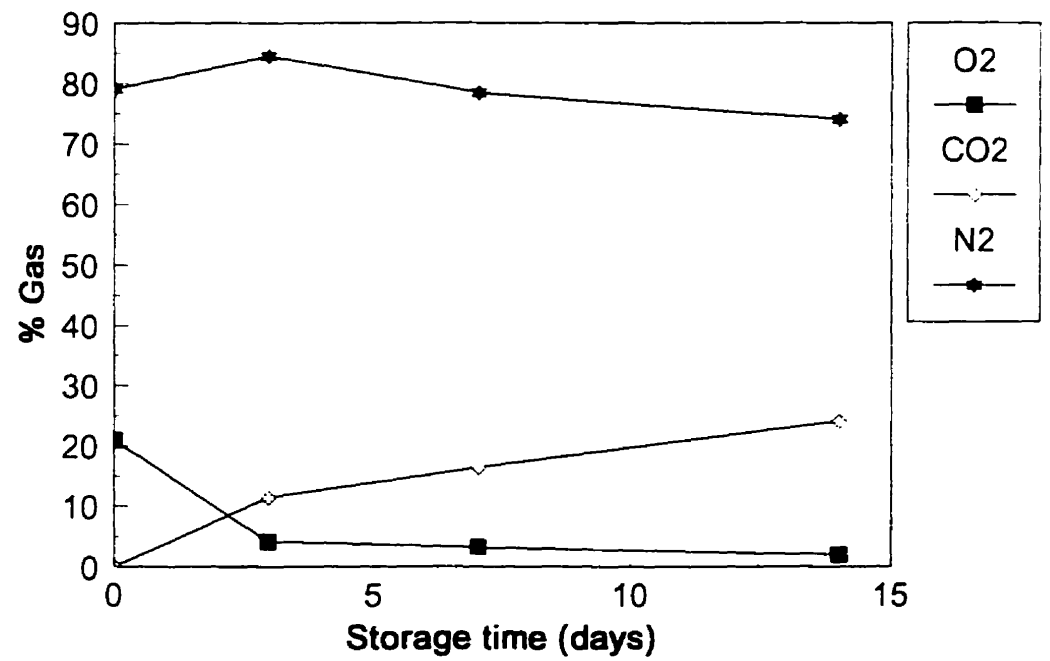


Fig.62a. Changes in headspace gas composition of water dipped (control) chicken thighs inoculated with S. enteritidis and stored in 60% CO2 at 4°C.

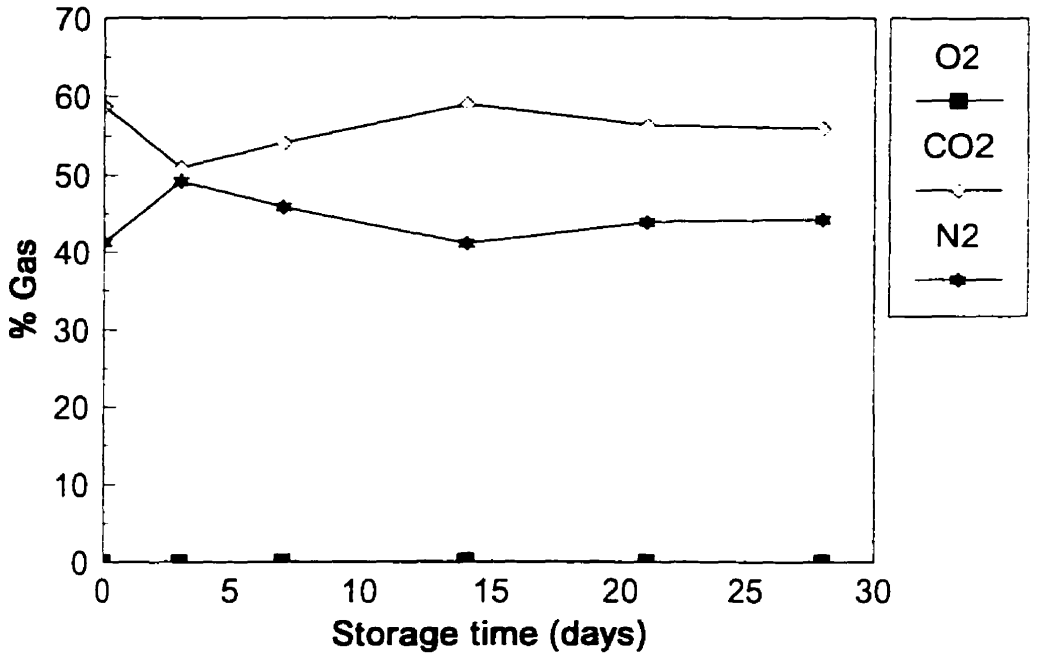


Fig.62b. Changes in headspace gas composition of KS dipped chicken thighs inoculated with S. enteritidis and stored in 60% CO2 at 4°C.

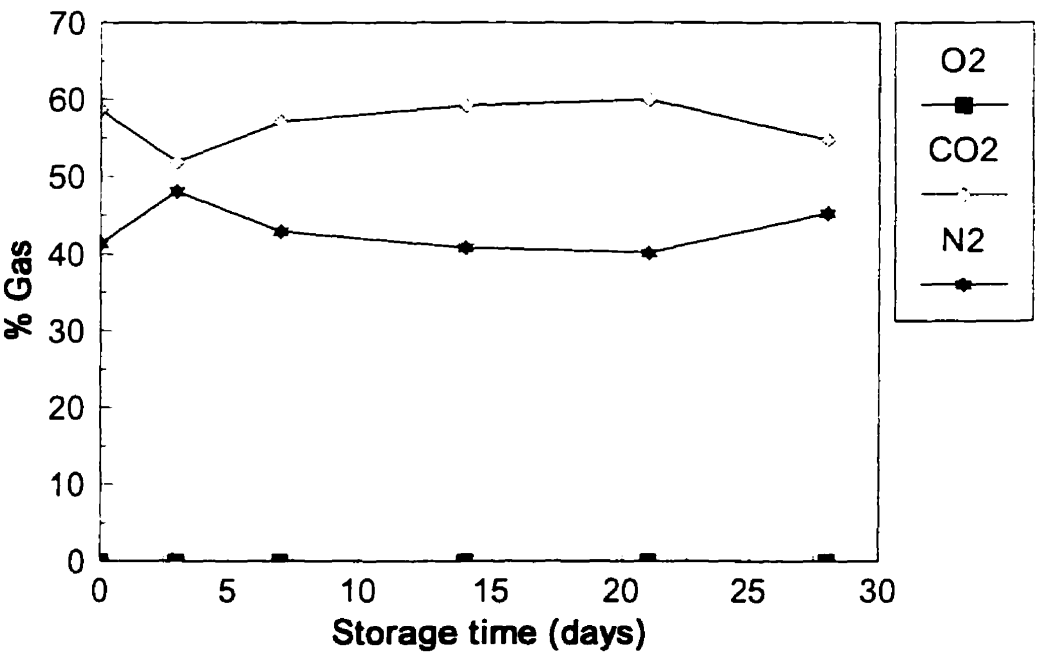


Fig.63a. Changes in headspace gas composition of water dipped (control) chicken thighs inoculated with S. enteritidis and stored in 60% CO2 at 12°C.

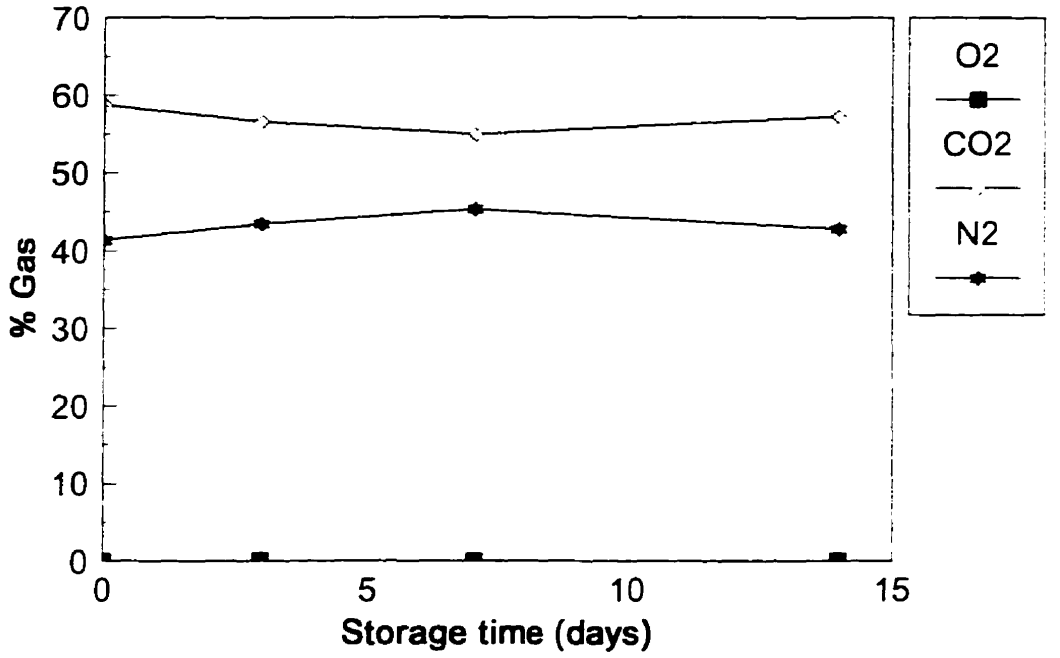


Fig.63b. Changes in headspace gas composition of KS dipped chicken thighs inoculated with S. enteritidis and stored in 60% CO2 at 12°C.

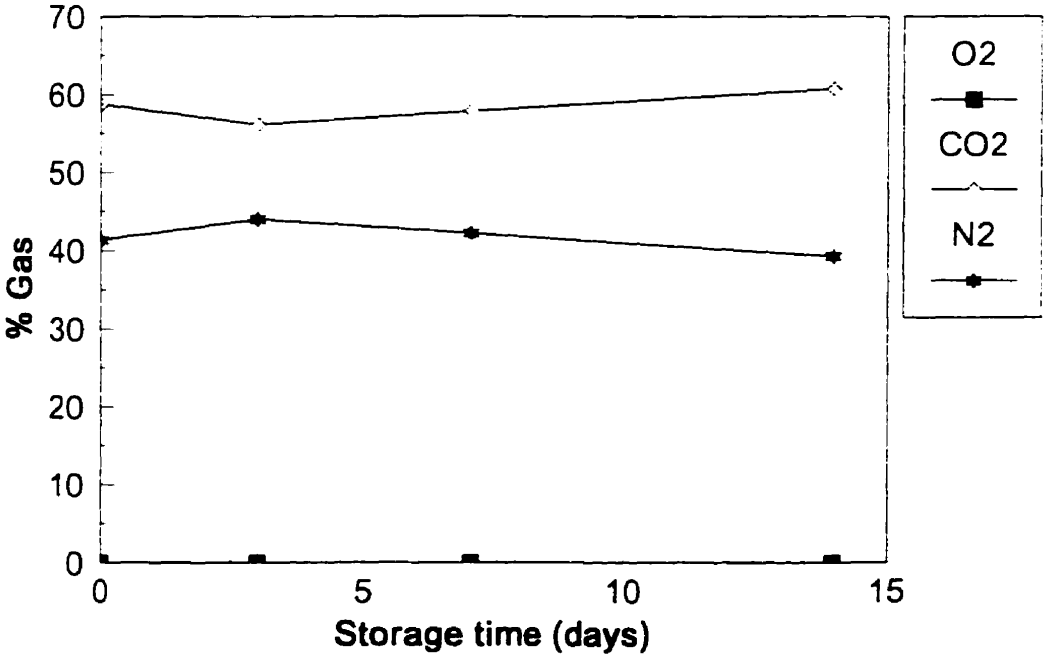


Fig.64a. Changes in headspace gas composition of water dipped (control) chicken thighs inoculated with S. enteritidis and stored in 100% CO2 at 4°C.

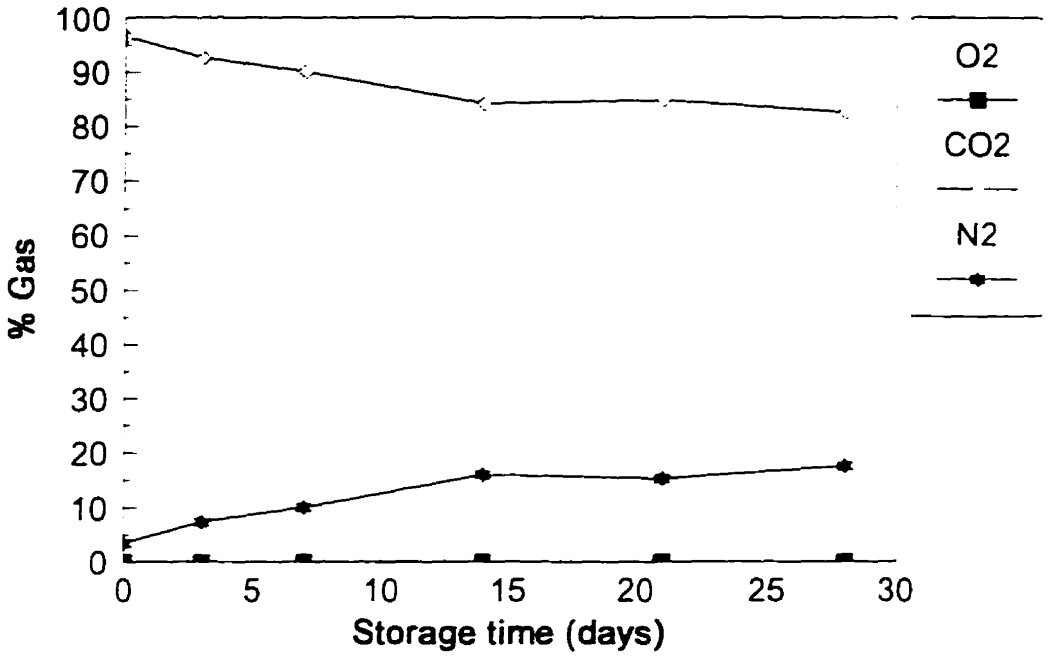


Fig.64b. Changes in headspace gas composition of KS dipped chicken thighs inoculated with S. enteritidis and stored in 100% CO2 at 4°C.

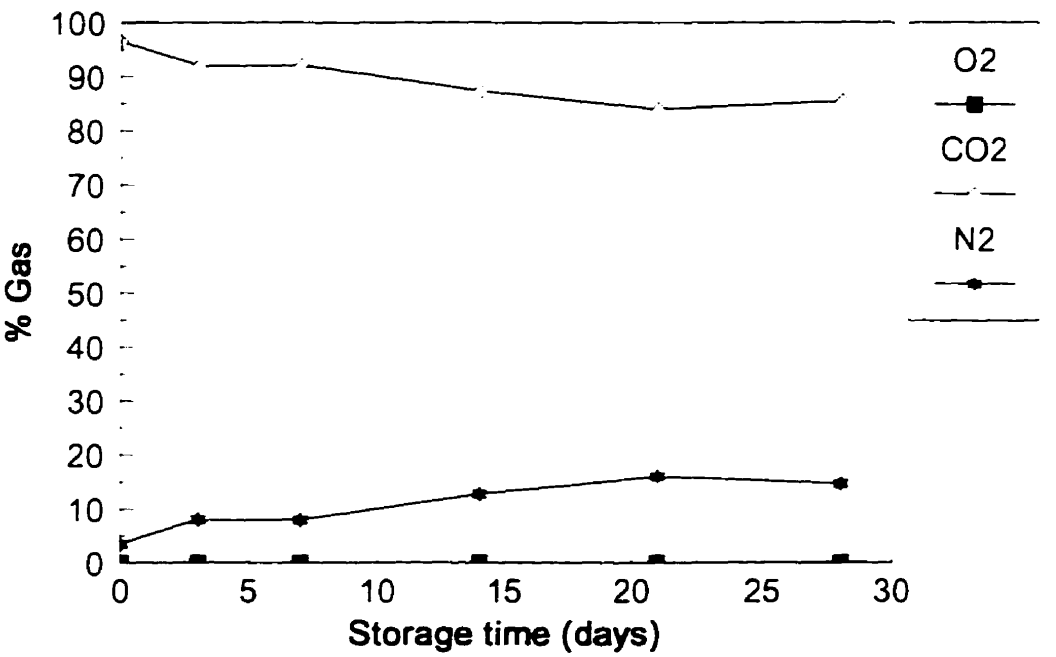


Fig.65a. Changes in headspace gas composition of water dipped (control) chicken thighs inoculated with S. enteritidis and stored in 100% CO2 at 12°C

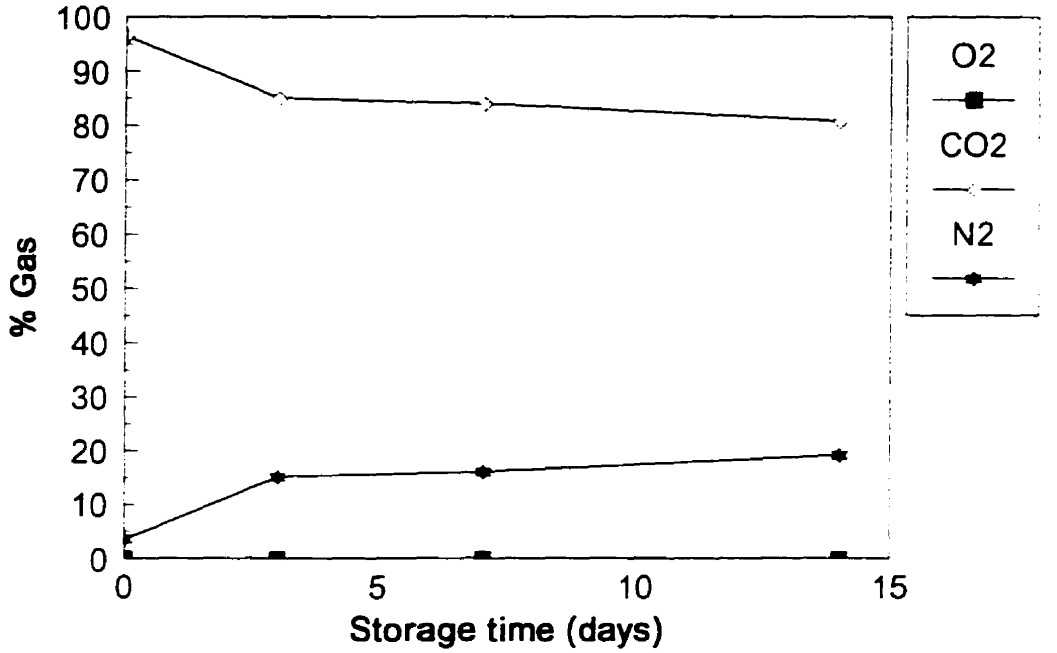
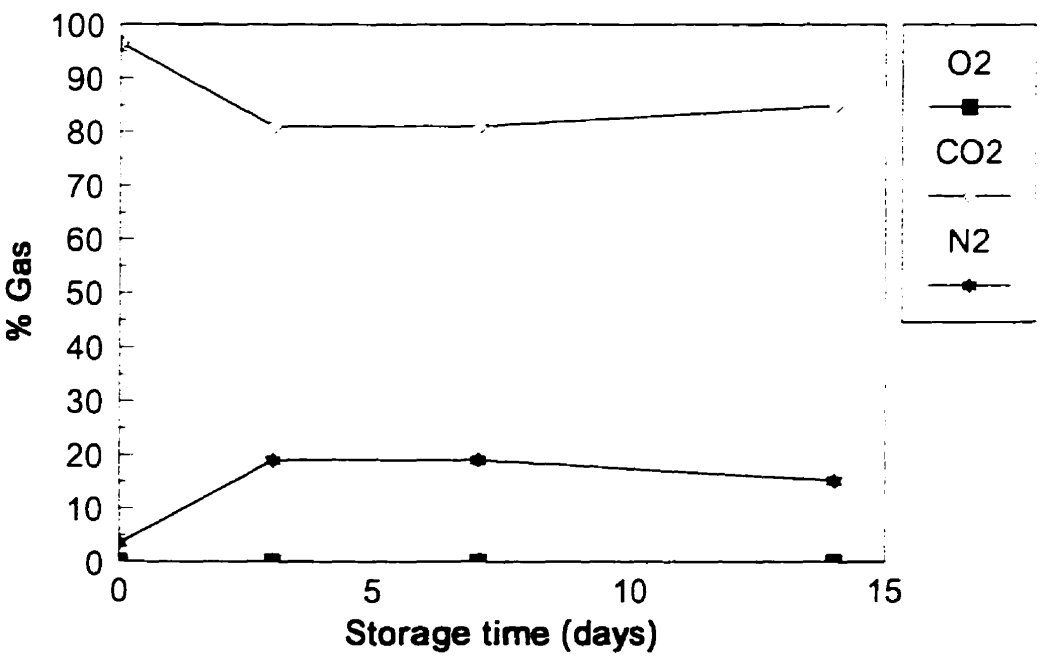


Fig.65b. Changes in headspace gas composition of KS dipped chicken thighs inoculated with S. enteritidis and stored in 100% CO2 at 12°C.



5.3.3. Color analyses

The L* (lightness), a* (redness) and b* (yellowness) values of chicken thighs pre-dipped either in deionized water (pH 5) or in potassium sorbate dipping solution (0.2 %w/v, pH 5) and packaged in various atmospheres and stored at 4 and 12°C are shown in Figures 66-68 a,b respectively. The L* values of chicken thighs increased irrespective of the dipping/packaging treatments indicating the lightness of chicken thighs was increasing i.e., a similar observation to previous studies with chitosan. At 4°C, the L* values increased steadily throughout storage and was relatively affected by the sorbate/packaging treatments. At 12°C, however, chicken thighs packaged in air (sorbate & control) had a higher ($P<0.05$) L* value than any of the other treatments after 7d (Figure 66 b). This increase in L* value reflects the high numbers of *Salmonella* present in the air packaged samples (sorbate & control) at 12°C. Lower ($P<0.05$) a* values were observed for the air treatments (sorbate & control) compared to the other treatments at both 4 and 12°C (Figure 67 a,b). The air:sorbate treated samples, in many cases, had a higher a* value than the air control, although this increase was not statistically significant ($P>0.05$).This decrease in a* value can be attributed not only to the presence of O_2 in the air packaged samples that oxidizes myoglobin into metmyoglobin but also to the enhanced oxidation of meat pigments in the presence of sorbates. Lipid oxidation catalyses the formation of metmyoglobin (Renerre, 1990). The unsaturated nature of potassium sorbate may add to the material to be oxidized and thus promote the oxidation of the meat pigment. Higher ($P>0.05$) b* values were also observed for the air treatments (sorbate & control) than those packaged in 60 or 100% CO_2 (sorbate & control). These observations could be largely attributed to the high drip present in the air packaged samples resulting in the dilution of the meat pigment.

Samples packaged in 60 or 100% CO_2 (sorbate & control) did not differ significantly ($P<0.05$) in the L* values which were more red than yellow (Figure 66 a,b). Changes in a* value were observed in all modified atmosphere treatments (60 & 100% CO_2) irrespective of the dipping solution. After 7d of storage, the 100% CO_2 treatments (sorbate & control) showed significantly higher ($P<0.05$) a* values compared to the other packaging treatments.

This increase in redness was also observed in 100% CO_2 treatments at 12°C after 3d (Figure 67 a,b). Higher concentrations of CO_2 have been reported to result in the discoloration of meat. Seideman et al. (1979) reported that CO_2 binds to the meat proteins and decreases their ability to bloom rapidly. However, the pronounced redness in all 100% CO_2 is believed to be a result of less microbial growth and spoilage compared to the air packaged samples. A general increase in b* values for all modified atmospheres treatments was also observed, however, this increase was not statistically significant ($P>0.05$) among treatments at both 4 and 12°C (Figure 68 a,b). It has been reported that potassium sorbate had no deleterious effect on the color stability of chicken thighs (Cunnigham, 1979). This study, confirms these results that potassium sorbate may act synergistically with modified atmosphere to increase the color stability of chicken thighs.

Fig.66a. Changes in L* coordinates of dipped chicken thighs and innoculated with S. enteritidis at 4°C.

