QUALITY ATTRIBUTES OF CURED SMOKED CHICKEN FILLETS AS INFLUENCED BY *CAPSICUM* SPP. EXTRACT AND CHLORIDE SALTS

Ayobami Temitope ADESHOLA

B. Agric (Hons.) Animal Production (Ago Iwoye), M.Sc. Animal Science (Ibadan)

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ABSTRACT

Sodium nitrite ($NaNO_2$) and sodium chloride (NaCl) are essential ingredients for meat curing. However, prolonged consumption of these salts could have deleterious health effects on consumers. *Capsicum* Extract (CE) has preservative and colour-fixing abilities which could replace $NaNO_2$, while NaCl could be substituted with combinations of safer chloride salts. There is paucity of information on utilisation of CE and selected chloride salts in meat product development. Hence, quality characteristics of cured smoked Chicken Fillets (CF) prepared with CE and chloride salts were investigated.

Four *Capsicum* spp.: Bell Pepper-BlP, Bird Pepper-BdP, Cayenne Pepper-CeP and Scotch Bonnet-SB were oven-dried at 60^{o}C and extracted with 95% methanol (1:5 w/v). Extracts were analysed for total phenol (mg/100g), Radical Scavenging Activity-RSA (%) and total carotenoids (mg/100g) using standard procedures. Chicken fillets obtained from 20-week old male *Funaab-α II* birds were cured using 0% nitrite (T1), 0.015% nitrite (T2); and BlP extract at: 0.150% (T3), 0.300% (T4), 0.450% (T5), 0.600% (T6) and 0.750% (T7) in a completely randomised design. New samples were cured with 3.500% NaCl (S1), 1.750% NaCl+1.750% calcium chloride-$CaCl_2$ (S2), 1.750% NaCl+1.750% potassium chloride-KCl (S3), 1.750% NaCl+1.750% magnesium chloride-$MgCl_2$ (S4), 1.750% NaCl+0.875% $CaCl_2$+0.875% KCl (S5), 1.750% NaCl+0.875% $CaCl_2$+0.875% $MgCl_2$ (S6), 1.750% NaCl+0.875% KCl+0.875% $MgCl_2$ (S7) and 0.875% NaCl+0.875% $CaCl_2$+0.875% KCl+0.875% $MgCl_2$ (S8) and then smoked, vacuum-packed and stored at 25^{o}C for 60 days. The best CF (0.450% BlP extract and 1.750% NaCl+0.875% KCl+0.875% $MgCl_2$) was prepared and stored at 4^{o}C and 25^{o}C for 90 days. The pH, Lipid Oxidation-LO (mg/100g), sodium (mg/kg), redness and Total Anaerobic Bacteria Count-TABC (log_{10}cfu/g) were determined at 15-day interval using standard procedures. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$.

Total phenol in extracts of BdP (0.48±0.16), 0.42±0.10 (CeP) and 0.35±0.06 (BlP) were similar but significantly higher than 0.29±0.07 in SB. The RSA of extracts ranged from 34.90±1.11 (BdP) to 85.17±2.00 (BlP). Total carotenoids ranged from 22.32±1.62 (BdP) to 43.63±1.67 (CeP). The pH significantly reduced from 6.06±0.06 (T6) to 5.12±0.02 (T1) during storage. Lipid oxidation of 0.91±0.30 was least in T6 on day 0, while 1.26±0.01 was highest in T7 on day 60. Lowest and highest redness of 13.82±1.12 and 23.20±3.74 were obtained in T5 and T1, respectively, while TABC ranged from 2.16±0.28 (T7) to 3.11±0.19 (T1). The pH of 4.93±0.25 was least in S1 while S7 had highest pH of 6.05±0.02. Lipid oxidation significantly increased during storage for all treatments from 0.07±0.06 (day 0) to 0.40±0.15 (day 60). Sodium content ranged from 15.09±1.03 (S7) to 28.20±1.06 (S2), while redness was highest in S8 (27.36±3.60) and least in S7 (11.27±2.45). The TABC ranged from 3.21±0.16 (S6) to 4.01±0.09 (S4). The CF stored at 25^{o}C had least LO of 0.59±0.06 indicating better stability. The TABC of 2.42±0.10 was least in CF stored at 4^{o}C while CF stored at 25^{o}C had higher TABC of 4.09±0.05.

Bell pepper extract at 0.45% inclusion in cured smoked chicken fillets and substitution of sodium chloride with 25% potassium chloride and 25% magnesium chloride maintained quality and reduced inherent sodium level in the product.

Keywords: Bell pepper, Sodium chloride replacement, Antimicrobial activity, Chicken fillets, Meat quality

Word count: 500

CERTIFICATION

I certify that this work was carried out by Ayobami Temitope ADESHOLA, in the Department of Animal Science, Faculty of Agriculture, University of Ibadan, Ibadan, Nigeria under my supervision.

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Supervisor Date

Professor Olubunmi O. Olusola
B.Sc. (Hons.), M.Sc., Ph.D. (Ibadan), RAS
Animal Products and Processing Unit,
Department of Animal Science,
University of Ibadan, Ibadan,
Nigeria.

DEDICATION

To my darling husband, Ademola Olugbenga Adeshola, for allowing me fly.

To my dear parents, Mr and Mrs Godwin Abayomi Oyesanwen, for all your sacrifices.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The world population growth rate is rapidly increasing alongside a rapid increase in the rate of urbanisation. Sixty-six (66) % of the world's population have been projected by 2050 to be living in urban centres. Currently, Nigeria, a developing country has about fifty-five (55) % of its population living in urban areas (DESA, 2014). Increased urbanisation leads to an increase in an urban-paced lifestyle where there is less available time for food preparation and better pay for a higher percentage of the population. This translates to an increase in consumption of fast or instant (ready-to-cook/ready-to-eat) meals. A large percentage of these foods are plant-based, such as flours of maize, yam, potato etc