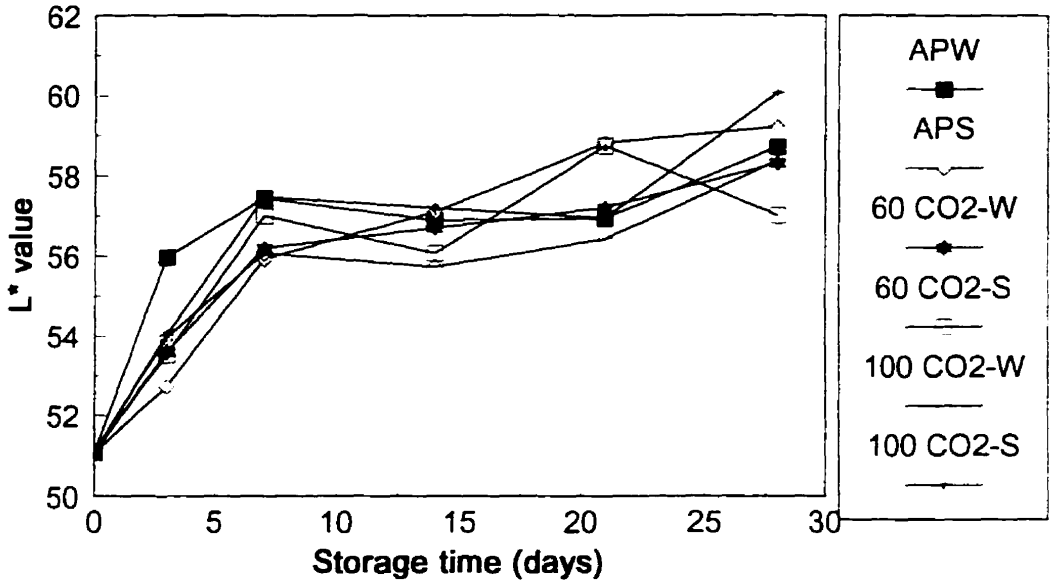
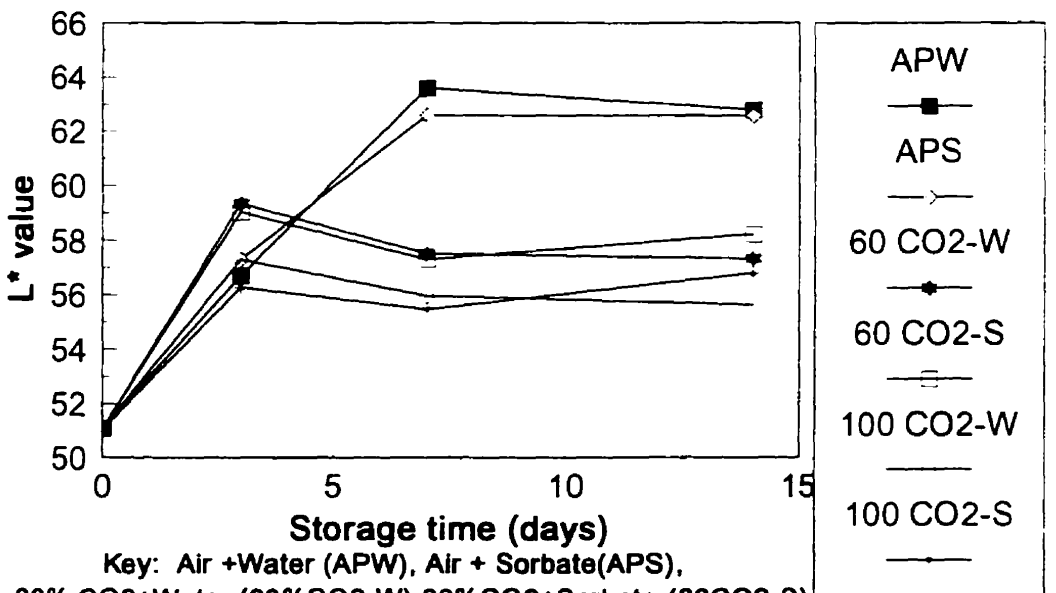


Fig.66b. Changes in L* coordinates of dipped chicken thighs and inoculated with S. enteritidis at 12°C.



Key: Air +Water (APW), Air + Sorbate(APS),
60% CO2+Water (60%CO2-W),60%CO2+Sorbate (60CO2-S)
100%CO2 + Water(100CO2-W), 100% CO2+Sorbate (100 CO2-S)

Fig.67a. Changes in a* coordinates of dipped chicken thighs and inoculated with S. enteritidis at 4°C.

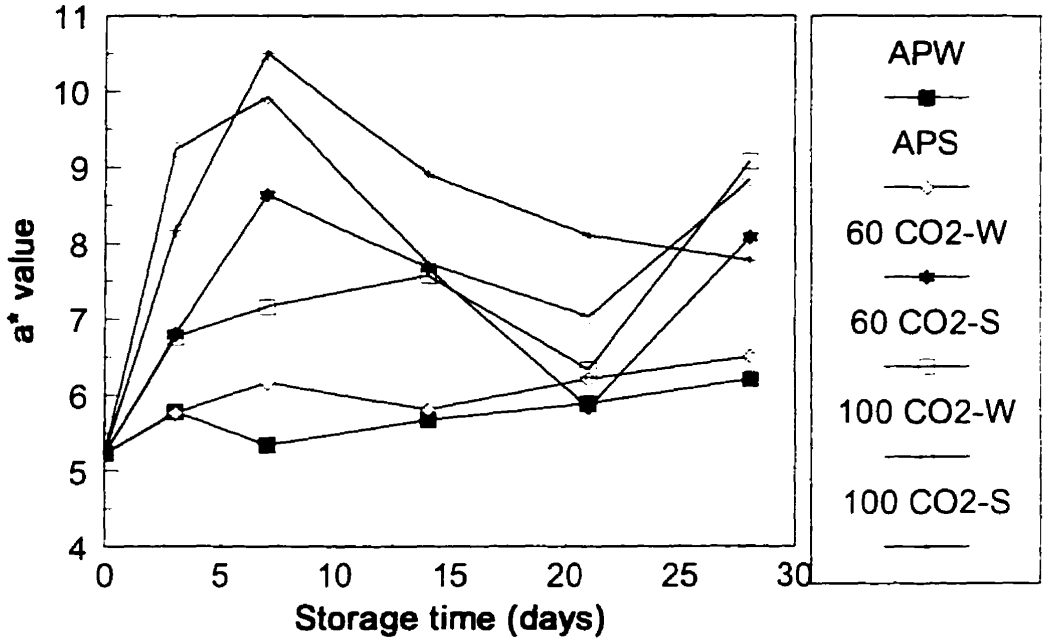
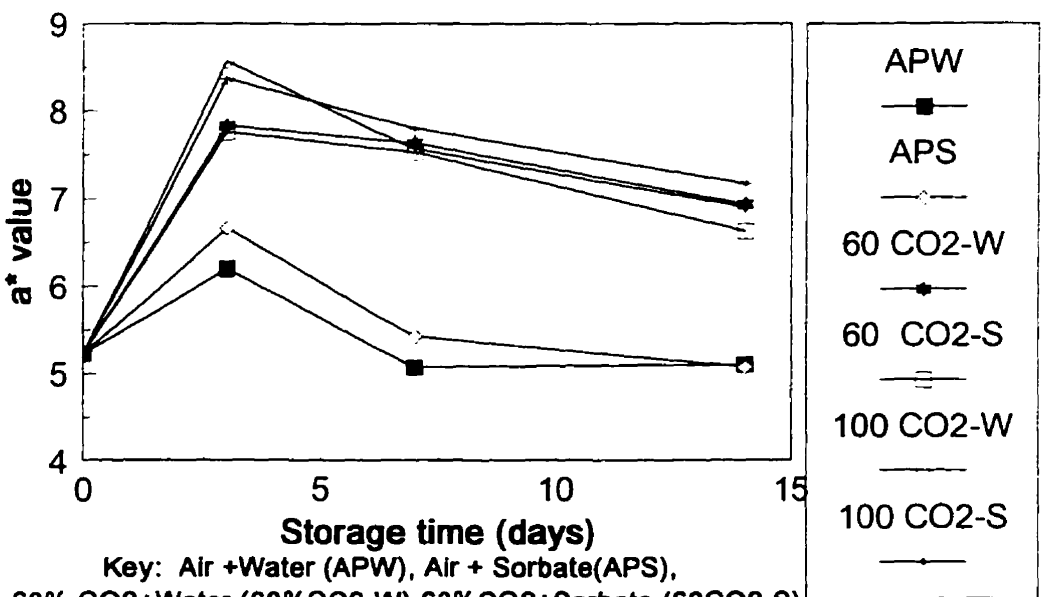


Fig.67b. Changes in a* coordinates of dipped chicken thighs and inoculated with S. enteritidis at 12°C.



Key: Air +Water (APW), Air + Sorbate(APS),
60% CO2+Water (60%CO2-W),60%CO2+Sorbate (60CO2-S)
100%CO2 + Water(100CO2-W), 100% CO2+Sorbate (100 CO2-S)

Fig.68a. Changes in b* coordinates of dipped chicken thighs and inoculated with S. enteritidis at 4°C.

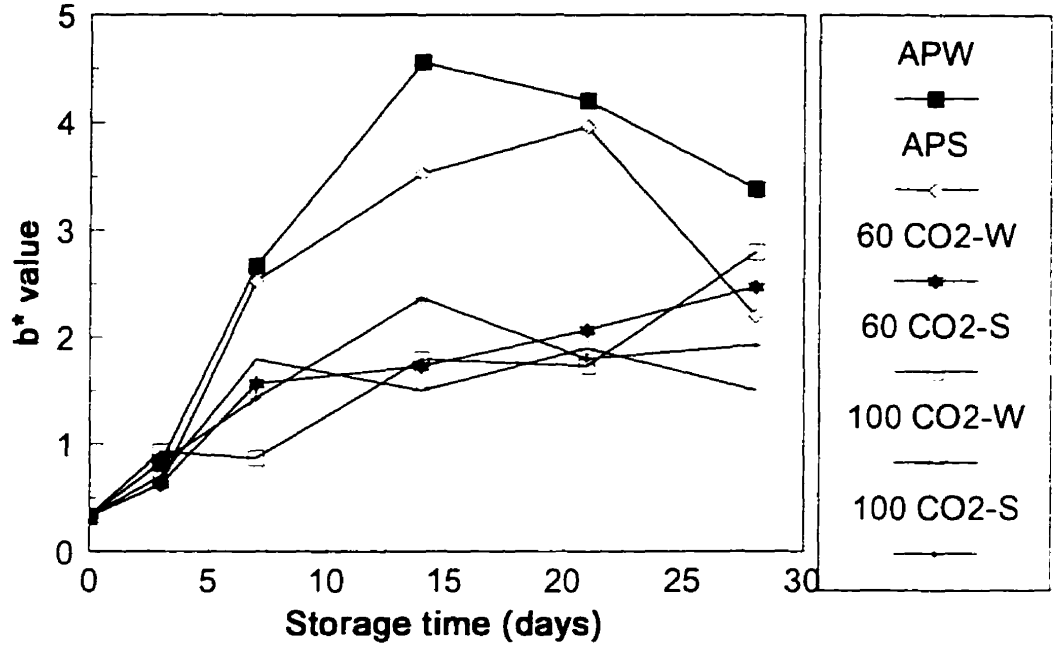
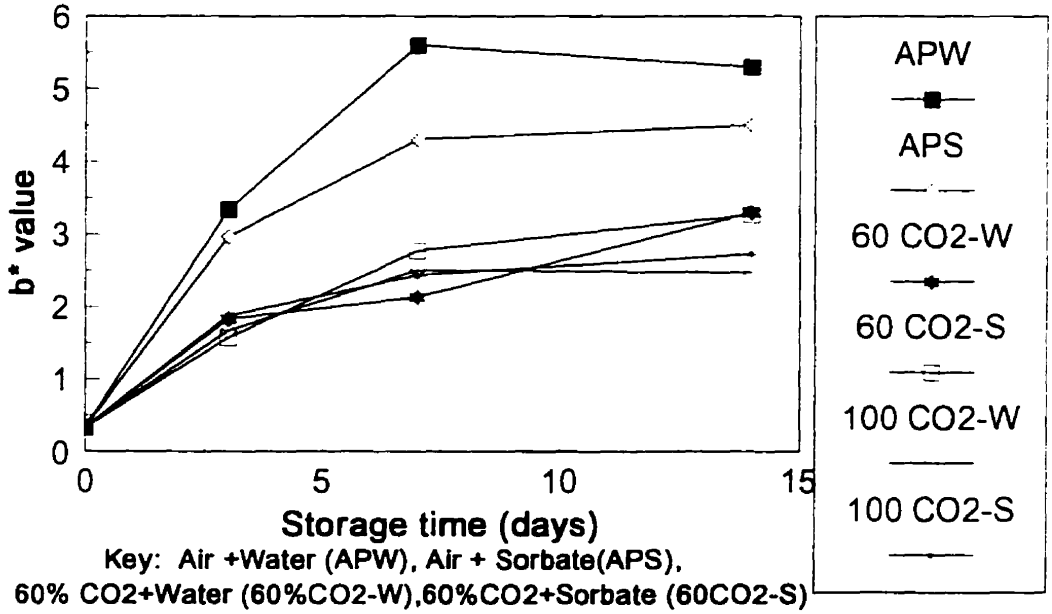


Fig.68b. Changes in b* coordinates of dipped chicken thighs and inoculated with S. enteritidis at 12°C.



Key: Air +Water (APW), Air + Sorbate(APS),
60% CO2+Water (60%CO2-W),60%CO2+Sorbate (60CO2-S)
100%CO2 + Water(100CO2-W), 100% CO2+Sorbate (100 CO2-S)

5.3.4. Drip loss

Changes in drip loss (%w/w) of chicken thighs pre-dipped in either deionized water or potassium sorbate prior to packaging in various atmospheres and storrage at 4 and 12°C are shown in Figure 69 a,b respectively. A positive correlation was found between increase in drip loss and growth of *S. enteritidis*. The air:sorbate samples showed significantly less drip ($P<0.05$) compared to the air:control samples after 3 and 7d of storage at 4°C (Figure 69 a). *S. enteritidis* counts were generally higher in the air:control than those packaged in air and dipped in potassium sorbate (0.2%, pH5). Furthermore, all MAP treatments (sorbate & control) showed significantly ($P<0.05$) less drip compared to the air:sorbate control at both 4 and 12°C (Figure 69 a,b), again reflecting the high number of *S. enteritidis* present in the air:sorbate control compared to MAP:sorbate treated samples. The 100% CO_2 water dipped control showed significantly less drip ($P<0.05$) compared to the other MAP:sorbate treated samples (Figure 69 a,b). This increase in drip loss observed in the modified atmosphere sorbate treated samples coincided with changes in pH of chicken thighs. This decrease in pH, due to dipping in sorbate and/or dissolution of CO_2 into the meat fluid has been previously reported (Gray et al., 1984). However, the formation of drip cannot only be explained in terms of high concentrations of CO_2 since the untreated 100% CO_2 had a significantly lower drip than the sorbate treated samples. Lawrie (1979) reported that precipitation of meat proteins usually occurs at or near the isoelectric point (pH 5.5). Lowering the pH by the addition of potassium sorbate may have affected the physical structure of the myofibril proteins and hence their water holding capacity. Thus, the addition of sorbate would appear to decrease the water holding capacity resulting in an increase in drip loss

Fig.69a. Changes in drip loss (%w/w) of dipped chicken thighs and inoculated with S. enteritidis at 4°C.

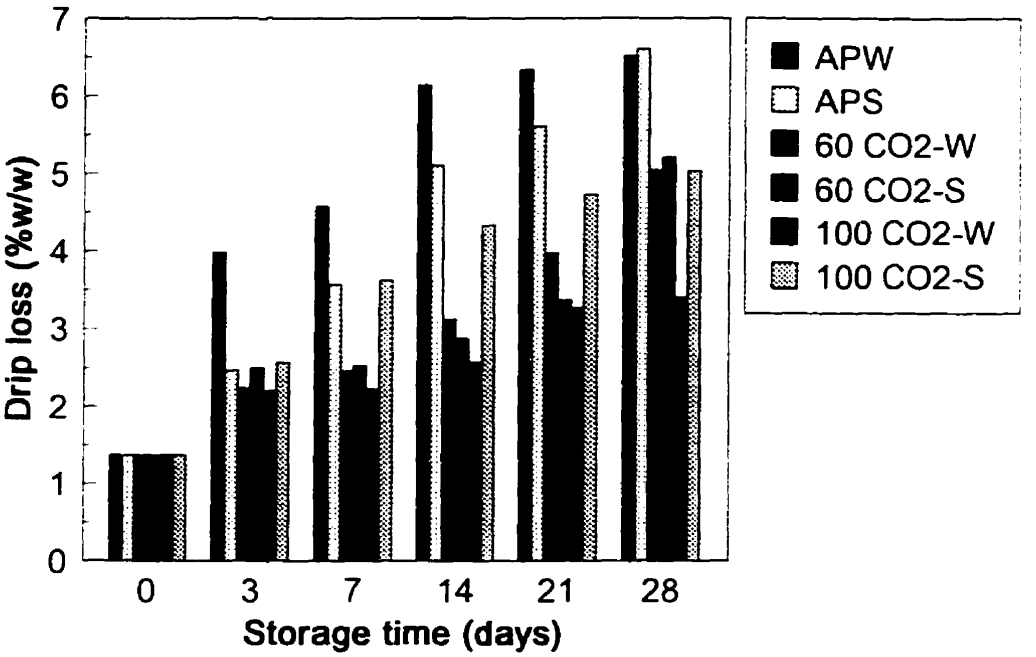
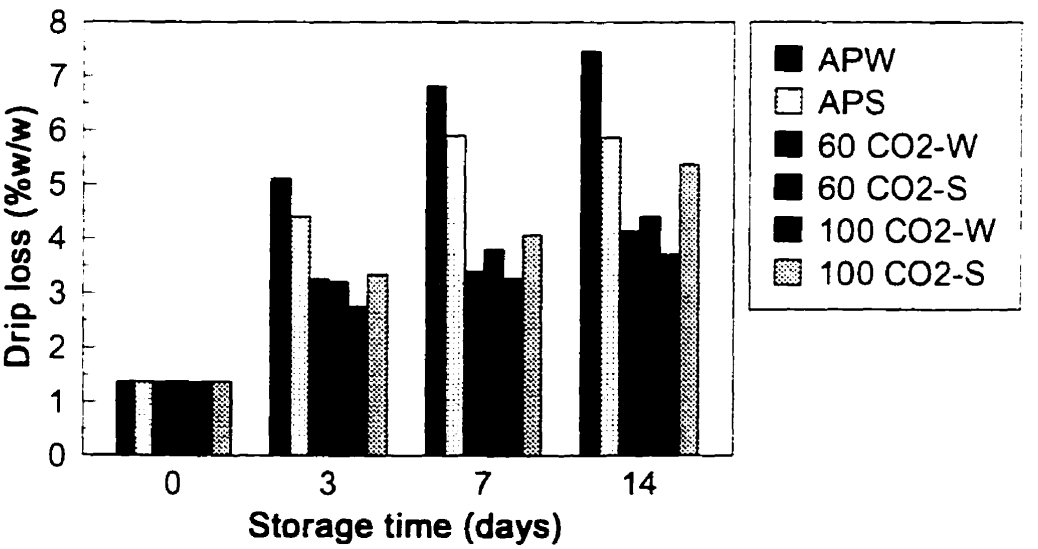


Fig.69b. Changes in drip loss (%w/w) of dipped chicken thighs and inoculated with S. enteritidis at 12°C.



Key: Air +Water (APW), Air + Sorbate(APS),
60% CO2+Water (60%CO2-W),60%CO2+Sorbate (60CO2-S)
100%CO2 + Water(100CO2-W), 100% CO2+Sorbate (100 CO2-S)

5.3.5. Sensory analyses

Changes in sensory (color & odor) attributes for the various dipping/packaging treatments of of chicken thighs and stored at 4 and 12°C are shown in Figures 70-71 a,b respectively. Samples packaged in the air:control were discolored after 14d at 4°C (Figure 70 a). For all other treatments, samples stored at 4°C did not show significant color deterioration and remained acceptable throughout the storage period (Figure 70 a). However, at 12°C, the air packaged samples (sorbate & control) were discolored after 7d while all other modified atmosphere treatments were discolored at the end of 14d storage (Figure 70 b). Surface discoloration was significantly higher ($P<0.05$) in air packaged samples (sorbate & control) after 7d compared tothe other modified atmosphere treatments at both 4 and 12°C (Figure 70 a,b). These results are closely related to the drip loss. Higher drip occurred in the air packaged samples compared to the other modified atmosphere treatments at day 7 (Figure 69 a,b). As drip loss increased, this generally detracted from the fresh appearance of meat and thus air samples (sorbate& control) received lower scores.

Odor deterioration was closely related with color deterioration as the temperature of storage increased (Figure 70-71 a,b). Odor deterioration occurred in the air:control by day 14 and 7 at 4 and 12°C respectively (Figure 71 a,b). Similarly, odor deterioration occured in the air:sorbate by 19 and 7d at 4 and 12°C respectively. These results indicate that microbial growth is primarily responsible for the high odor scores in the air packaged samples. Robach and Ivey (1978) reported that members of the genus *Pseudomonas* have shown to be the predominant organisms at the time of spoilage in chicken treated with 5% potassium sorbate. Thus, the observed difference in off-odor development between the air:sorbate and the MAP:sorbate treatments can be attributed to the rapid growth of the spoilage flora in these samples. Elliott et al. (1985) reported that a combination of 100%CO_2 and 2.5% potassium sorbate extended shelf-life of poultry, but had no selective effect on the growth of lactic acid bacteria at 6°C. This was apparent in our study in the slight sour odor in the modified atmosphere treatments dipped in potassium sorbate (0.2%w/v, pH5) after 28 and 14d at 4 and 12°C respectively (Figure 71 a,b).

Fig.70a. Changes in sensory color of dipped chicken thighs and inoculated with S. enteritidis at 4°C.

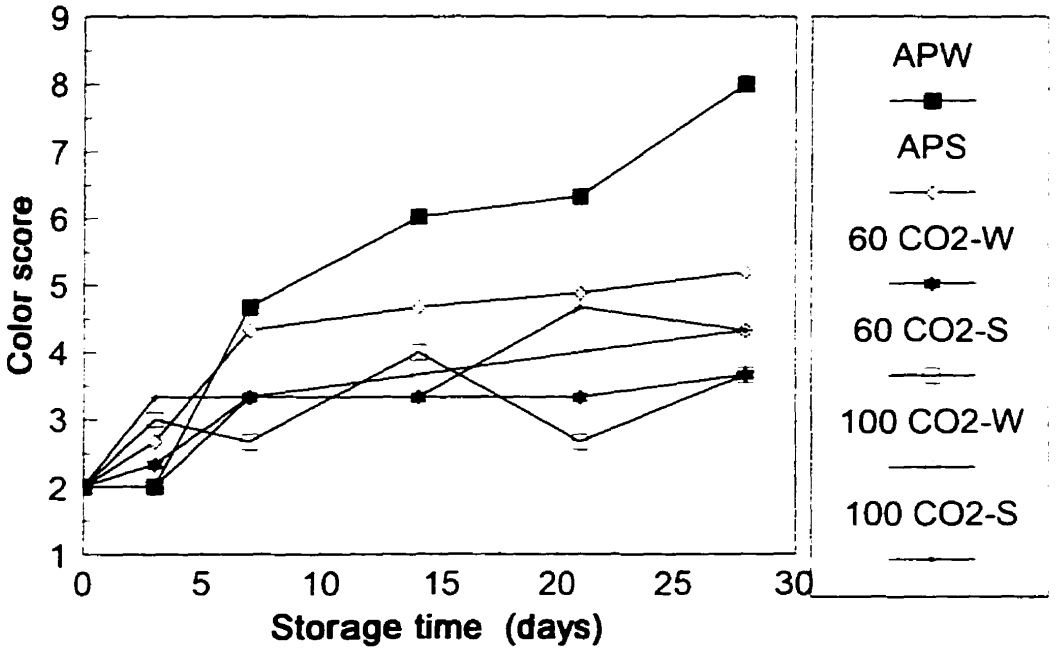
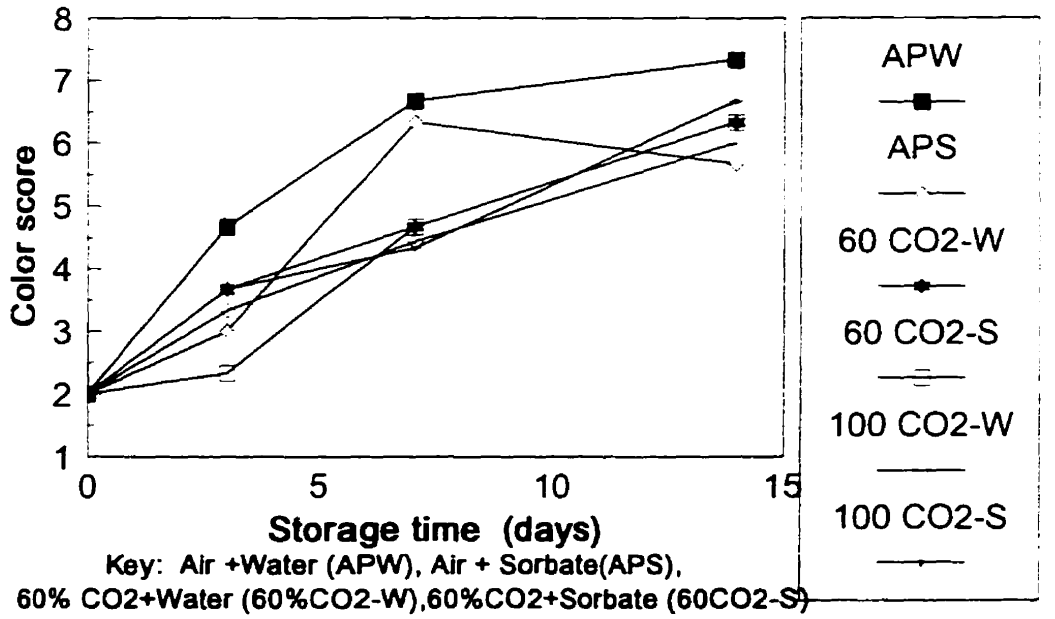


Fig.70b. Changes in sensory color of dipped chicken thighs and inoculated with S. enteritidis at 12°C.



Key: Air +Water (APW), Air + Sorbate(APS),
60% CO2+Water (60%CO2-W),60%CO2+Sorbate (60CO2-S)
100%CO2 + Water(100CO2-W), 100% CO2+Sorbate (100 CO2-S)

Fig.71a. Changes in sensory odor of dipped chicken thighs and inoculated with S. enteritidis at 4°C.

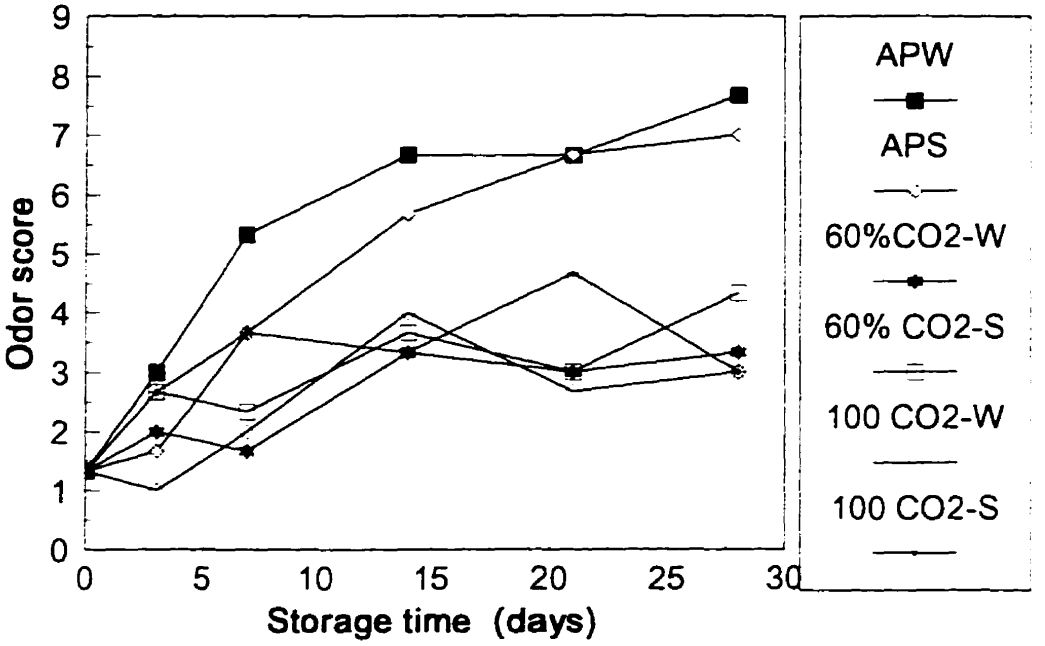
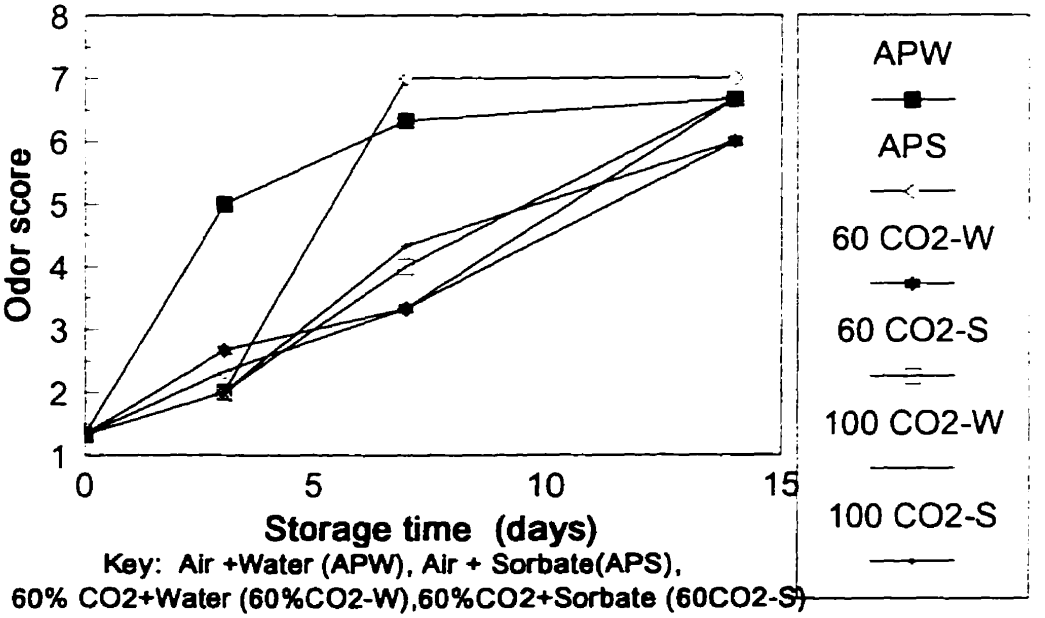


Fig.71b. Changes in sensory odor of dipped chicken thighs and inoculated with S. enteritidis at 12°C.



Key: Air +Water (APW), Air + Sorbate(APS),
60% CO2+Water (60%CO2-W),60%CO2+Sorbate (60CO2-S)
100%CO2 + Water(100CO2-W), 100% CO2+Sorbate (100 CO2-S)

5.3.6. pH

Changes in pH of chicken thighs for the various dipping/packaging treatments and stored at 4 and 12°C are shown in Figure 72 a,b respectively. The initial pH of chicken thighs was 6.12 and remained within 0.3 pH units throughout the storage period at both temperatures, irrespective of the dipping/packaging treatments (Figure 72 a,b). At 4°C, there was a noticeable decrease in pH for the air:sorbate control after 7d of storage (Figure 72 a), the pH decreasing from 6.12 to 5.98. This decrease in pH was accompanied by an increase in drip loss (Figure 69 a). After day 7, the surface pH of air:sorbate control increased towards the end of the storage period. This increase in pH could be due to bacterial proteolysis. The pH of the modified atmosphere treatments (sorbate & control) was slightly reduced at day 7 compared to the air:sorbate control, after which growth increased steadily throughout storage (Figure 72 a). Similar trends in pH decrease were also observed for the air sorbate:control at day 3 of storage at 12°C (Figure 72 b). This decrease in pH at both temperatures is probably due to the activity of lactic acid bacteria rather than the dissolution of CO_2 in the meat tissue.

5.3.7. Microbial analyses

The effect of the various dipping/packaging treatments on the growth of *S. enteritidis* on chicken thighs stored at 4 and 12°C are shown in Figure 73 a,b. In general, numbers of *S. enteritidis* increased more rapidly at 12°C than at 4°C, irrespective of the dipping/packaging treatment. The addition of potassium sorbate (0.2%w/v, pH 5) resulted in a sharp decrease in *S. enteritidis* by day 3 of storage in air:sorbate control at 4°C (Figure 73 a). By day 7, growth of *S. enteritidis* in the air:sorbate control increased rapidly and reached 10^6 CFU/g after 14d at 4°C (Figure 73 a). This initial decrease in *Salmonella* counts were not observed in the air:sorbate control at 12°C (Figure 73b). These results are in agreement with the work of Gray et al. (1984) who found that in air packaged samples stored at 10°C, 1% potassium sorbate (pH 6) was not initially bactericidal to the growth of *S. enteritidis* and did not affect

its subsequent rate of growth. It is therefore evident that with storage time and higher storage temperature, the inhibitory effect of sorbate was reduced. This loss in inhibition may be a result of sorbate degradation with time indicating that potassium sorbate alone is ineffective in controlling the growth of *S. enteritidis*.

Similarly, a sharp decrease in *S. enteritidis* was also observed for the 60% and the 100% CO_2 treatments at day 3 with inhibition being greater in the 100% CO_2 treatments even in the absence of sorbate (Figure 73 a). In all 100% CO_2 treatments, onset of growth was delayed for 4d, after which the growth was markedly slower compared to thighs packaged in 60% CO_2. This indicates that the inhibition of *S. enteritidis* after day 3 is primarily due to high concentrations of CO_2 in the package headspace. Gray et al. (1984) concluded that a combination of 100% CO_2 and potassium sorbate were most inhibitory to the growth of *S. enteritidis* on chicken thighs. These results appear to confirm the combined inhibitory effect of high CO_2 (100% CO_2) and dipping in potassium sorbate (0.2% w/v, pH5) prior to packaging on the growth of the pathogen in poultry.

Fig.72a. Changes in pH of dipped chicken thighs and inoculated with S. enteritidis at 4°C.

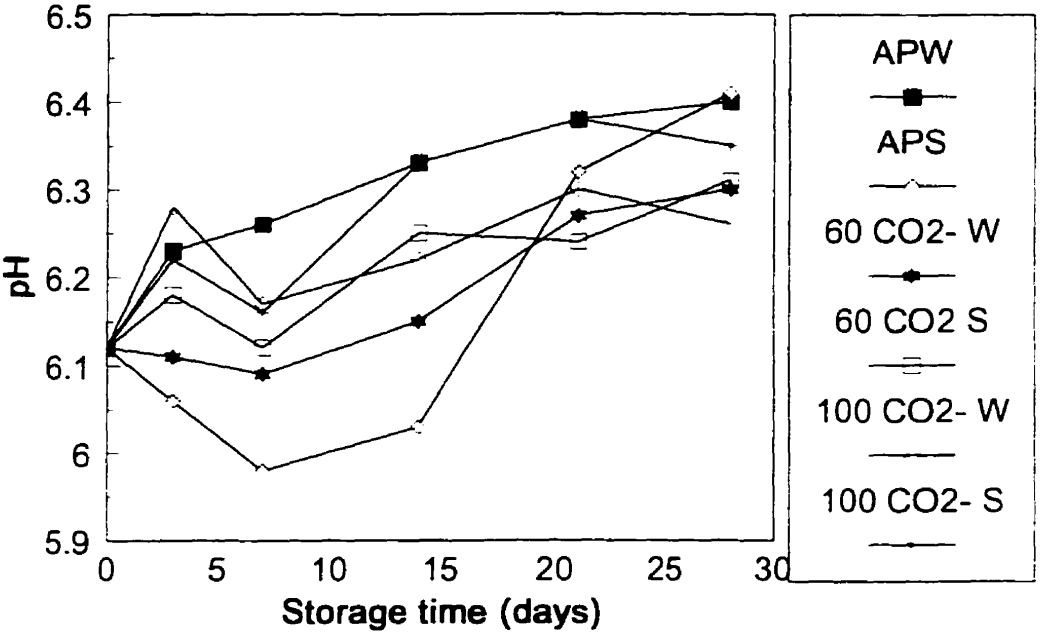
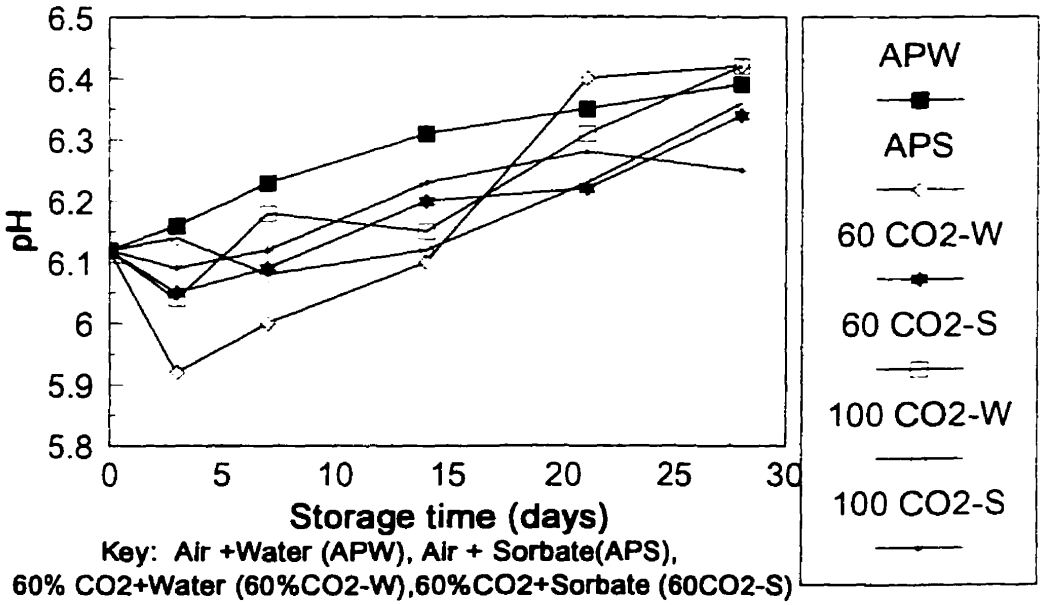


Fig.72b. Changes in pH of dipped chicken thighs and inoculated with S. enteritidis at 12°C.



Key: Air +Water (APW), Air + Sorbate(APS),
60% CO2+Water (60%CO2-W),60%CO2+Sorbate (60CO2-S)
100%CO2 + Water(100CO2-W), 100% CO2+Sorbate (100 CO2-S)

Fig.73a. Effect of sorbate pretreatment and packaging atmosphere on the growth of S. enteritidis at 4°C.

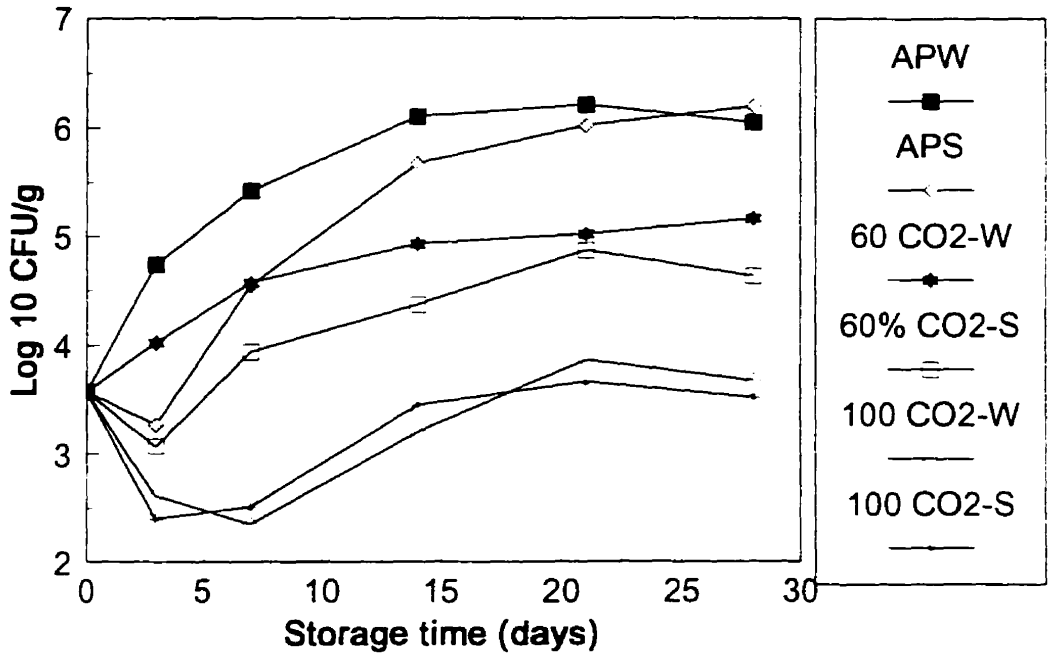
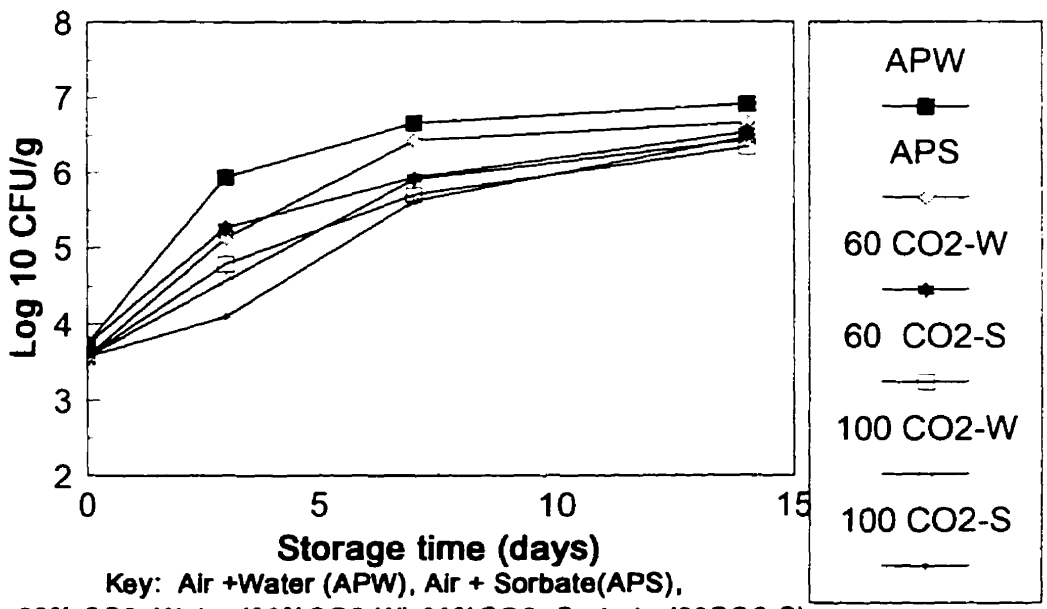


Fig.73b. Effect of sorbate pretreatment and packaging atmosphere on the growth of S. enteritidis at 12°C.



Key: Air +Water (APW), Air + Sorbate(APS),
60% CO2+Water (60%CO2-W),60%CO2+Sorbate (60CO2-S)
100%CO2 + Water(100CO2-W), 100% CO2+Sorbate (100 CO2-S)

5.3.8. Shelf-life

The sensory shelf-life of chicken thighs for the various dipping/packaging treatments of chicken thighs stored at 4 and 12°C are shown in Table 16. Packaging of chicken thighs in various modified atmospheres after dipping with 0.2% potassium sorbate for 1min. substantially retarded the growth of *S. enteritidis* and increased shelf-life of chicken thighs to more than 28d compared to a 14d for the air:sorbate control at 4°C. Thus, modified atmosphere alone, and in combination with potassium sorbate, was able to inhibit the growth of *S. enteritidis* and extend the shelf-life of chicken thighs. The inhibitory effect of modified atmosphere, with or without dipping, on the growth of *S. enteritidis* was negligible at higher temperatures (i.e., 12°C). All samples stored at 12°C were rejected on the basis of microbial shelf-life rather than quality deterioration again indicating the importance of strict temperature control.

Storage in high concentrations of CO_2 did not have a deleterious effect on the sensory characteristics of chicken thighs. Jurdi et al. (1980) reported that the use of 100% CO_2 resulted in meat discoloration. However in this study, most samples were sensorilly rejected (color & odor) at the same time for CO_2 concentrations and for both temperatures used in this study.

Table 16. Estimated shelf-life of pre-dipped chicken thighs inoculated with S. *enteritidis* in various packaging treatments stored at 4 and 12°C.

Packaging Treatment[a]	Rejection point/color[b]		Rejection point/odor[b]		Microbial shelf life[c]		Shelf life[d]	
	Control	Sorbate	Control	Sorbate	Control	Sorbate	Control	Sorbate
Storage at 4°C								
A	19	>28	11	17	13	21	13	17
B	>28	>28	>28	>28	>28	>28	>28	>28
C	>28	>28	>28	>28	>28	>28	>28	>28
Storage at 12°C								
A	6	8	6	6	4	6	4	6
B	13	13	12	13	8	13	8	13
C	14	13	13	14	8	12	8	12

[a] A, Air B, 60% CO_2:40% N_2 C, 100% CO_2

[b] Time to reach a score of six

[c] Time to reach a *Salmonella* count of 10^6 CFU/g

[d] Earliest rejection point on terms of odor,color and microbial load

Conclusion

Pre-dipping of chicken thighs, with or without 0.2% potassium sorbate, prior to packaging under a modified atmosphere substantially inhibited the growth of *S. enteritidis*. This effect was greater in thighs packaged with 100% CO_2 compared to the 60% CO_2 treatments.

In conclusion, although the food industry is trying to avoid the addition of preservatives due to consumer resistance to preservatives, low concentrations of potassium sorbate were used in this study. Furthermore, potassium sorbate is generally regarded as safe and there should be no restriction on its use by the poultry industry. Thus, predipping in 0.2 % potassium sorbate (pH5) and gas packaging in not less than 60% CO_2 and, preferably in 100% CO_2, in conjunction with low temperatures storage, could be employed to ensure the safety and quality of packaged chicken thighs.

CHAPTER 6

MODIFIED ATMOSPHERE PACKAGING AND IRRADIATION CHALLENGE STUDIES

6.1. INTRODUCTION

The use of low dose irradiation i.e., <10 kGy have been used succesfully to inhibit the growth of spoilage bacteria as well as pathogens on meat. Lambert et al. (1992a) concluded that the combined effect of irradiation (1kGy) and a modifed atmosphere (100%N_2) retarded microbial spoilage without adversely affecting the sensory, and chemical characteristics of pork. Therefore, this study was undertaken to determine the combined effect of irradiation and modified atmospheres involving elevated levels of CO_2, on the growth of *S. enteritidis* inoculated onto chicken thighs.

6.2. MATERIAL & METHODS

6.2.2. Inoculum preparation

Storage, subculturing and preparation of *S. enteritidis*^{NAST} inoculum was as described previously **(See Section 5.2.1.)**.

6.2.3. Preparation of chicken thighs and packaging.

Chicken thighs were again obtained frozen from Club Price, Montreal. After thawing under controlled conditions (4°C), thighs (~50 gram each) were placed individually in high

gas barrier bags (Cryovac, Canada) .Chicken thighs were then inoculated with 0.5 ml of *S. enteritidis*^{NAST} prepared previously **(See Section 5.2.1.)** to give an inoculum level of ~10^3 CFU/g. All samples were then packaged in the following treatments: air (AP), 60% CO_2 + 40% N_2 and 100% CO_2 as described previously **(See Section 5.2.3.3.)**.

6.2.4. Irradiation and storage

Samples were transported to the irradiation facility in Styrofoam coolers with ice packs to prevent termperature abuse. Samples were kept in the styrofoam coolers until they were irradiated. Samples were irradiated to average doses of 1.5 and 3.0 kGy at 22°C using a Gamma-Cell (Model 220, Atomic Energy of Canada, Limited, Ottawa). The dose rate was 3.95 kGy/hr, and the duration, 22.8 min and 45.6 min for the 1.5 and 3.0 doses respectively. All packaged products were stored at 4 and 12°C for 28d.

6.2.5. Headspace gas composition

Headspace gas composition was determined as previously described **(See Section 3.1.4.)**

6.2.6. Color analyses

Color analysis was measured as previously described **(See Section 2.2.2.2.)**.

6.2.6. Drip loss

Drip loss was measured as previously described **(See Section 2.2.2.3.)**.

6.2.7. Sensory, pH And microbiological analyses

Sensory, pH and microbiological analyses were carried out as previously described **(See Section 3.2.8.)**.When *S.enteritidis* counts were below the detection limit i.e., $<10^1$ CFU/g, the presence or absence of *S.enteritidis* was determined by a series of pre-enrichment and selective enrichment techniques as previously described **(See Section 2.2.2.6.)**

6.2.8. Statistical analyses

Data were analyzed using a split plot design in which storage time was the main plot effect and the storage temperature and the packaging treatment the subplot effect. Bacterial counts were expressed as $\log_{10}$ CFU/g and the reported means were the average of three replicates per treatment. Differences between means were analyzed using the least significant difference (LSD) and the Duncan multiple range test. Probability (P) of $P<0.05$ was considered to be significantly different.

6.3. RESULTS & DISCUSSION

6.3.1. Changes in headspace gas composition

Changes in headspace gas composition are shown in Figures 74-82 a,b respectively. There was a significant changes in the headspace gas composition between treatments at 4°C. In the aerobically packaged samples, headspace O_2 decreased and CO_2 increased but more rapidly in the non-irradiated samples than the irradiated samples. In the non-irradiated aerobically packaged samples, O_2 decreased to less than 1% and CO_2 increased to approximately 25% after 21d storage (Figure 74 a). However, for aerobically packaged samples irradiated at 1.5 and 3 kGy, O_2 decreased to approximately 10% and CO_2 increased to 15% after 28d storage at 4°C (Figures 75-76 a). Similar changes occurred in the headspace gas composition of aerobically packaged samples at 12°C. In the non-irradiated samples, headspace O_2 rapidly decreased to less than 1% and CO_2 increased to 28% after 14d storage, while for samples irradiated at 1.5 and 3 kGy, O_2 decreased to approximately 9.1% and 7.2% and CO_2 increased to 12 and 13.3% after 14d storage (Figures 75-76 b). These changes in headspace gas composition could be attributed to the partial inhibitory effect of low dose irradiation on the microbial population.

For samples packaged in 60%CO_2, no significant difference in headspace gas composition was observed between treatments. An initial decrease in headspace CO_2 was observed after 3d at both 4°C and 12°C, after which the concentration of CO_2 increased between day 3 and 7 and then remained fairly constant throughout storage (Figure 77-79 a,b). Similar changes were observed for the 100% CO_2 treatments, i.e., an initial decreased in CO_2 at day 3 and then the concentration of CO_2 remained fairly constant throughout storage at both 4 and 12°C (Figures 80-82 a,b).

Fig.74a. Changes in headspace gas composition of non-irradiated chicken thighs inoculated with S. enteritidis and stored in air at 4°C.

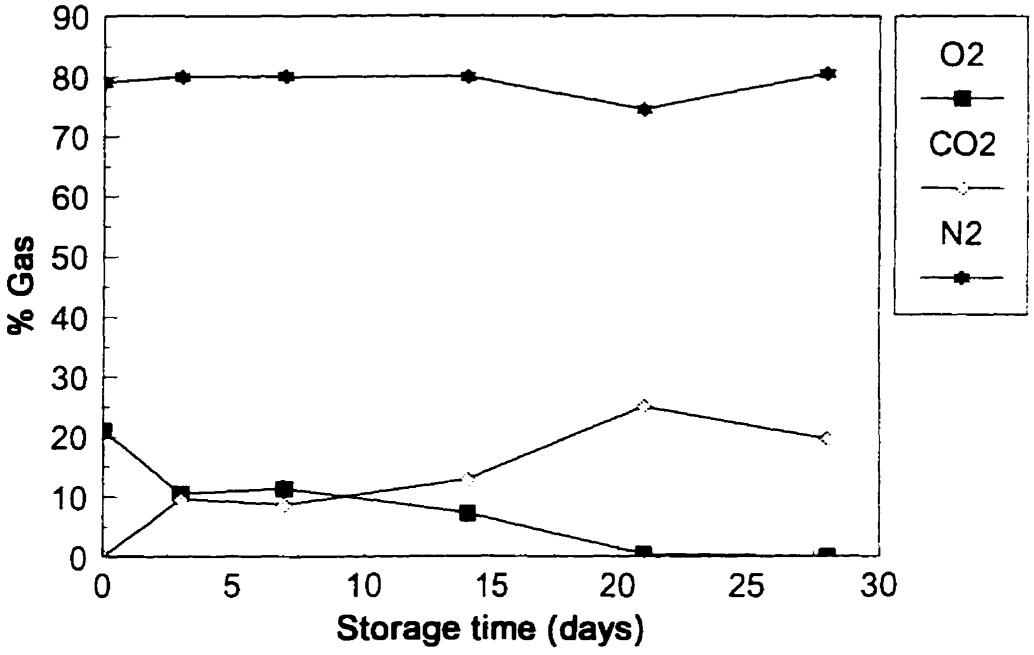


Fig.74b. Changes in headspace gas composition of non-irradiated chicken thighs inoculated with S. enteritidis and stored in air at 12°C.

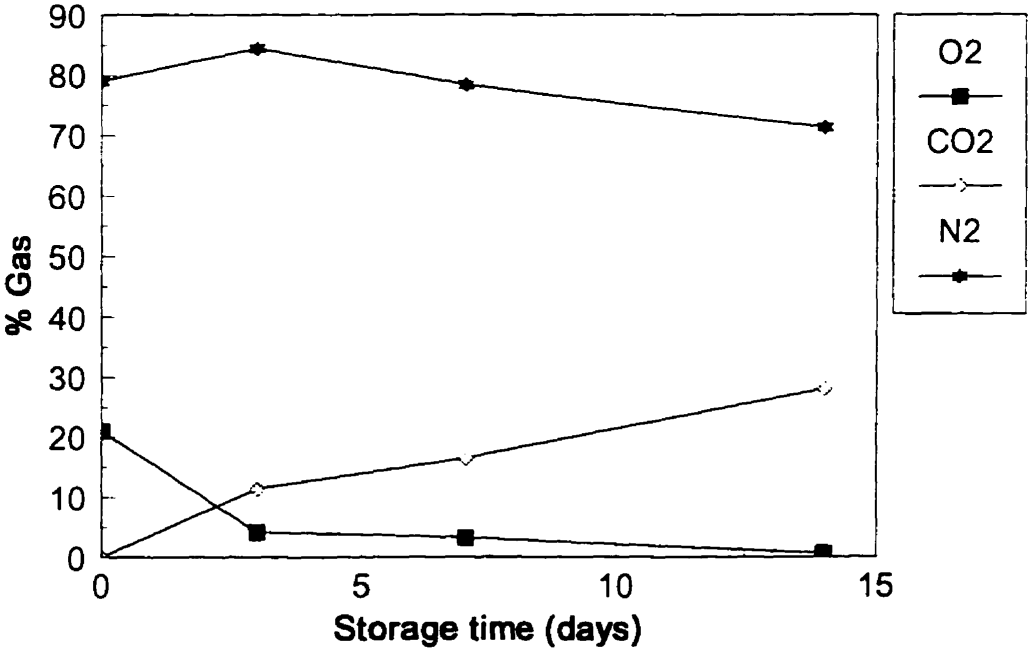


Fig.75a. Changes in headspace gas composition of irradiated (1.5 kGy) chicken thighs inoculated with S. enteritidis and stored in air at 4°C.

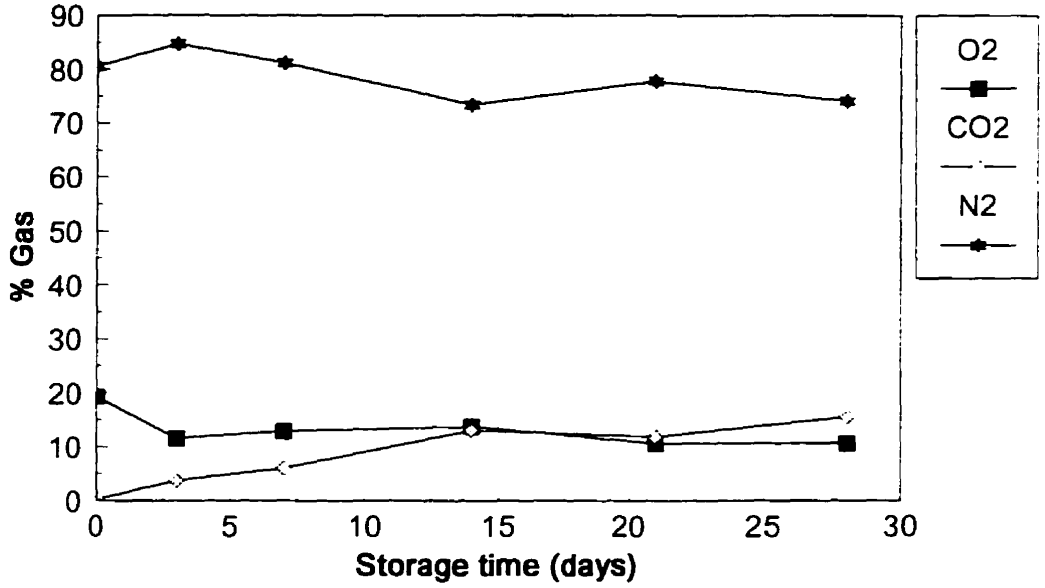


Fig.75b. Changes in headspace gas composition of irradiated (1.5 kGy) chicken thighs inoculated with S. enteritidis and stored in air at 12°C.

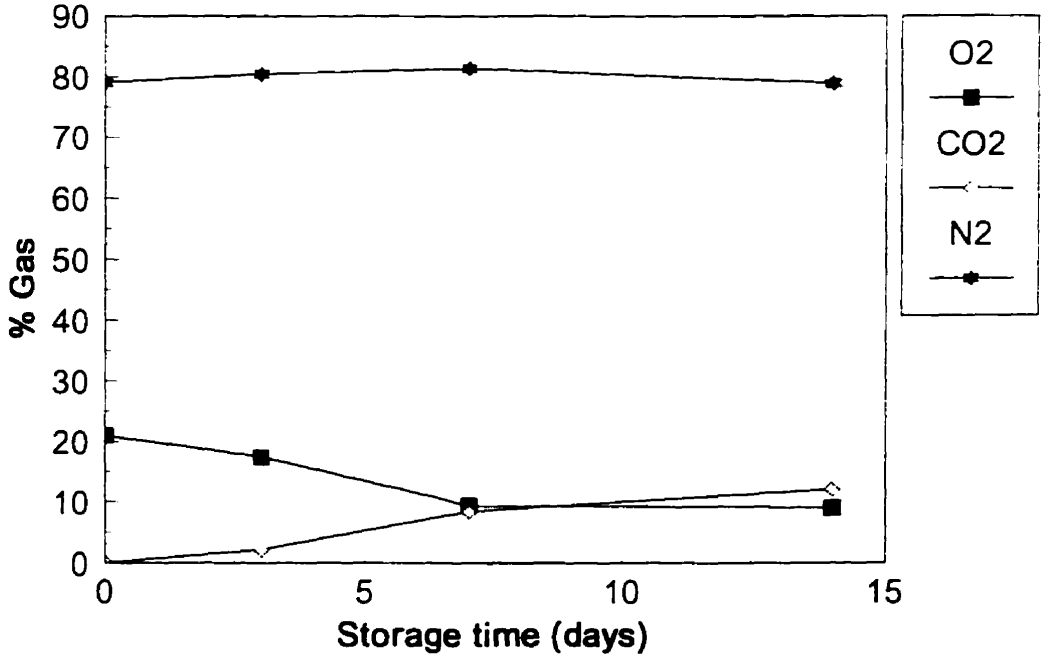


Fig.76a. Changes in headspace gas composition of irradiated (3 kGy) chicken thighs inoculated with S. enteritidis and stored in air at 4°C.

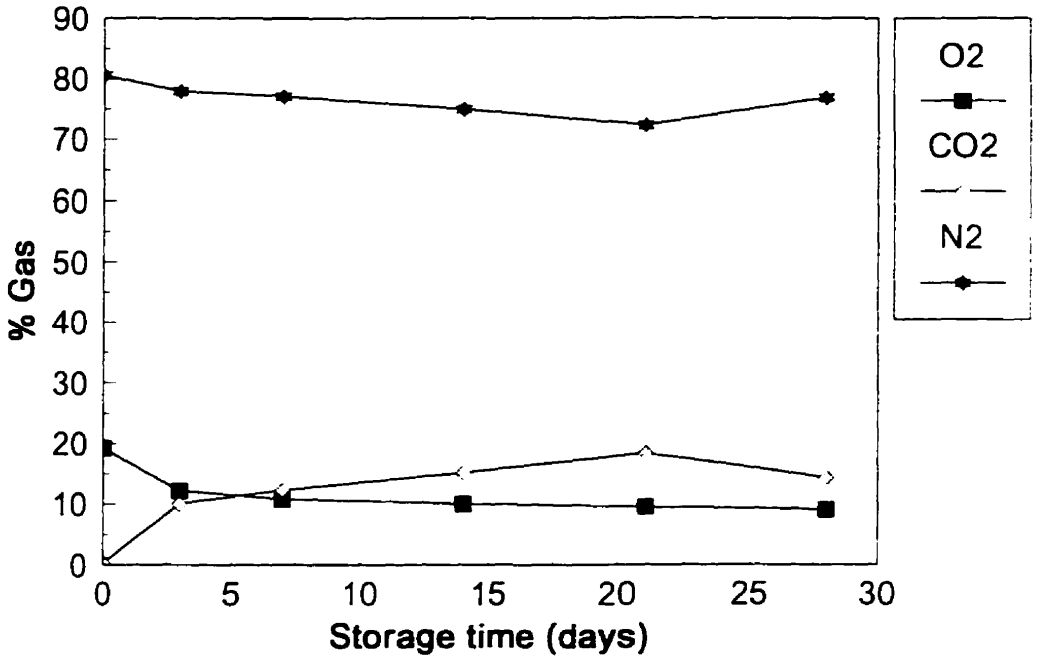


Fig.76b. Changes in headspace gas composition of irradiated (3 kGy) chicken thighs inoculated with S. enteritidis and stored in air at 12°C.

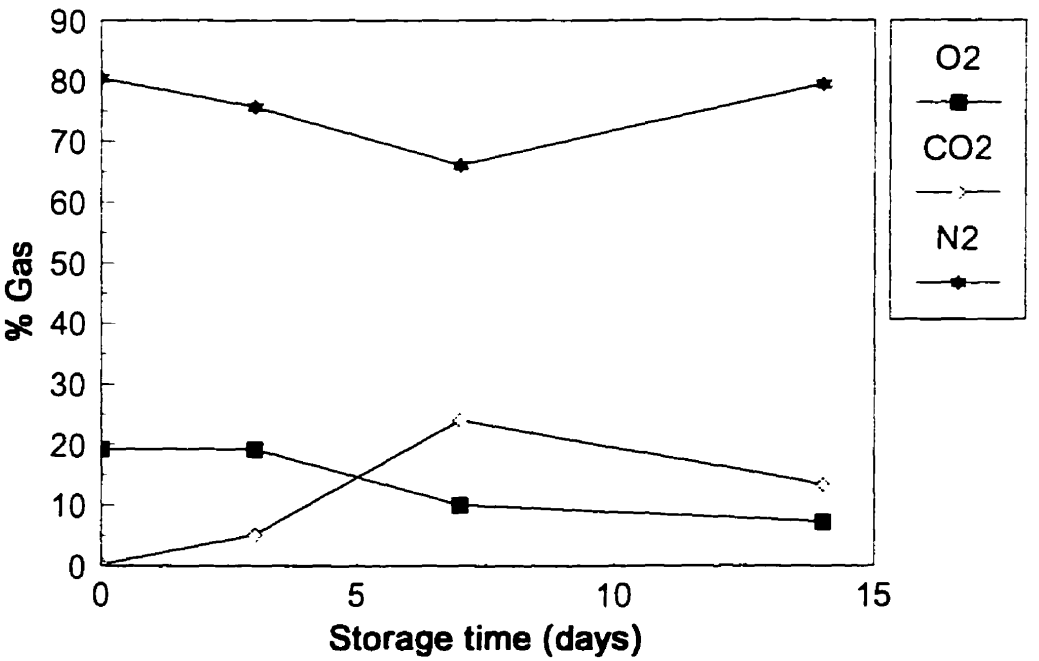


Fig.77a. Changes in headspace gas composition of non-irradiated chicken thighs inoculated with S. enteritidis and stored in 60% CO2 at 4°C.

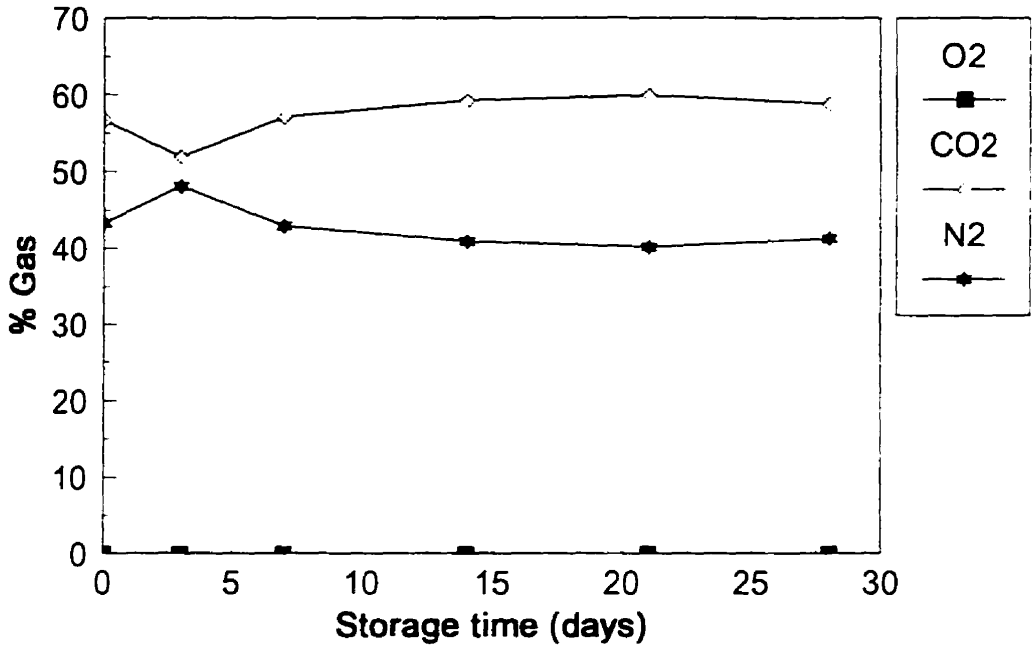


Fig.77b. Changes in headspace gas composition of non-irradiated chicken thighs inoculated with S. enteritidis and stored in 60% CO2 at 12°C.

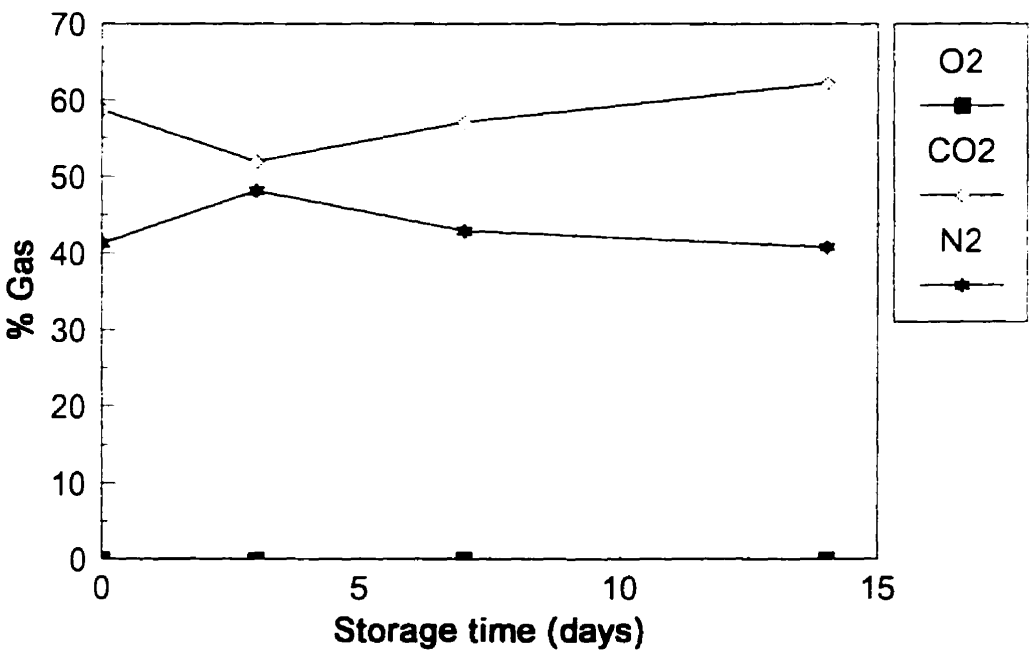


Fig.78a. Changes in headspace gas composition of irradiated (1.5 kGy) chicken thighs inoculated with S. enteritidis and stored in 60% CO2 at 4°C.

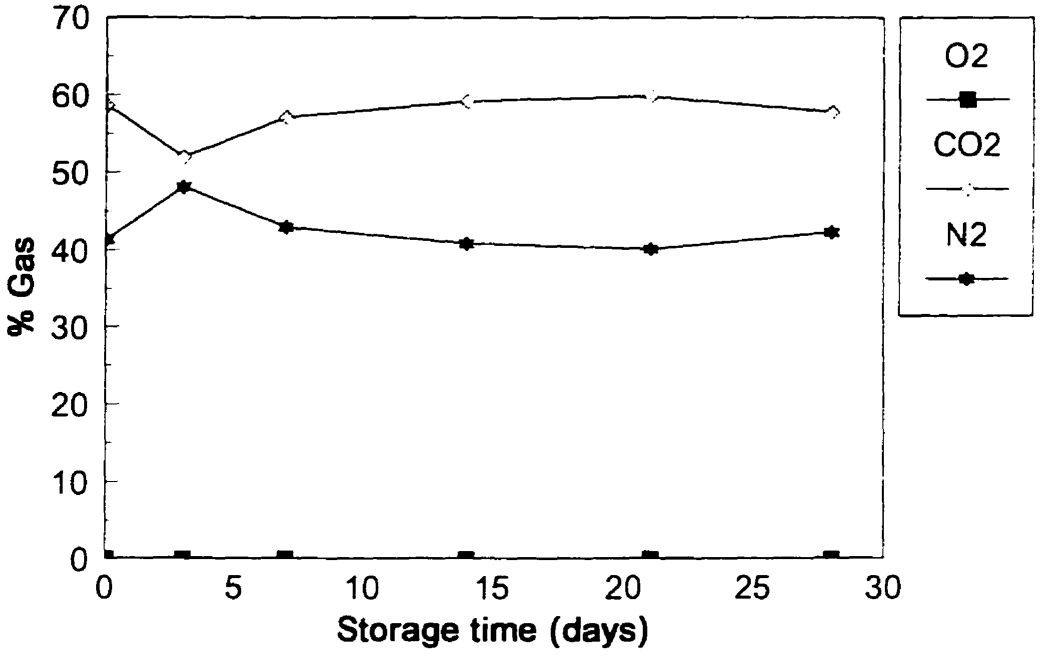


Fig.78b. Changes in headspace gas composition of irradiated (1.5 kGy) chicken thighs inoculated with S. enteritidis and stored in 60% CO2 at 12°C.

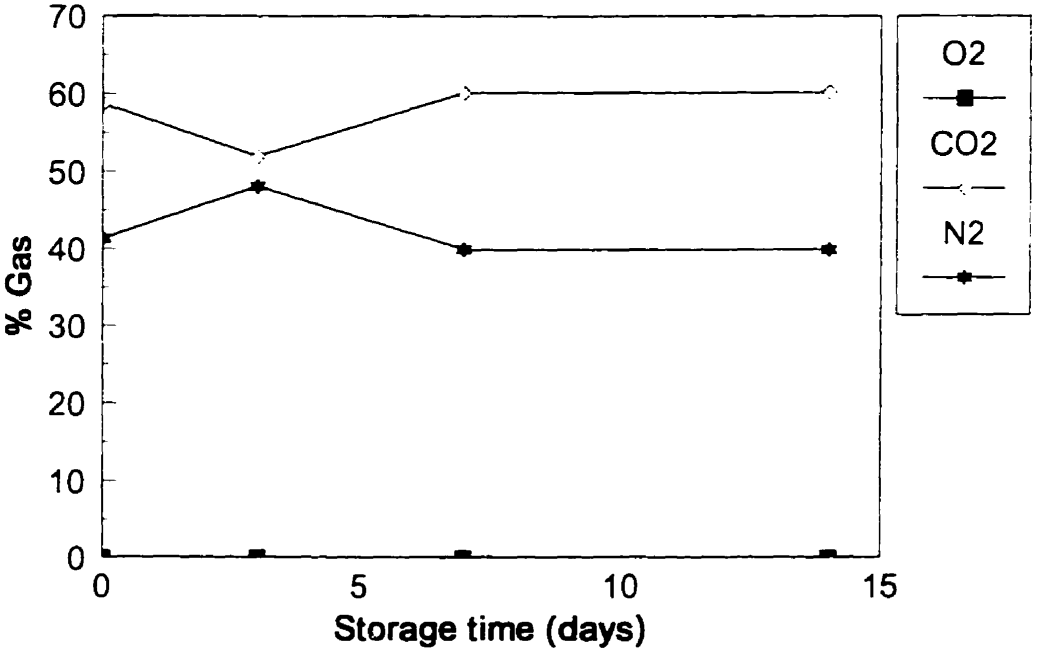


Fig.79a. Changes in headspace gas composition of irradiated (3 kGy) chicken thighs inoculated with S. enteritidis and stored in 60% CO2 at 4°C.

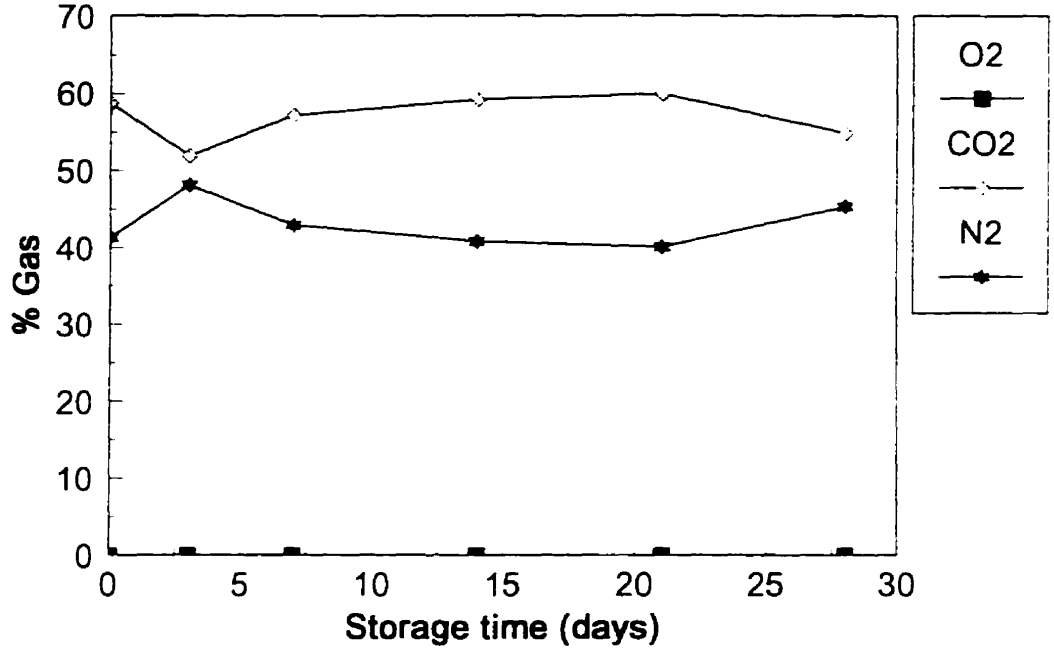


Fig.79b. Changes in headspace gas composition of irradiated (3 kGy) chicken thighs noculated with S. enteritidis and stored in 60% CO2 at 12°C.

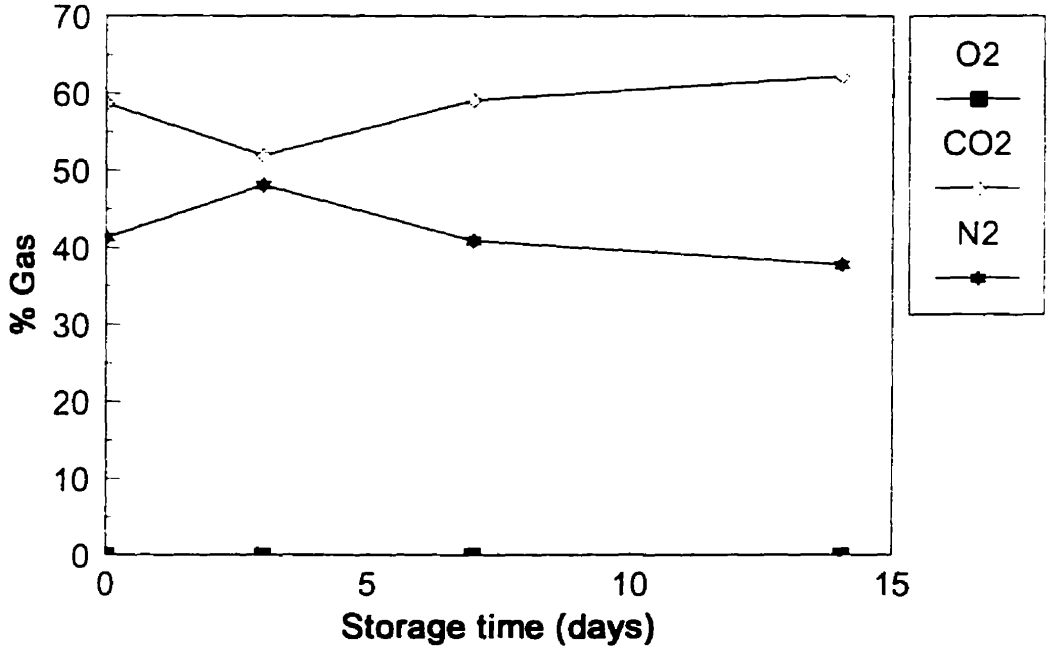


Fig.80a. Changes in headspace gas composition of non-irradiated chicken thighs inoculated with S. enteritidis and stored in 100% CO2 at 4°C.

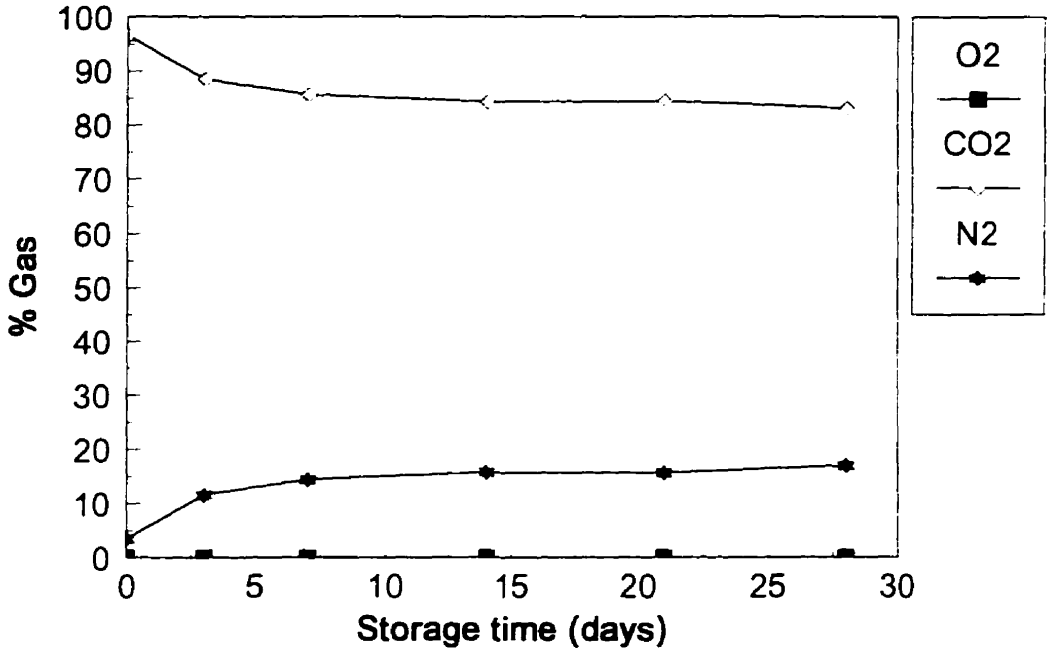


Fig.80b. Changes in headspace gas composition of non-irradiated chicken thighs inoculated with S. enteritidis and stored in 100% CO2 at 12°C.

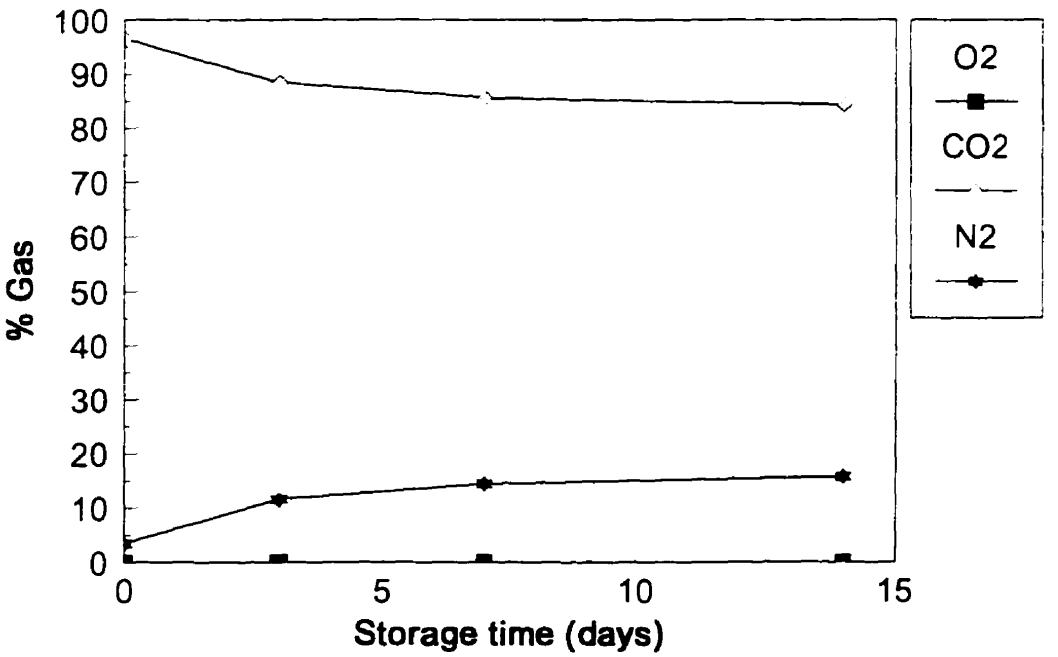


Fig.81a. Changes in headspace gas composition of irradiated (1.5 kGy) chicken thighs inoculated with S. enteritidis and stored in 100% CO2 at 4°C.

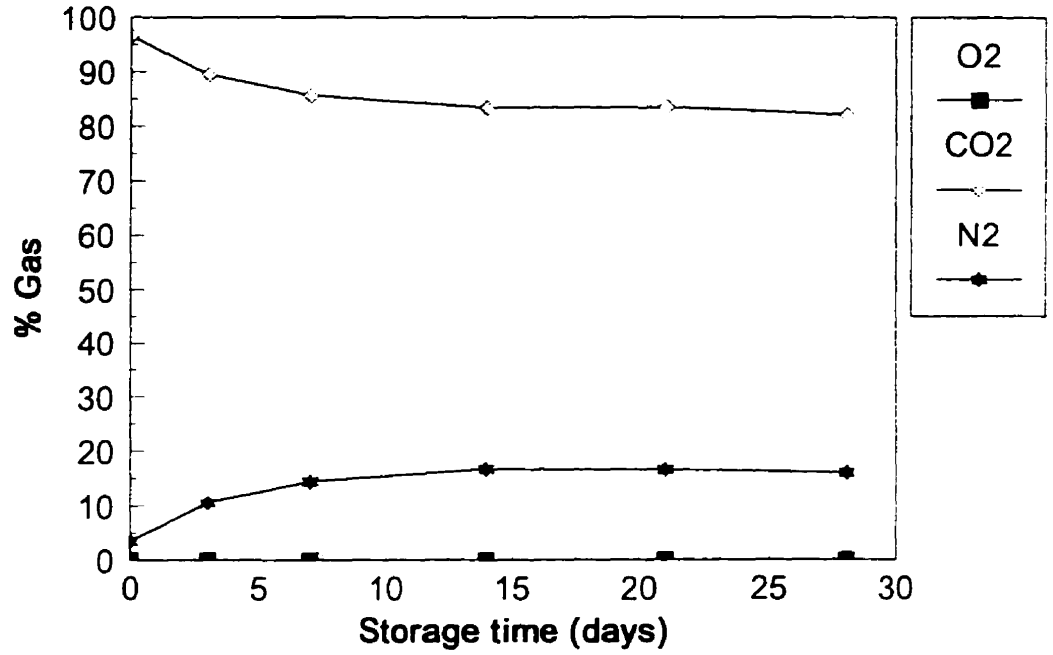


Fig.81b. Changes in headspace gas composition of irradiated (1.5 kGy) chicken thighs inoculated with S. enteritidis and stored in 100% CO2 at 12°C.

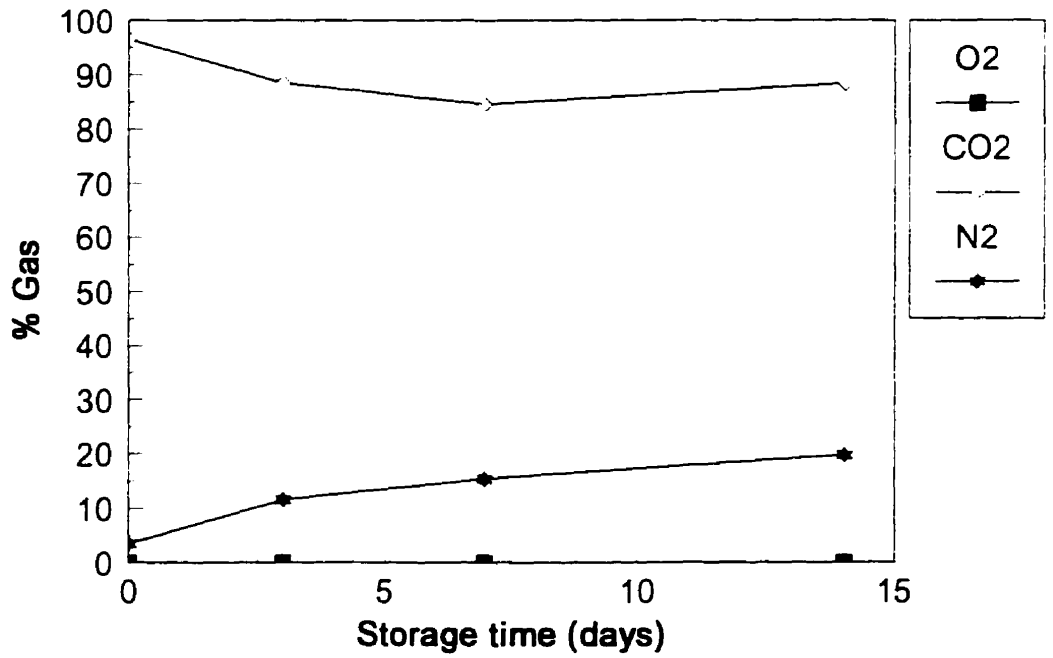


Fig.82a. Changes in headspace gas composition of irradiated (3 kGy) chicken thighs inoculated with S. enteritidis and stored in 100% CO2 at 4°C.

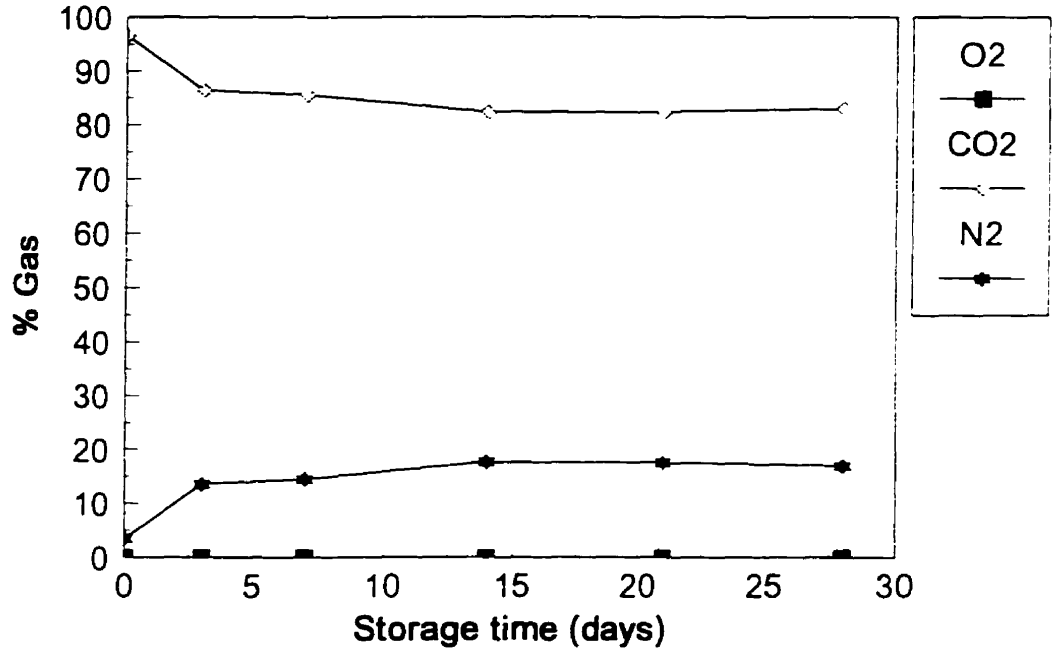
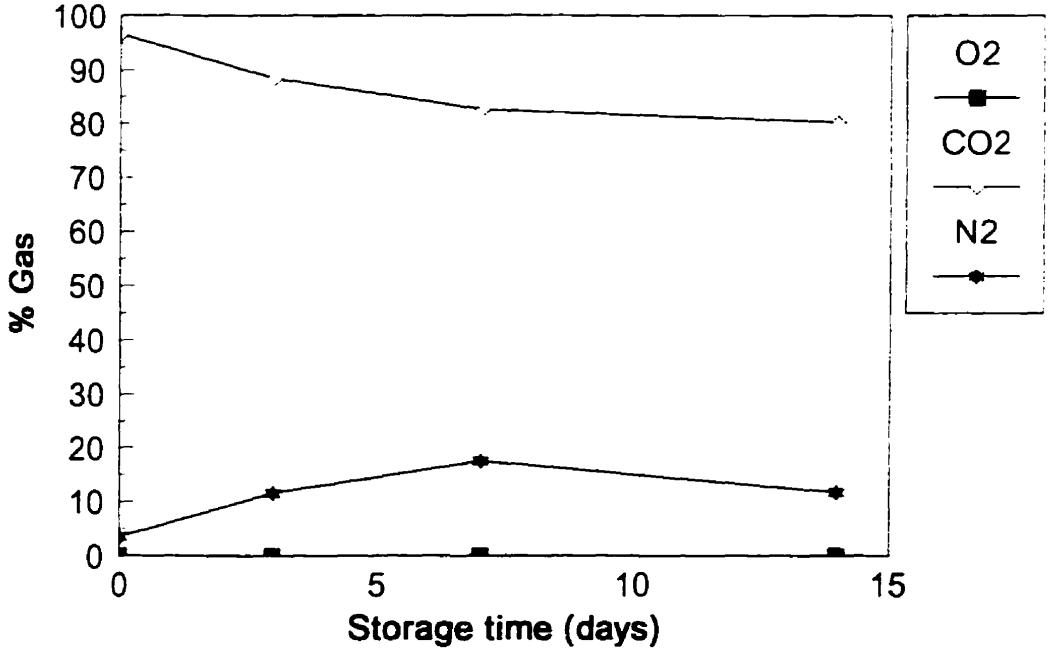


Fig.82b. Changes in headspace gas composition of irradiated (3 kGy) chicken thighs inoculated with S. enteritidis and stored in 100% CO2 at 12°C.



6.3.2. Color analyses

Changes in L*values (lightness), a* values (redness) and b* values (yellow) are shown in Figures 83-88 a,b respectively. Generally, the L* and b* values increased where as the a* values decreased indicating that the thighs were becoming lighter, less red and more yellow. The L* values increased significantly ($P<0.05$) with storage time and temperature. In general, samples stored at 12°C were more pale (higher L*values) than those stored at 4°C (Figure 84 a,b). Furthermore, both irradiation and the packaging treatment ($P>0.05$) significantly affected the L*values. The L* values in the non-irradiated samples increased readily and then remained constant throughout the storage period at 4°C (Figure 83 a,b). In the non-irradiated treatments, the aerobically packaged samples showed lower L* values ($P<0.05$) compared to modified atmosphere treatments after 14 and 7d at both 4 and 12°C respectively. Similarly, irradiated aerobically packaged samples (1.5 & 3.0 kGy) showed lower L* values ($P<0.05$) compared to the irradiated modified atmosphere samples after 7d at 12°C (Figure 84 a,b). The differences in L* values between the air treatments (irradiated& non-irradiated) and the other treatments could be attributed to the presence of O_2 that enhances oxidative changes in the muscles and also the synergistic effect of O_2 and irradiation that produces free radicals resulting in oxidation of myoglobin into metmyoglobin (Lambert et al., 1992a).

Changes in a* values are shown in Figures 85-86 a,b respectively. Initially, all samples showed an increase in a* values after 7 and 3d at both 4 and 12°C respectively (Figures 85-86 a,b) . For the remainder of storage the a* values either decreased or remained constant regardless of the packaging treatment. Irradiation and modified atmosphere had a significant effect ($P>0.05$) on the a* values. In the non-irradiated samples, both the 60 and 100% CO_2 samples, had significantly higher a* values ($P<0.05$) than aerobically packaged samples after 7-21 d at 4°C (Figure 85 a,b) . Furthermore, irradiated modified atmosphere samples (1.5& 3.0 kGy) had higher a* values ($P<0.05$) than the similarly packaged non-irradiated samples after 14 d at 4°C (Figure 85 a,b). These results agree with the higher L* values observed for the irradiated modified atmosphere samples after 14d (Figure 83 a,b). The aerobically

packaged samples (irradiated & non-irradiated) showed the greatest decrease in a* values compared to the other treatments at both 4 and 12°C (Figures 85- 86 a,b). These results are in close agreement with those reported by Lambert et al., (1992a) that irradiation (1 kGy) and modified atmosphere did not adversely affect the redness of meat.

Changes in b* values are shown in Figures 87-88 a,b respectively. There was a general increase in b* values in all treatments. After 14d of storage at 4°C, all aerobically packaged samples (irradiated & non-irradiated) showed significantly higher b* values ($P<0.05$) compared to the other treatments (Figure 87 a,b). Similar changes were observed for the aerobically packaged samples (irradiated & non-irradiated) after 7d at 12°C (Figure 88 a,b). Overall, aerobically packaged samples had a higher b* values followed by the irradiated aerobically packaged samples (1.5 & 3.0 kGy) with the modified atmosphere samples (irradiated &non-irradiated) having the lowest b* values. This increase in b* values in the aerobically packaged samples could be attributed to the growth of spoilage bacteria which may have survived the low irradiation doses resulting in lower O_2 tension and the conversion of myoglobin into metmyoglobin.

Fig.83a. Changes in L* coordinates of chicken thighs and inoculated with S. enteritidis at 4°C.

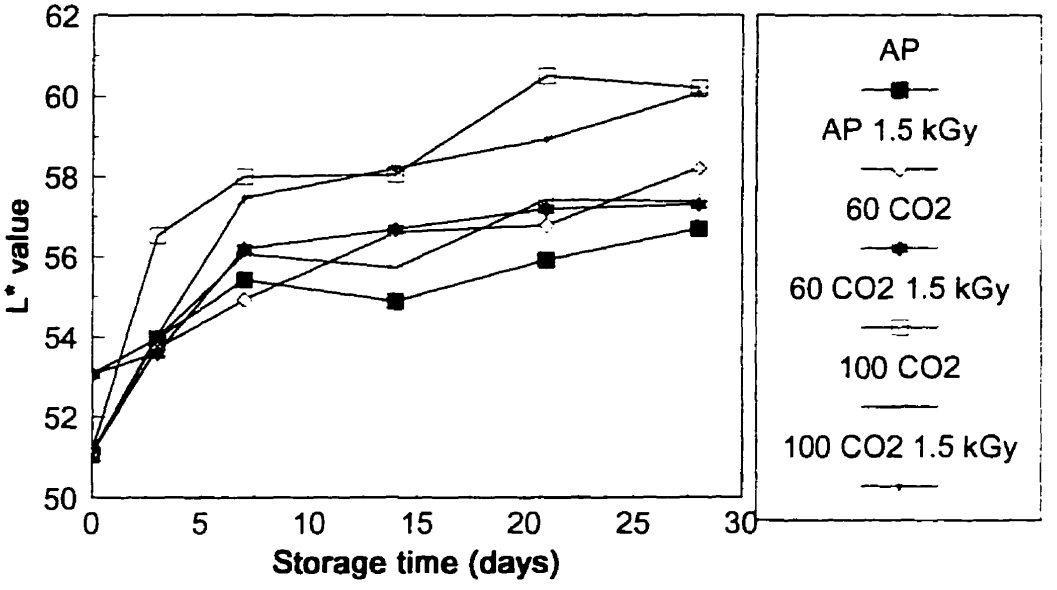


Fig.83b. Changes in L* coordinates of chicken thighs and inoculated with S. enteritidis at 4°C.

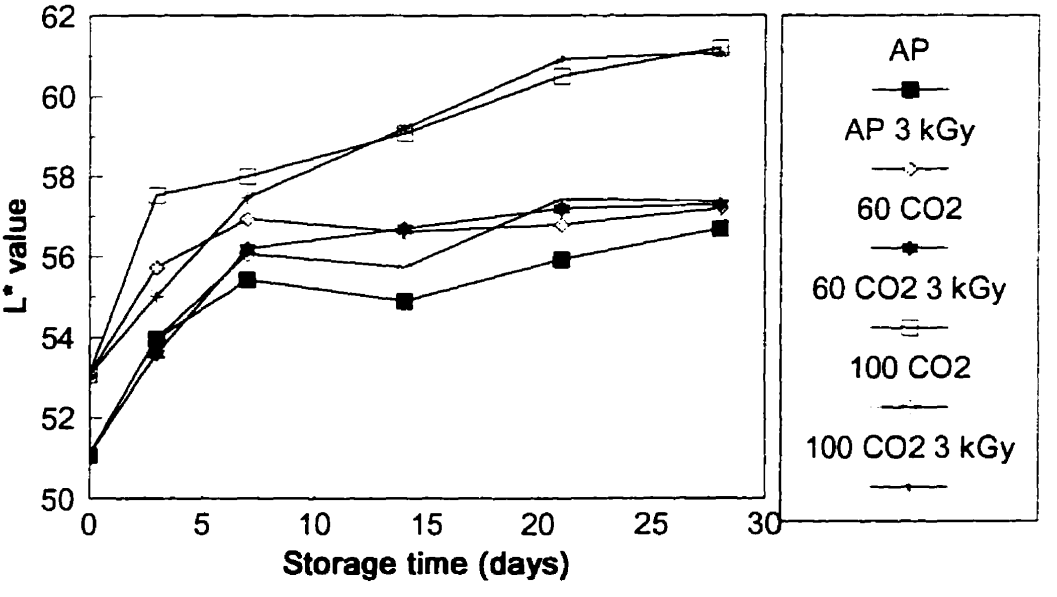


Fig.84a. Changes in L* coordinates of chicken thighs and inoculated with S. enteritidis at 12°C.

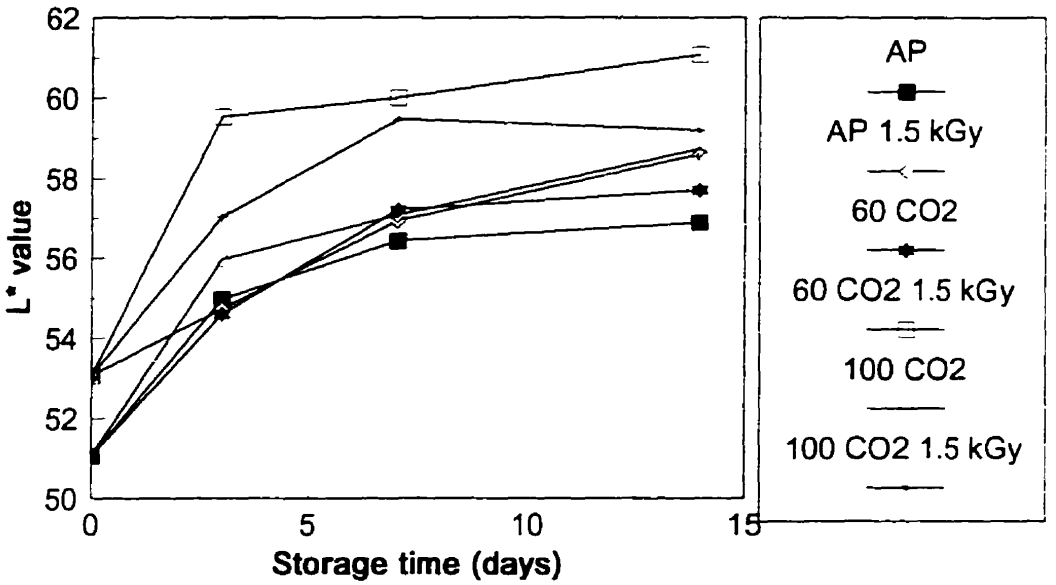


Fig.84b. Changes in L* coordinates of chicken thighs and inoculated with S. enteritidis at 12°C.

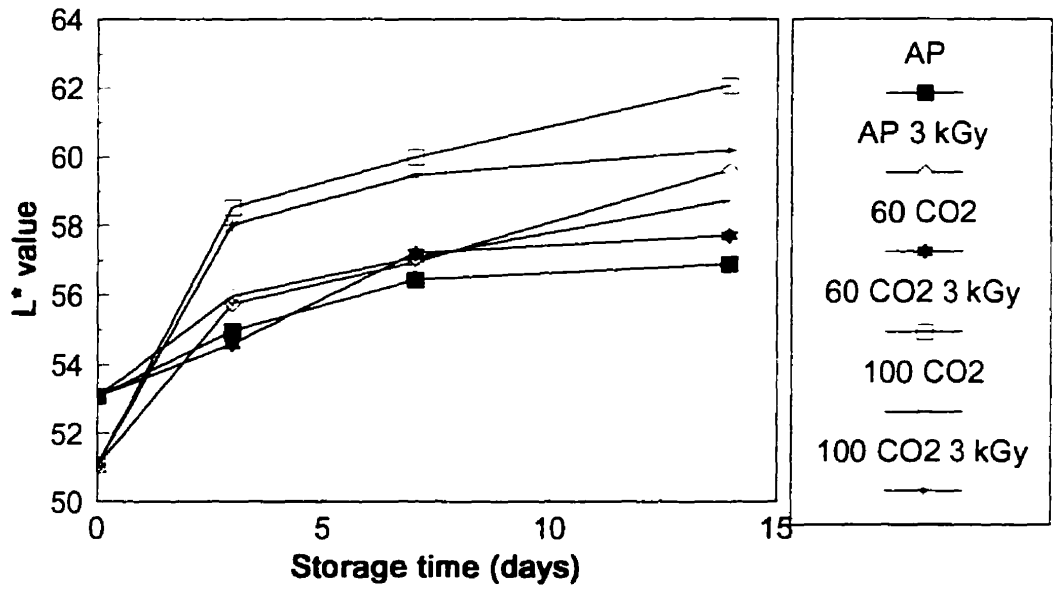


Fig.85a. Changes in a* coordinates of chicken thighs and inoculated with S. enteritidis at 4°C.

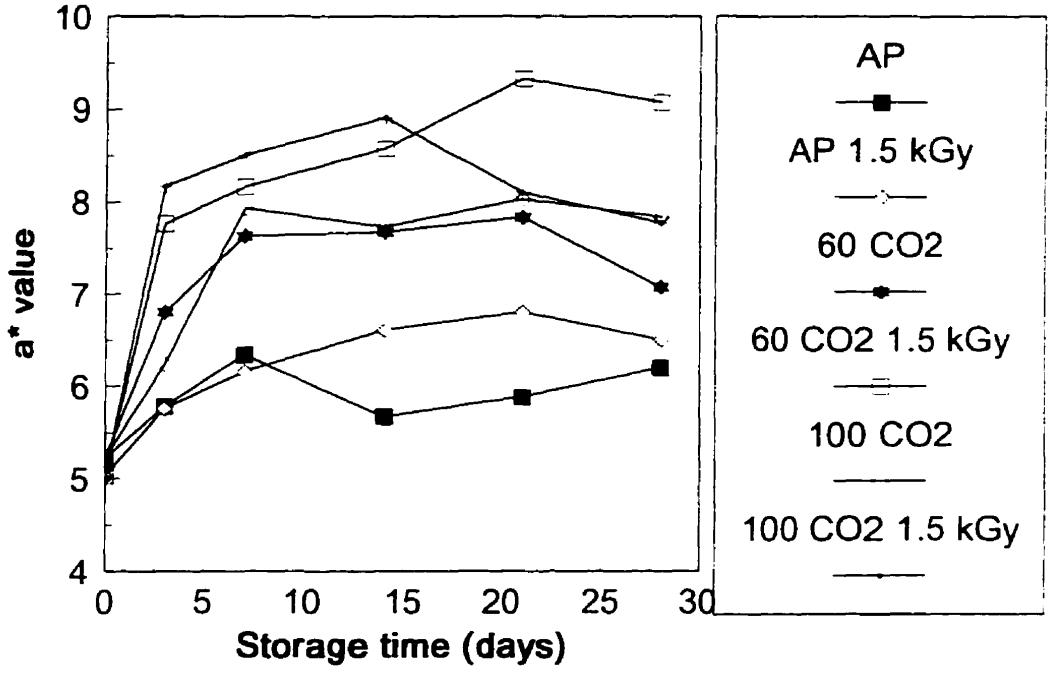


Fig.85b. Changes in a* coordinates of chicken thighs and inoculated with S. enteritidis at 4°C.

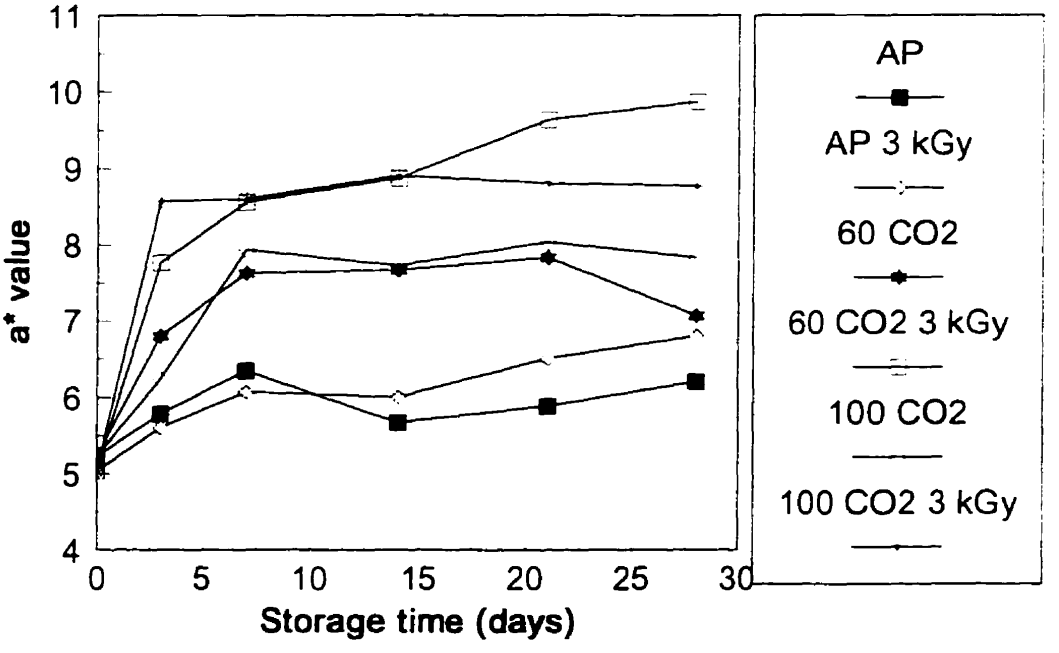


Fig.86a. Changes in a* coordinates of chicken thighs and inoculated with S. enteritidis at 12°C.

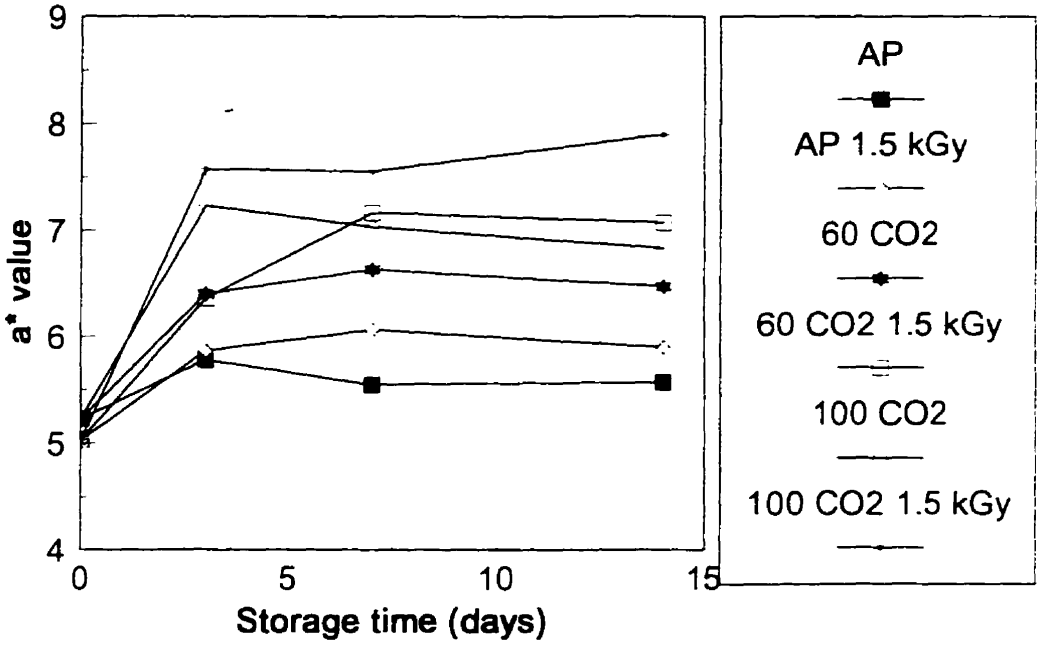


Fig.86b. Changes in a* coordinates of chicken thighs inoculated with with S. enteritidis at 12°C.

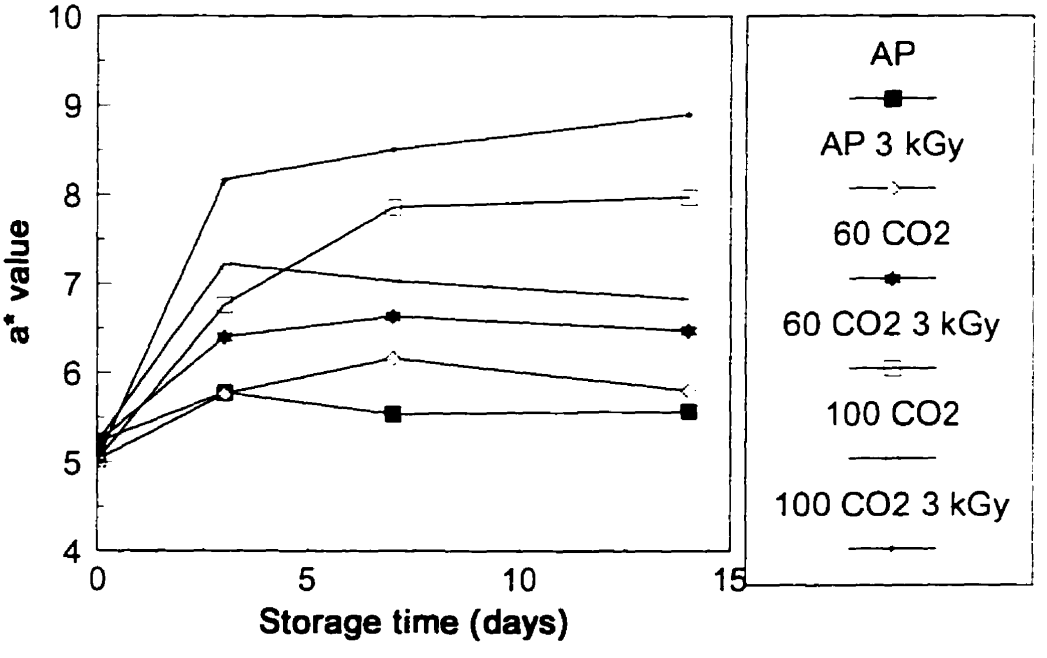


Fig.87a. Changes in b* coordinates of chicken thighs and inoculated with S. enteritidis at 4°C.

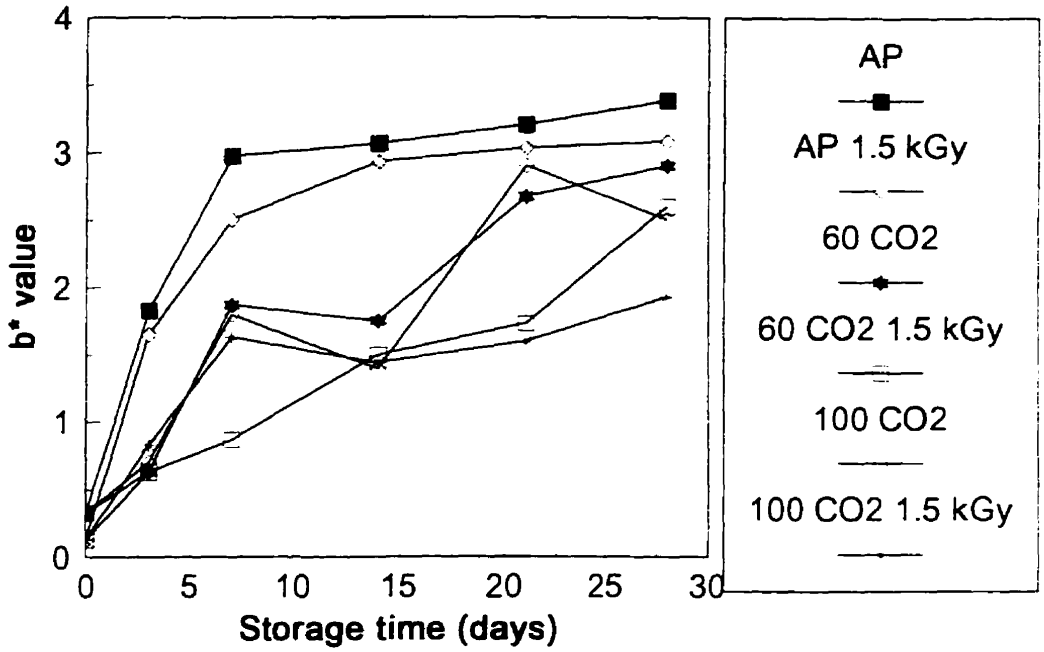


Fig.87b. Changes in b* coordinates of chicken thighs and inoculated with S. enteritidis at 4°C.

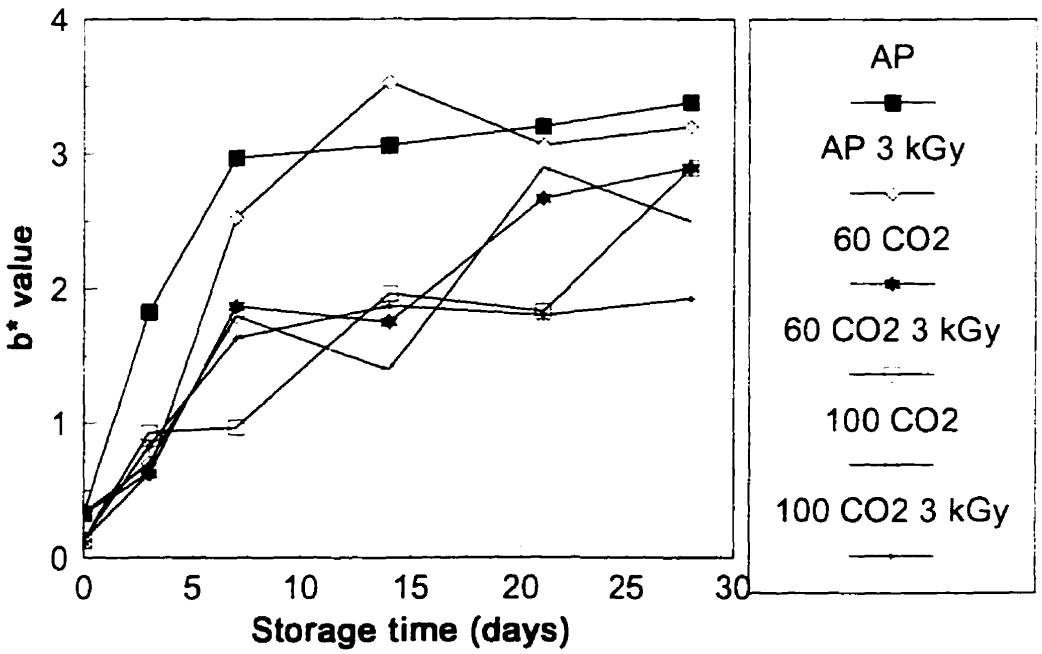


Fig.88a. Changes in b* coordinates of chicken thighs and inoculated with S. enteritidis at 12°C.

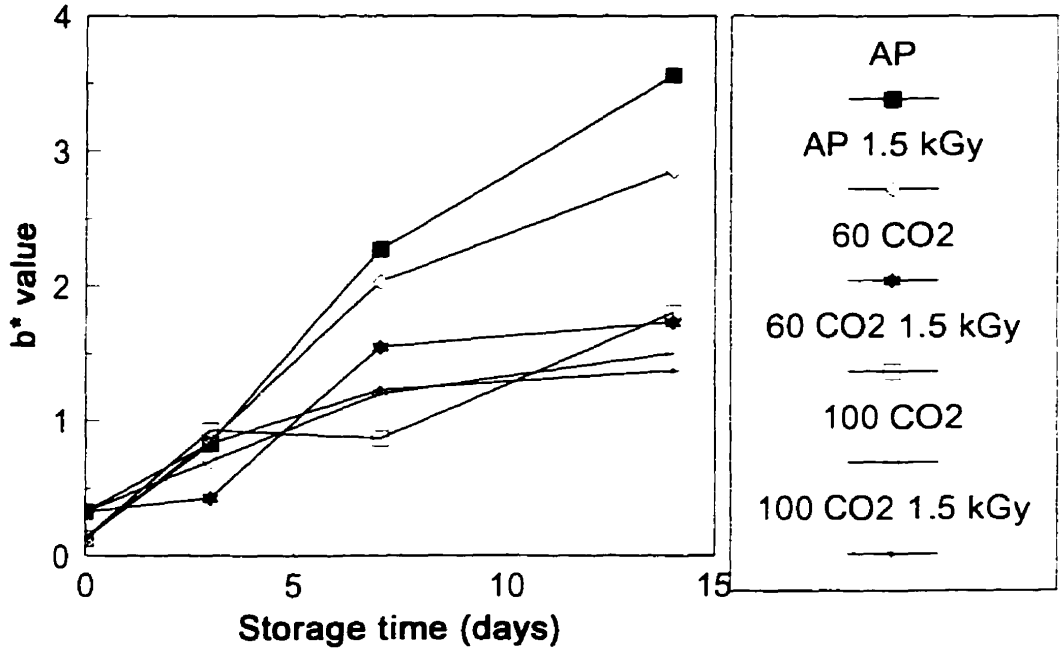
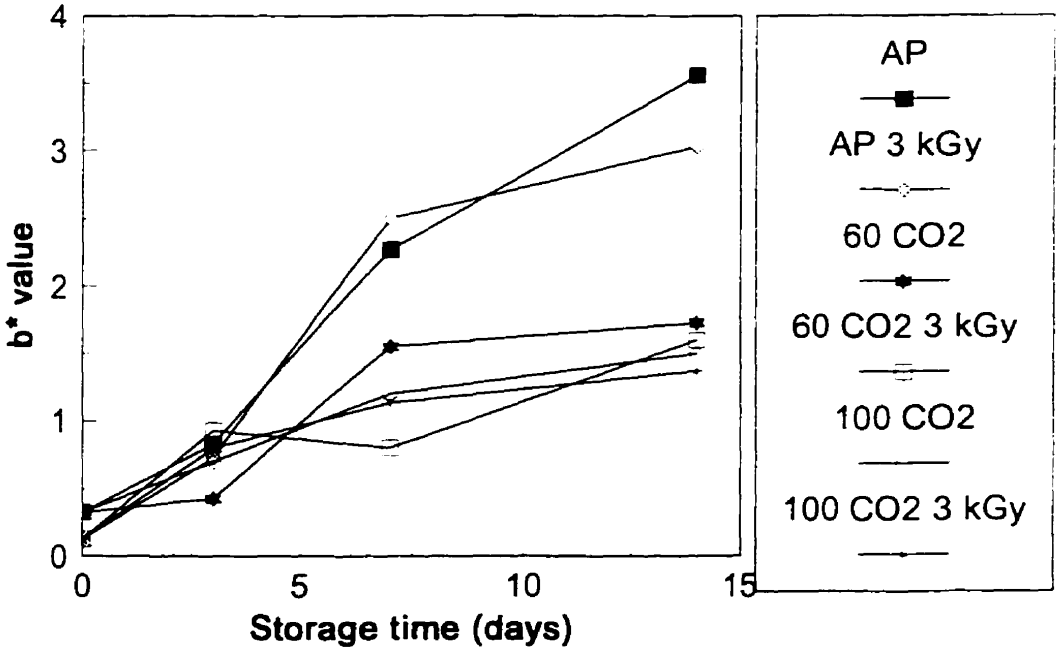


Fig.88b. Changes in b* coordinates of chicken thighs and inoculated with S. enteritidis at 12°C.



6.3.3. Drip loss

The drip loss of irradiated chicken thighs is shown in Figures 89-90 a,b respectively. Irradiation significantly affected ($P<0.05$) drip loss. Irradiated samples had consistently higher drip ($P<0.05$) compared to the non-irradiated samples (Figures 89-90 a,b). This effect was more pronounced at a higher irradiation dose (3.0 kGy) and temperature (12°C). Results also show that drip loss was significantly different ($P<0.05$) between the irradiated samples. After 14-21d storage at 4°C, irradiated aerobically packaged samples showed significantly higher drip ($P<0.05$) compared to the irradiated modified atmosphere samples regardless of the irradiation dose (Figure 89 a,b). Samples packaged in air and irradiated at 1.5 and 3.0 kGy reached a value of 6.2 and 6.6% respectively after 21d of storage at 4°C (Figure 89 a,b). Similar changes in drip loss were observed for the irradiated aerobically packaged samples (1.5 & 3.0 kGy) at 12°C (Figure 90 a,b). After 14d of storage at 12°C, a drip loss of 7.0 and 7.6% was recorded for the 1.5 and 3.0 kGy samples (Figure 89 a,b). These results suggest that O_2 and irradiation markedly affect the water holding capacity and thus increase drip loss. Lambert et al. (1992a) reported that irradiation (1kGy) increased the exudate loss of pork. Higher doses of irradiation in the presence of O_2 have been also known to enhance lipid oxidation (Lambert et al.,1992a). These oxidized products of the unsaturated fatty acids can react with the muscle proteins leading to the destruction of their functional properties (Lea, 1962). Our results confirm these observations and those of Lambert et al.(1992a) that irradiation, in the presence of O_2, increases the drip loss of muscle proteins.

Fig.89a. Changes in drip loss (%w/w) of chicken thighs and inoculated with S. enteritidis at 4°C.

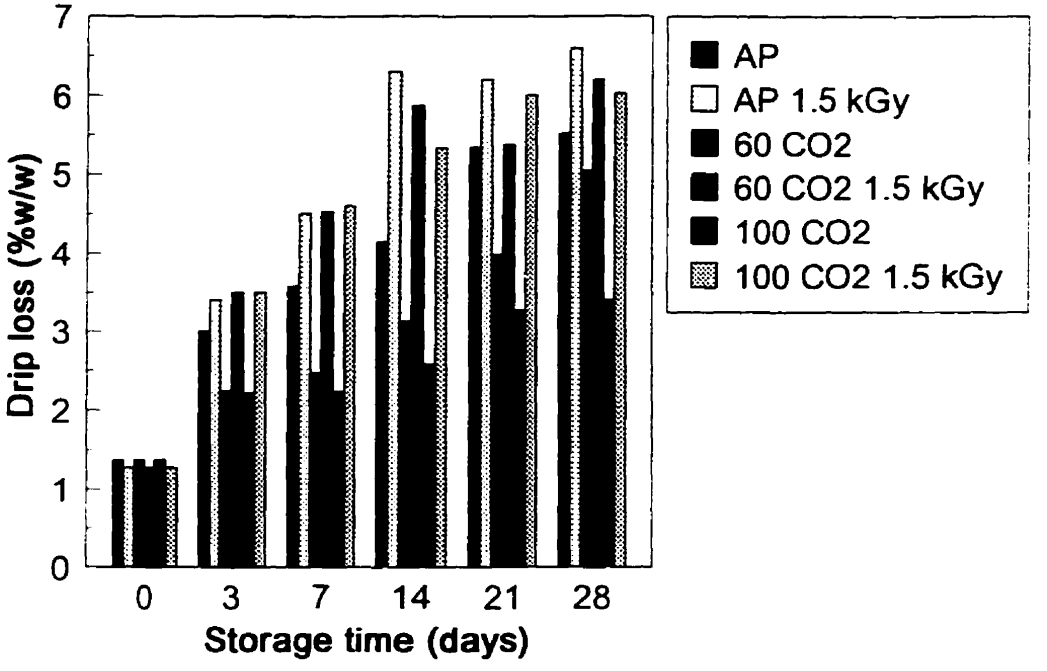


Fig.89b. Changes in drip loss (%w/w) of chicken thighs and inoculated with S. enteritidis at 4°C.

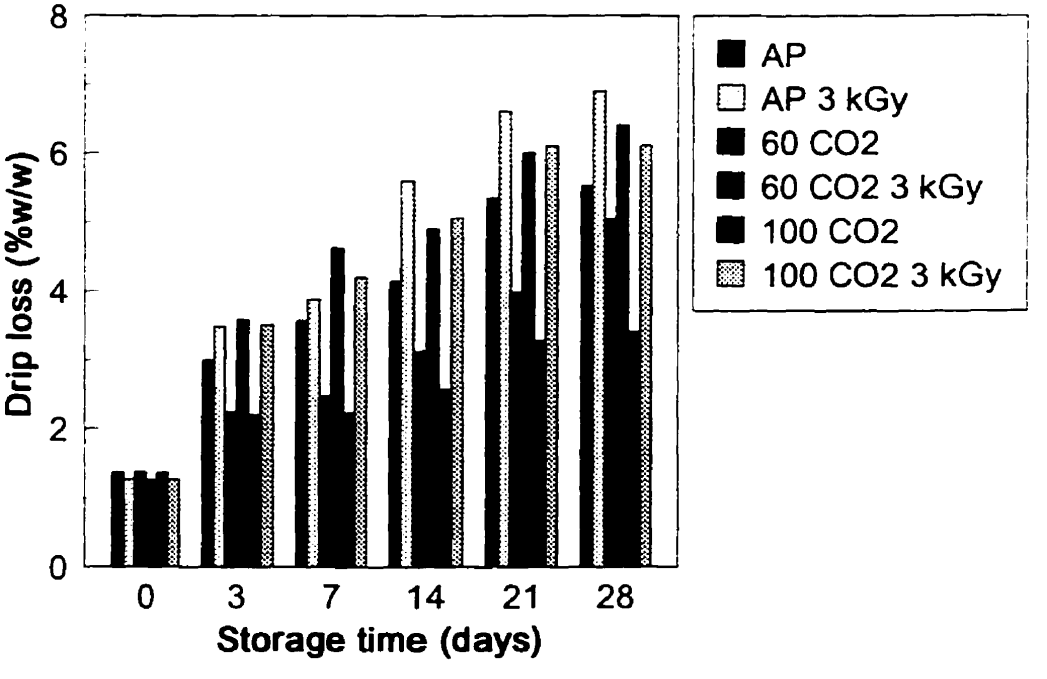


Fig.90a. Changes in drip loss (%w/w) of chicken thighs and inoculated with S. enteritidis at 12°C.

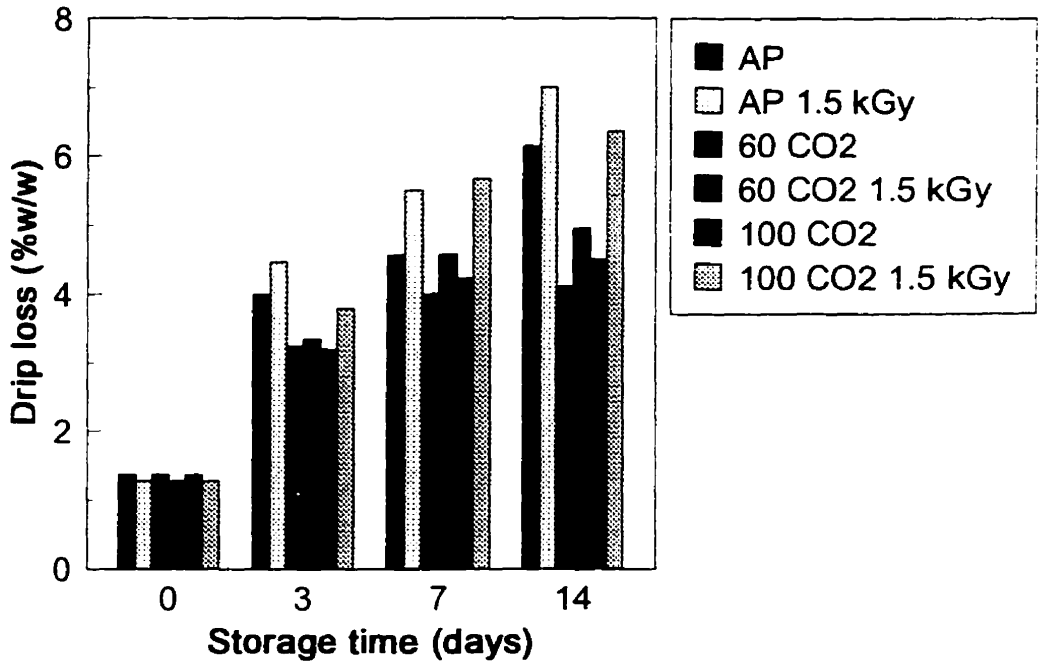
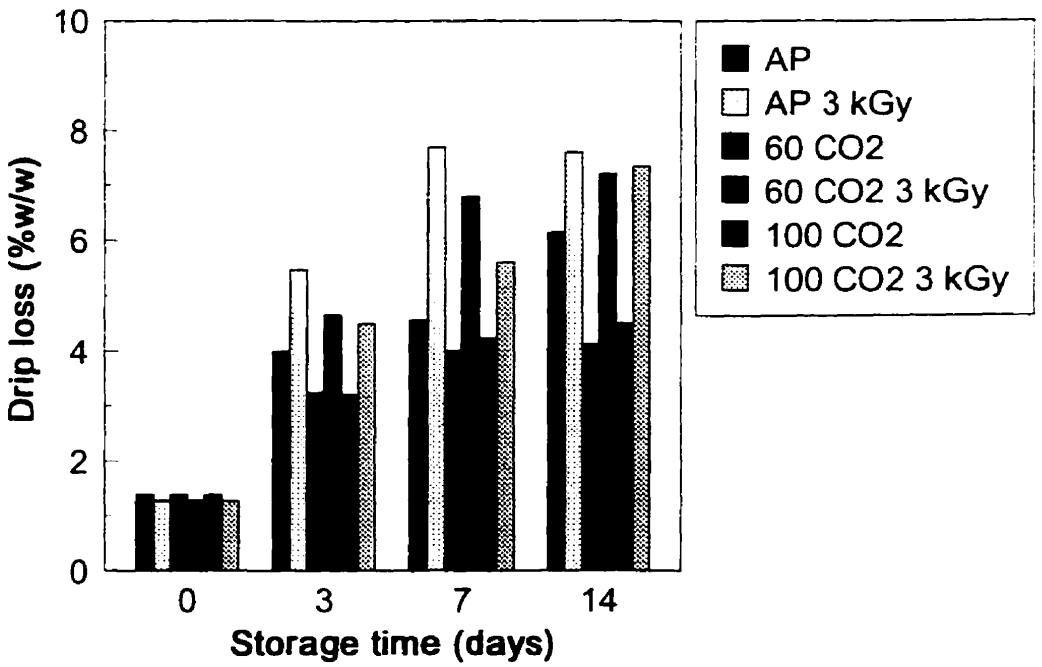


Fig.90b. Changes in drip loss (%w/w) of chicken thighs inoculated with with S. enteritidis at 12°C.



6.3.4. Sensory analyses

Changes in sensory attributes (color &odor) are shown in Figures 91-94 a,b respectively. Irradiation, modified atmosphere and the combined effect of irradiation and modified atmosphere had an important effect on the color/odor of packaged chicken thighs. Initially (day 0), no color difference was detected immediately after irradiation between the irradiated and non-irradiated samples. After 7d at 4°C, aerobically packaged samples were more discolored ($P<0.05$) compared to the other treatments (Figure 91 a,b). Similarly, the aerobically packaged samples (1.5 &3.0 kGy) were more discolored ($P<0.05$) than all the treatments after 14d of storage at 4°C (Figure 91 a,b). After 3-14d at 4°C, no significant difference ($P<0.05$) was observed among the modified atmosphere treatments (irradiated & non-irradiated). However, after 21d, non-irradiated modified atmosphere samples ($P<0.05$) were more discolored compared to the similarly packaged irradiated samples (Figure 91 a,b). At 12°C, similar but more dramatic color changes occurred. Aerobically packaged samples were discolored after 3d (Figure 92 a,b). After 7-9d, the irradiated aerobically packaged samples (1.5& 3.0 kGy) were more discolored compared to the irradiated modified atmosphere samples (1.5 & 3.0 kGy) but not from the non-irradiated modified atmosphere packaged samples (60% &100%CO_2). The non-irradiated modified atmosphere samples increased as storage progressed and were discolored after 13d (Figure 92 a,b). Overall, our results show that the presence of O_2 had a detrimental effect on the color of chicken thighs. Lambert et al. (1992a) concluded that factors affecting the discoloration of pork included O_2, irradiation and the combined action of O_2 /irradiation. In this study, the presence of O_2 in the aerobically packaged samples enhanced the growth of aerobic spoilage bacteria in the early stages of the storage period, thereby reducing the O_2 tension with consequent formation of metmyoglobin. Irradiation in the presence of O_2, had no detrimental effect on color in the early stages of the storage period probably due to the sensitivity of aerobic spoilage bacteria to irradiation. However, as storage progressed, irradiation resistant bacteria such as *Moraxella/Acinetobacter* and the lactic acid bacteria may predominate, resulting in a low O_2 tension and thus favoring the formation of metmyoglobin.

Changes in sensory odor are shown in Figures 93-94 a,b respectively. No significant differences were observed between irradiated and non-irradiated samples ($P>0.05$) immediately after irradiation. However, some panellists noted off-odors in the aerobically packaged samples irradiated at 3.0 kGy. This off-odor dissipated quickly and was considered to have no impact on the wholesomeness of the samples. Aerobically packaged samples had strong off-odors after 7d at 4°C (Figure 93 a,b). After 3-14d at 4°C, no significant differences ($P>0.05$) were observed among treatments until day 21 of storage, at which time, the irradiated aerobically packaged samples (1.5 & 3.0 kGy) showed higher odor scores ($P<0.05$) compared to the modified atmosphere samples (irradiated & non-irradiated). The modified atmosphere samples (irradiated & non-irradiated) showed no significant changes in odor throughout the 28d storage period at 4°C (Figure 93 a,b). However at 12° C, odor scores increased significantly ($P>0.05$) towards the end of the storage period with samples stored at 12°C having strong off-odors compared to those stored at°4 C (Figures 93-94 a,b). irrespective of the packaging treatment. Lambert et al. (1992a) reported a decrease in off-odors for pork samples packaged in N_2 and stored at 5°C. These results show that modified atmosphere, irradiation and low temperature favor the growth of lactic acid bacteria which usually do not produce off-odor.

Fig.91a. Changes in sensory color of chicken thighs and inoculated with S. enteritidis at 4°C.

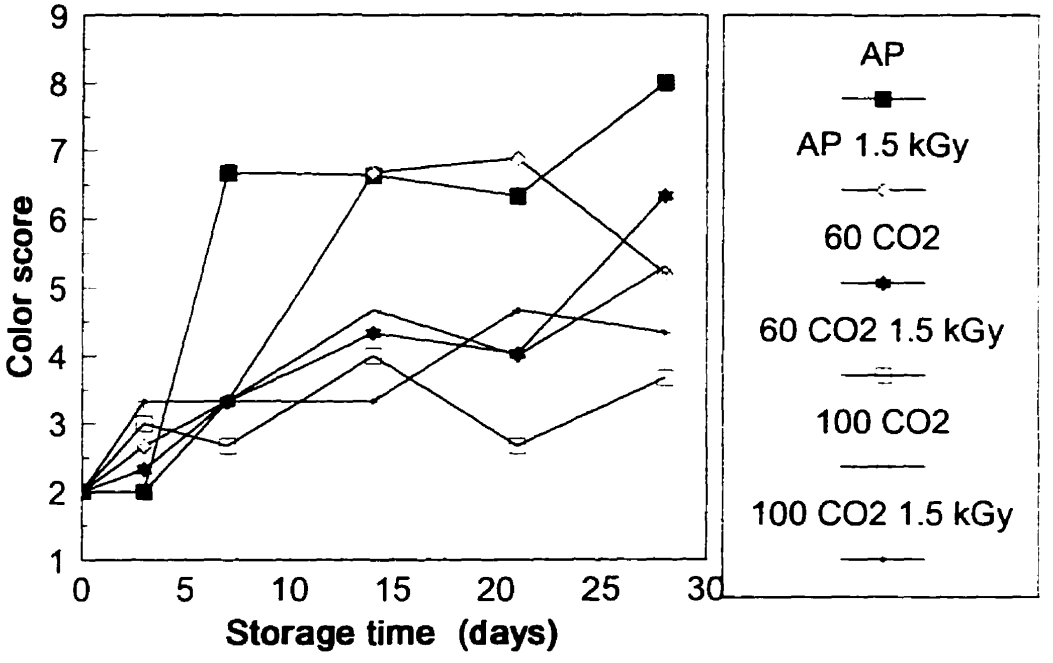


Fig.91b. Changes in sensory color of chicken thighs and inoculated with S. enteritidis at 4°C.

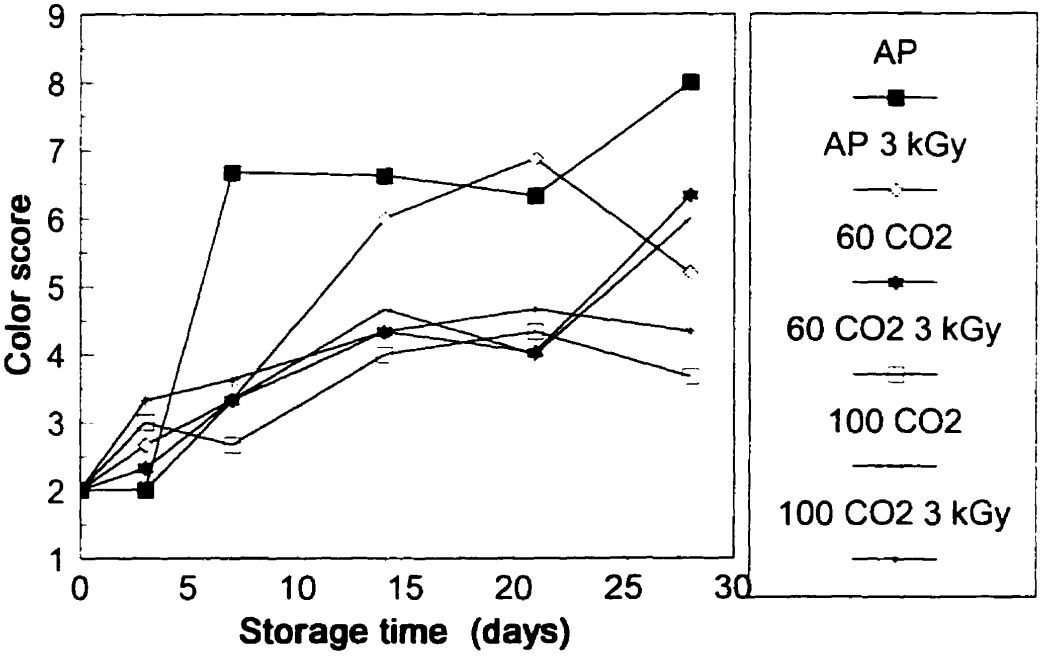


Fig.92a. Changes in sensory color of chicken thighs and inoculated with S. enteritidis at 12°C.

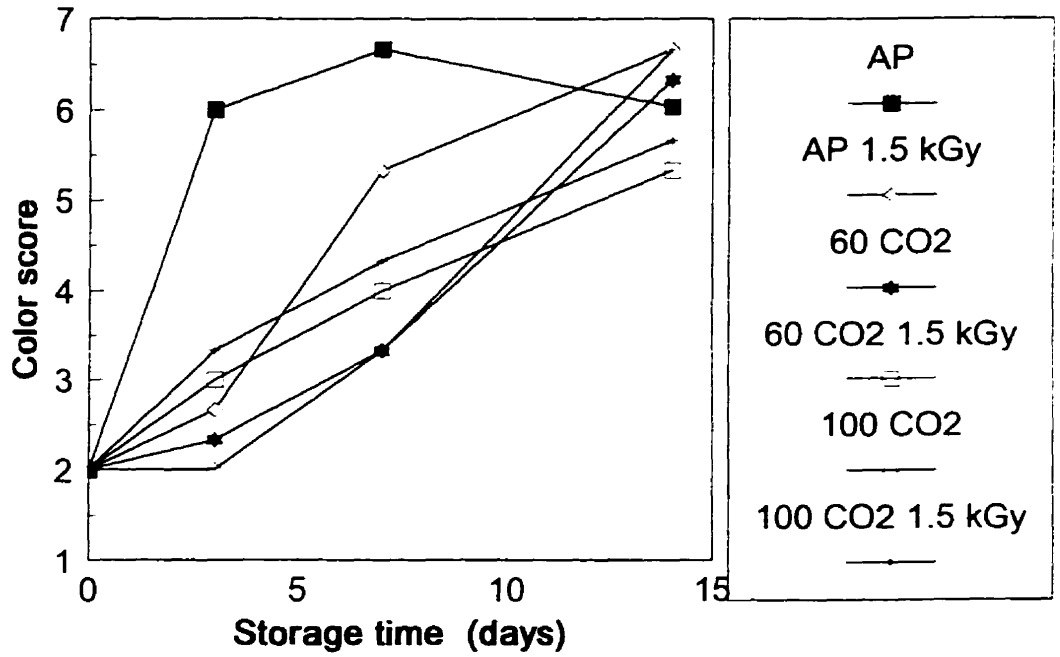


Fig.92b. Changes in sensory color of chicken thighs and inoculated with S. enteritidis at 12°C.

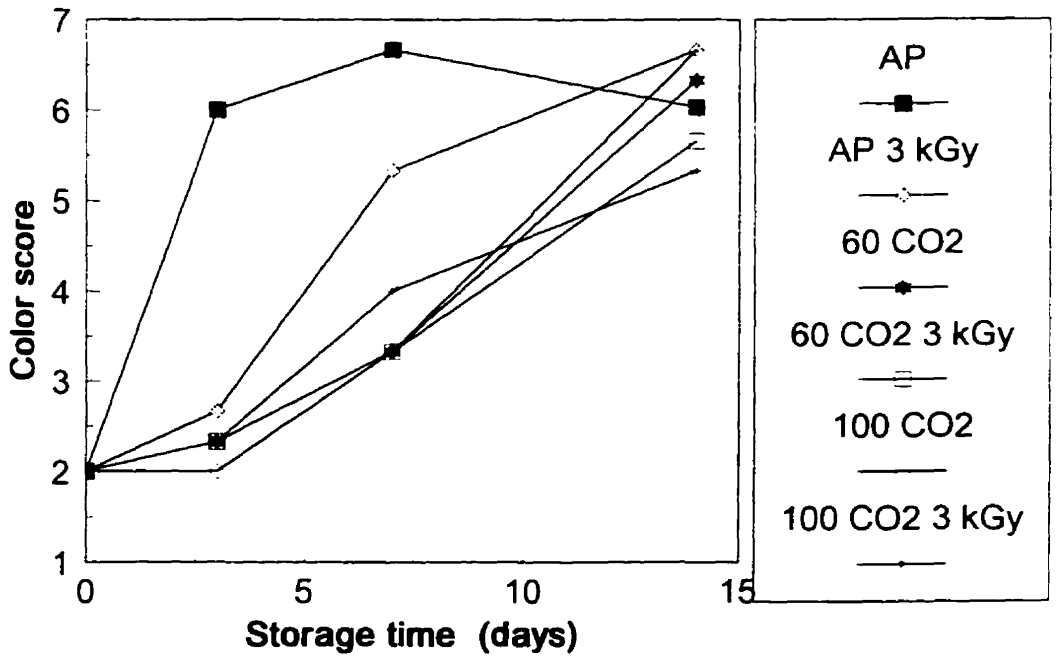


Fig.93a. Changes in sensory odor of chicken thighs and inoculated with S. enteritidis at 4°C.

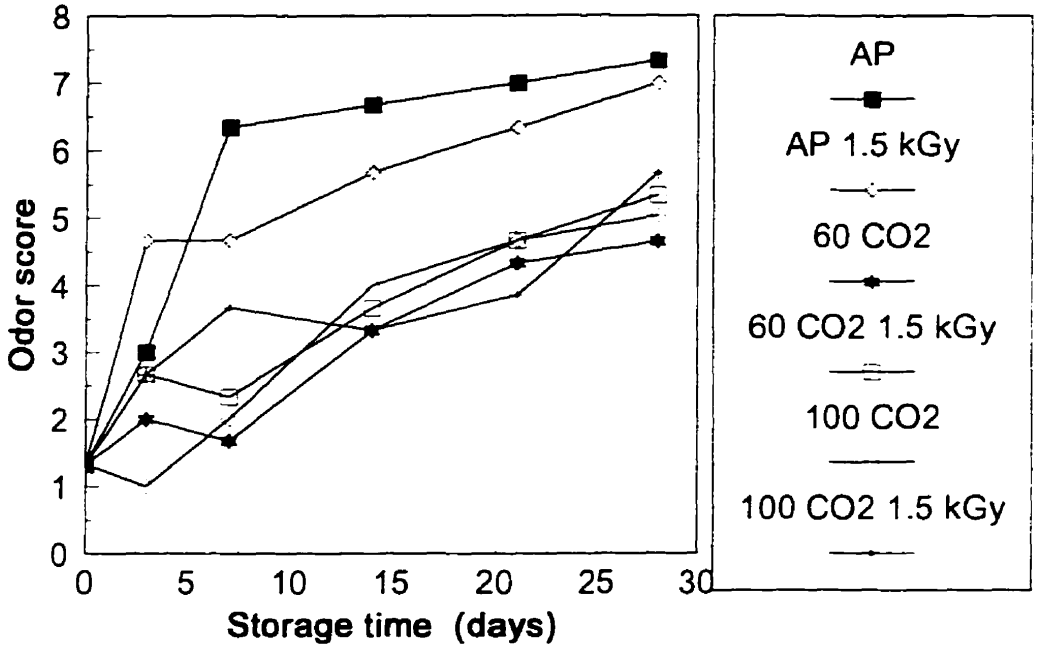


Fig.93b. Changes in sensory odor of chicken thighs and inoculated with S. enteritidis at 4°C.

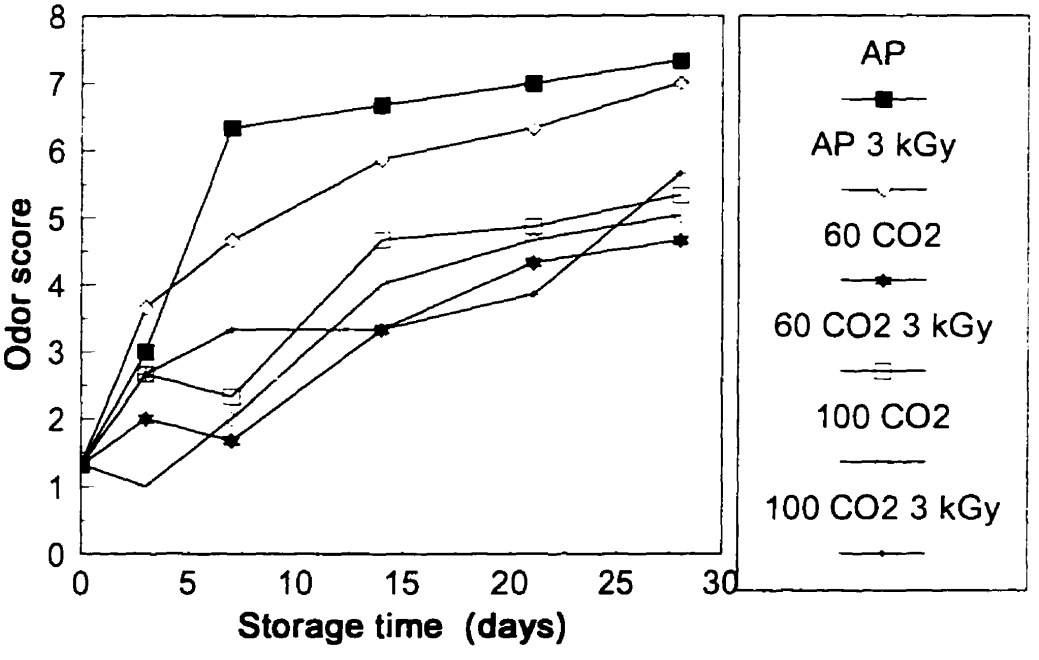


Fig.94a. Changes in sensory odor of chicken thighs and inoculated with S. enteritidis at 12°C.

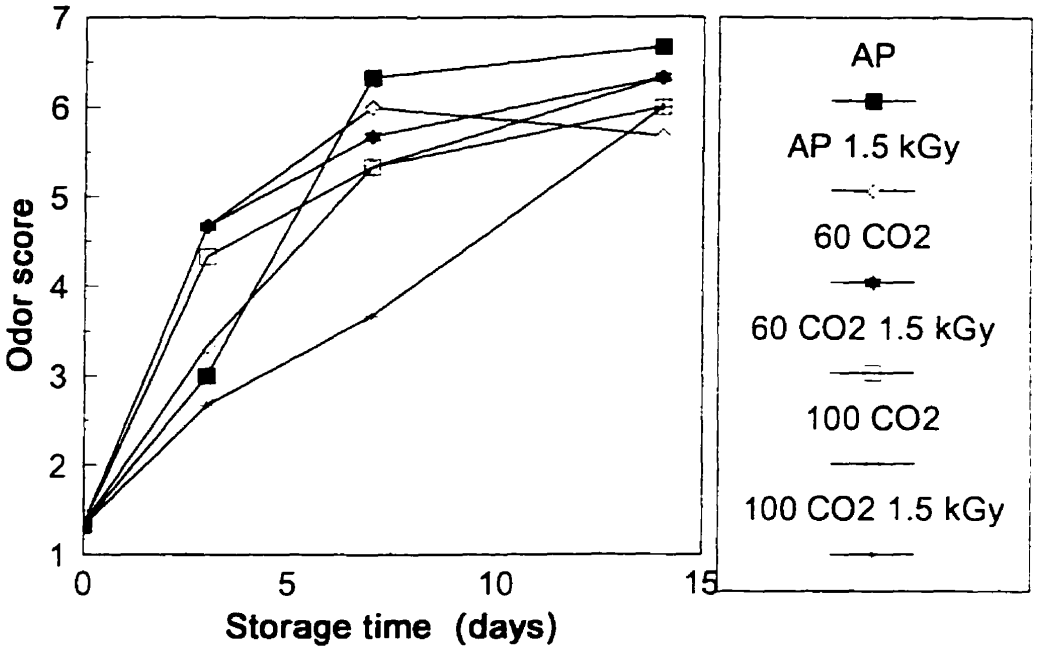
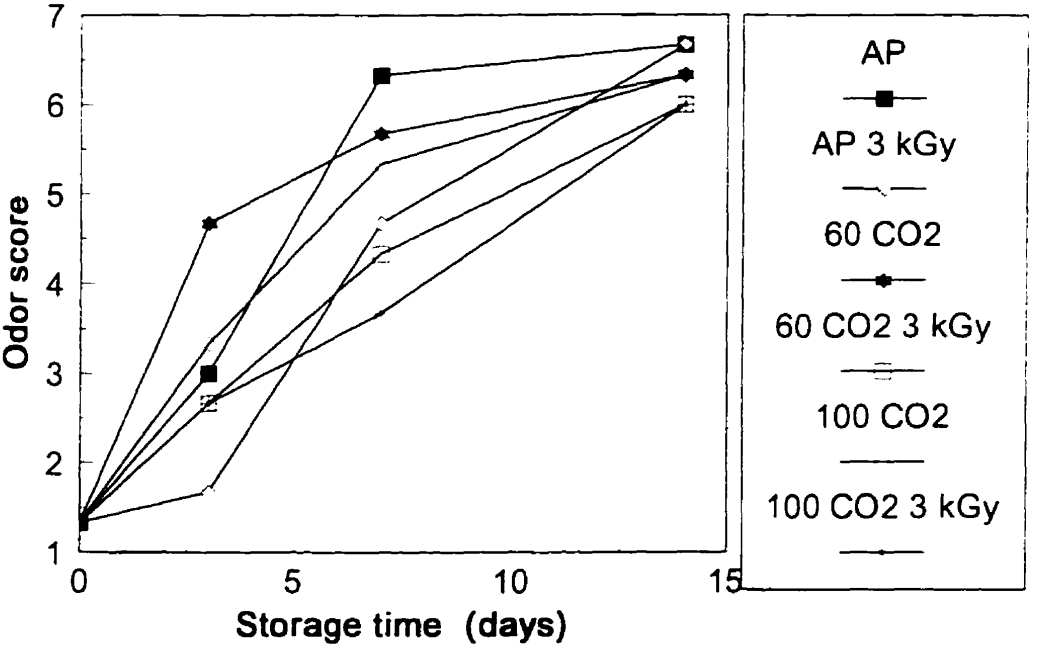


Fig.94b. Changes in sensory odor of chicken thighs and inoculated with S. enteritidis at 12°C.



6.3.5. pH

Changes in pH of irradiated chicken thighs packaged in various atmospheres and stored at 4 and 12°C are shown in Figures 95-96 a,b respectively. At both 4 and 12°C, there was no significant difference ($P>0.05$) in pH regardless of the packaging treatment/irradiation dose. After 7d at 4°C, a decrease in pH was observed for the modified atmosphere irradiated samples (1.5 & 3.0 kGy). Similarly, a decrease in pH was also observed for the irradiated aerobically packaged samples (1.5 & 3.0 kGy) after 14d at 4°C (Figure 95 a,b). Fu et al. (1995a) reported no pH changes in irradiated steak and ground beef until after 9d at 7°C. This decrease, observed in all irradiated samples compared to the non-irradiated samples, could be a result of the growth of lactic acid bacteria and the production of organic acids.

6.3.6. Microbial analyses

The growth of *S. enteritidis* in irradiated chicken thighs packaged in various atmospheres and stored at 4 and 12°C are shown in Table 18. The initial inoculum level of *S. enteritidis* was 3.77 $\log_{10}$CFU/g.Treatment of thighs with a dose of 1.5 and 3.0 kGy eliminated *S. enteritidis* inoculated onto chicken thighs irrespective of the storage temperature/ packaging treatment. On selective enrichment with 0.1% buffered peptone water, *S. enteritidis* was only present in the 1.5 kGy treatments, however, they were present in low numbers (<1 $\log_{10}$ CFU/g). At higher irradiation level (3.0 kGy) *S. enteritidis* was not detected even after selective enrichment.The low survival rate of *S. enteritidis* could be probably due to the sensitivity of *S. enteritidis* to irradiation.The bactericidal effect of irradiation on *S. enteritidis* has been previously reported by Monk et al. (1995). The effectiveness of irradiation on the growth of *S. enteritidis* in vacuum canned mechanically deboned chicken meat was also studied by Thayer et al. (1995). An initial inoculum level of 3.86 $\log_{10}$CFU/g was reduced to less than 1.22 CFU/g with 1.5 kGy at 5°C. The authors also observed that *S. enteritidis* did not recover and remained below detectable limits after 2 weeks of storage. Fu et al. (1995b) reported a similar reduction (~ 3 $\log_{10}$CFU/g) of *S.*

typhimurium inoculated onto ham with no significant increase in *Salmonella* counts with 0.75 or 0.95 kGy after 7d at 7°C. Furthermore, *Salmonella spp.* have a D_{10} value ranging from 0.38-0.77 min. at 2°C (Thayer, 1993). Thus a dose of 1.5-3.0 kGy would be expected to completely inactivate the pathogen. The microbiological effect of irradiation on the microbial composition of meat has been previously reported (Lambert et al., 1992c). *Moraxella/Acinetobacter spp.* were the major spoilage microorganisms associated with meat stored under aerobic conditions. However, under anaerobic conditions, lactic acid bacteria predominated and caused an atypical spoilage odor. Lambert et al. (1992c) observed a reduction in mesophilic, psychrotrophic counts by 2 log cycles in pork irradiated with 1 kGy, whereas the lactic acid bacteria were unaffected. Similar results were observed by Ehioba et al. (1987) in irradiated vacuum packaged pork. These results show that the combined effect of irradiation and modified atmosphere will not only eliminate the growth of *S. enteritidis* but would favor the growth of lactic acid bacteria during storage and would account for these changes.

Fig.95a. Changes in pH of chicken thighs and inoculated with S. enteritidis at 4°C.

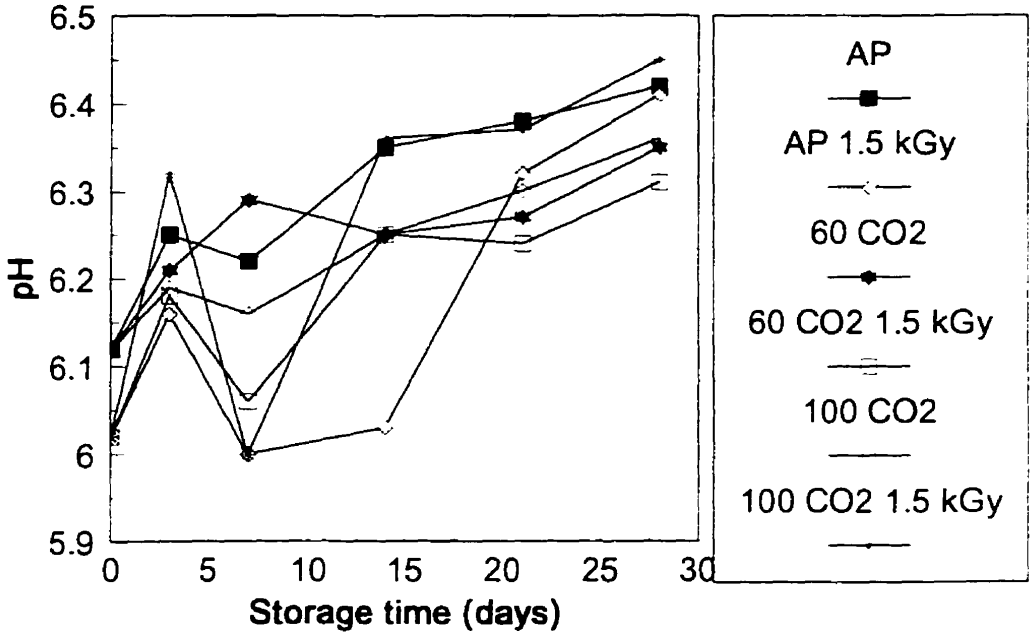


Fig.95b. Changes in pH of chicken thighs and inoculated with S. enteritidis at 4°C.

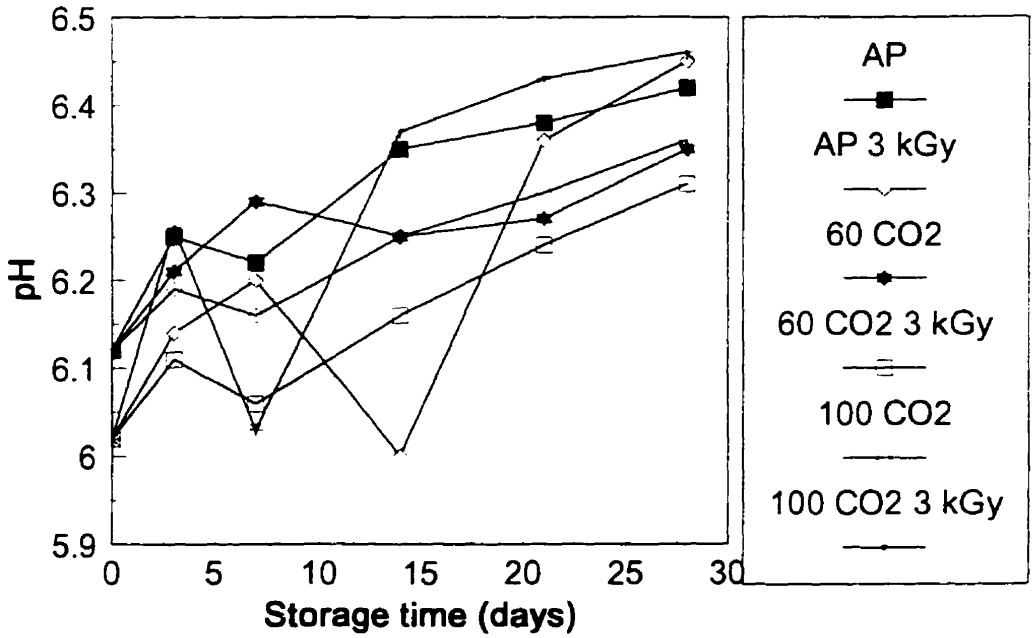


Fig.96a. Changes in pH of chicken thighs and inoculated with S. enteritidis at 12°C.

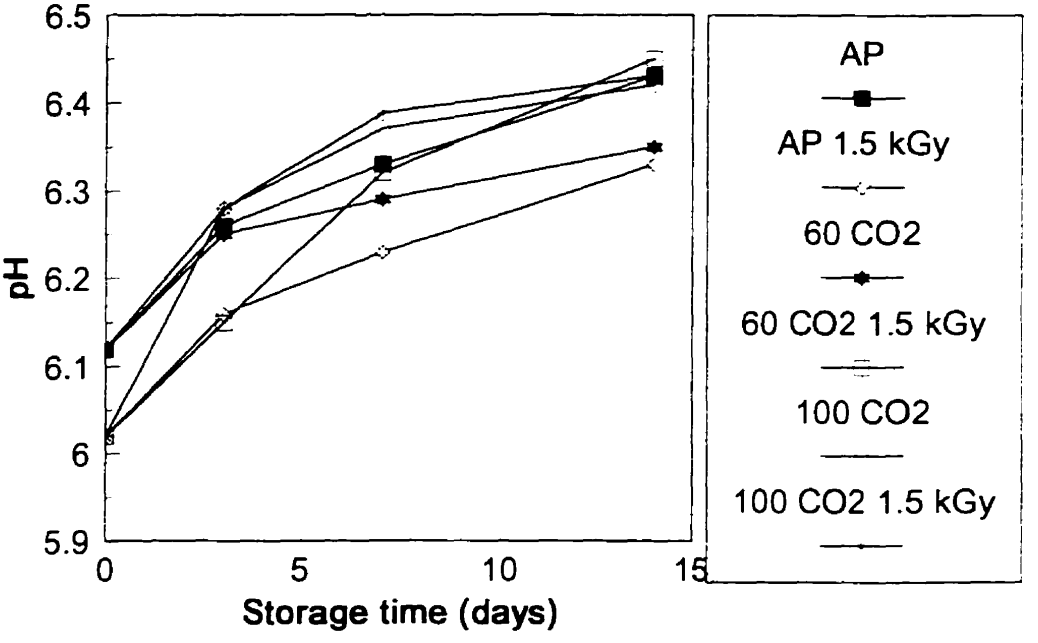


Fig.96b. Changes in pH of chicken thighs and inoculated with S. enteritidis at 12°C.

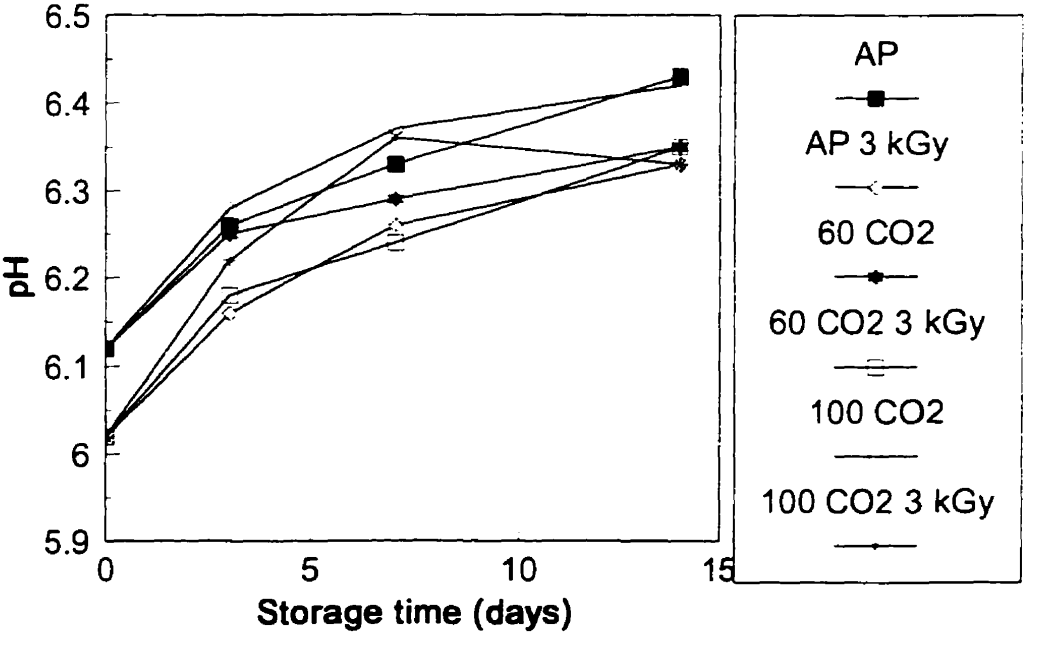


Table 17. Growth of *S. enteritidis* in irradiated chicken thighs in various modified atmospheres and stored at 4 and 12°C

Storage time (days)	Temp (°C)	Irradiation dose (kGy)								
		Air			0% CO_2			100%CO_2		
		0[b]	1.5	3	0	1.5	3	0	1.5	3
0	4	3.77[a]	ND[e] (-)[c]	ND (-)	3.77	ND (-)	ND (-)	3.77	ND (-)	ND (-)
3		4.77	ND (-)	ND (-)	3.2	ND (-)	ND (-)	2.35	ND (-)	ND (-)
7		5.5	ND (-)	ND (-)	3.9	ND (-)	ND (-)	2.8	ND (-)	ND (-)
14		6.1	ND (+)[d]	ND (-)	4.2	ND (-)	ND (-)	2.6	ND (-)	ND (-)
21		6.27	ND (+)	ND (-)	4.6	ND (+)	ND (-)	2.9	ND (+)	ND (-)
28		6.18	ND (+)	ND (-)	4.8	ND (+)	ND (-)	3.2	ND (-)	ND (-)
0	12	3.77	ND (-)	ND (-)	3.77	ND (-)	ND (-)	3.73	ND (-)	ND (-)
3		5.9	ND (+)	ND (-)	4.6	ND (+)	ND (-)	4.5	ND (-)	ND (-)
7		6.06	ND (+)	ND (-)	4.9	ND (+)	ND (-)	4.8	ND (+)	ND (-)
14		6.89	ND (+)	ND (-)	5.03	ND (+)	ND (-)	5.2	ND (+)	ND (-)

[a] Results expressed as Log_{10} CFU/g

[b] Non irradiated samples

[c,d] Presence (+) or absence (-) of *S. enteritidis* following enrichment in 0.1% buffered peptone water of 25 grams of chicken

[e] Not detected (lower limit of detection 10^1 CFU/g)

6.3.6. Shelf-life

The estimated shelf-life of irradiated chicken thighs are shown in Table 18. The storage shelf-life was based on a sensory score of six or a *S. enteritidis* count of 10^6 CFU/g. Our results show that irradiation with or without modified atmosphere (60 or 100%CO_2) eliminated *S. enteritidis*. Furthermore, irradiation and modified atmosphere didnot adversely affect the sensory characteristics of packaged samples resulting in extension of shelf-life to more than 28d compared to 12d for the irradiated, aerobically packaged samples at 4°C. The bactericidal effect of irradiation and modified atmosphere on the growth of *S. enteritidis* was also observed at higher temperatures (i.e., 12°C) indicating that irradiation can ensure the safety of the packaged samples even under temperature abuse conditions. However, shelf-life was terminated after 14d of storage on the basis of strong off-odors possibly due to protein breakdown or lipid oxidation.

Conclusion

Packaging of chicken thighs in 60% or 100% CO_2, followed by irradiation with 1.5 or 3 kGy, was found to extend the shelf-life to more than 28d at 4°C without adversely affecting the sensory properties of packaged chicken thighs. Packaging in air, followed by irradiation (1.5 & 3.0 kGy), completely inactivated *S. enteritidis* but adversely affected the sensory properties of the packaged samples. Therefore, for maximum extension of shelf-life, thighs should be packaged in 60% or 100% CO_2 followed by irradiation with 1.5 or 3 kGy and storage at 4°C.

Table 18. Estimated shelf-life of irradiated chicken thighs inoculated with *S. enteritidis* in various packaging treatments stored at 4 and 12°C.

Packaging Treatment[a]	Rejection point/color[b]			Rejection point/odor[b]			Microbial shelf life[c]			Shelf life[d]		
	Irradiation dose (kGy)											
	0[e]	1.5	3	0	1.5	3	0	1.5	3	0	1.5	3
Storage at 4°C												
A	6	12	12	6	21	21	12	>28	>28	6	12	12
B	26	>28	>28	>28	>28	>28	>28	>28	>28	26	>28	>28
C	>28	>28	>28	>28	>28	>28	>28	>28	>28	>28	>28	>28
Storage at 12°C												
A	3	13	13	7	7	6	7	>14	>14	3	8	6
B	12	>14	>14	12	14	14	>14	>14	>14	12	14	14
C	13	>14	>14	13	14	14	>14	>14	>14	13	14	14

[a] A, Air B, 60%CO_2:40%N_2 C, 100%CO_2

[b] Time to reach a score of six

[c] Time to reach a *S. enteritidis* count of 10^6 CFU/g.

[d] Earliest rejection point on terms of odor,color and microbial load

[e] Non irradiated samples

GENERAL CONCLUSION

The key factor for any improvement in the quality and shelf-life of perishable products, such as poultry meat, is to preserve the product under the most convenient environment to minimize the growth of spoilage bacteria and ensure the safety of the product. From this study, several approaches could be recommended for the overall improvement of the quality and shelf-life of packaged chicken thighs.

I) It is highly recommended that storage of chicken thighs be at low temperatures preferably at 4°C or less. This was found to extend the shelf-life of air packaged thighs to 7d compared to 3d for similarly packaged samples at 12°C.

II) Gas packaging (60% CO_2+40% N_2), in conjunction with low temperature storage was found to extend the shelf-life to more than 28d compared to 7d for the air packaged samples at 4°C.

III) Pre-dipping of gas packaged samples with 0.2% chitosan increased the shelf-life to 20d compared to 7d for the aerobically packaged samples and inhibited the growth of *S. enteritidis* at 4°C.

IV) Pre-dipping of gas packaged samples with 0.2% potassium sorbate increased the shelf-life to more than 28d and inhibited the growth of *S. enteritidis*. Likewise, packaging of thighs in 100% CO_2 with or without pre-dipping with 0.2% potassium sorbate improved shelf-life and inhibited the growth of *S. enteritidis*, with degree of inhibition being greater in sorbate treated samples than in the untreated samples.

V) Gas packaging samples (60 or 100% CO_2) followed by low dose irradiation with 1.5 or 3 kGy completely eliminated *S. enteritidis* and improved the shelf-life to more than 28d compared to 6d for the aerobically packaged samples at 4°C.

Therefore, from the summation of the results shown, the shelf-life and safety of chicken thighs could be extended by storage at low temperatures, and by packaging in 60 or 100% CO_2 with or without pre-dipping in potassium sorbate. For maximum shelf-life of

packaged chicken thighs, thighs can be irradiated with low dose irradiation (1.5 or 3 kGy). The use of these methods by the poultry industry for shelf-life extension of its products is greatly dependent on cost and the extra cost will ultimately be passed on to the consumer. Furthermore, there is still consumer resistance to the use of low dose irradiation although sales of irradiated poultry in the U.S. appear to be strong. This extra cost should result in increase in sales for these preservative techniques to be feasible. If improvement of the products quality induces consumer confidence in the product and leads to an increase in sales, and in revenues, and profits and the decision lies with the poultry industry to implement such alternative methods to freezing for shelf-life extension of products.

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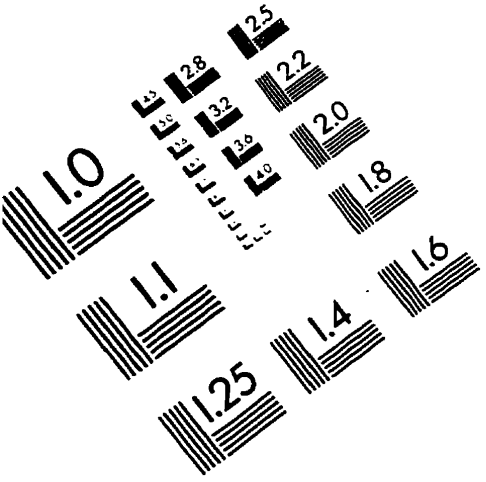
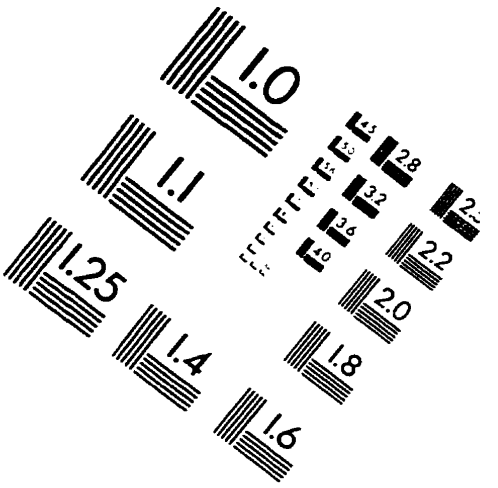
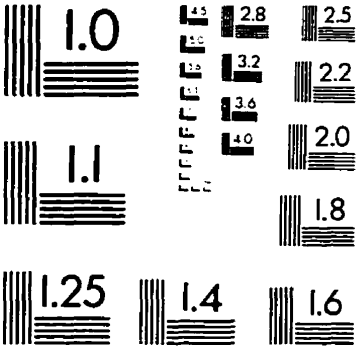
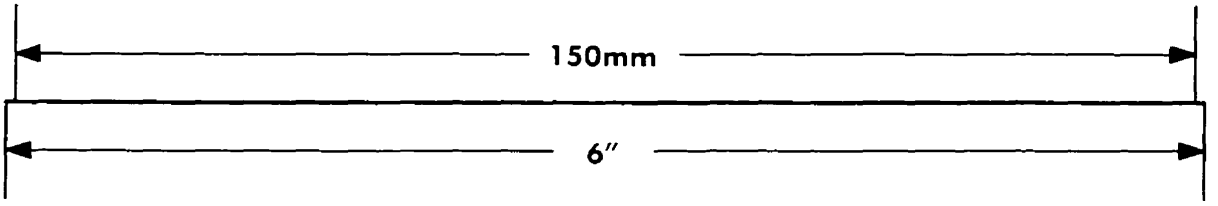
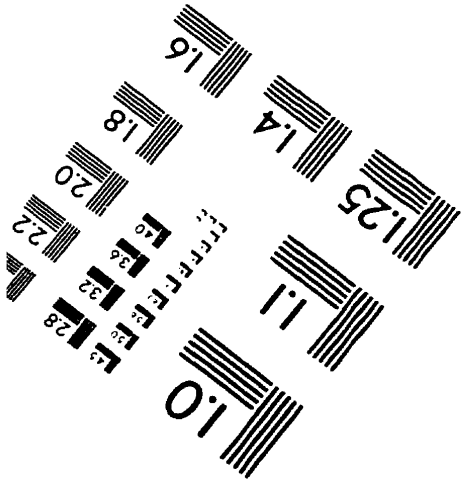
IMAGE EVALUATION TEST TARGET (QA-3)

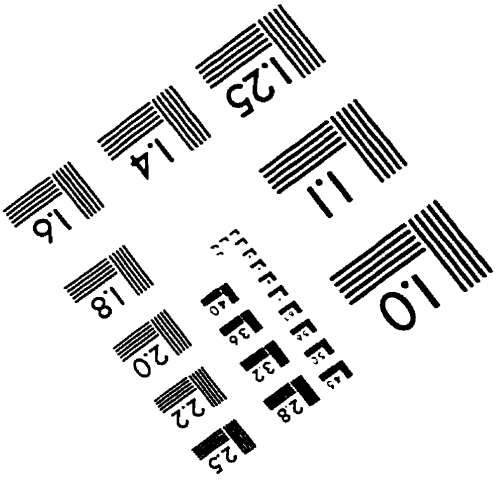












APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989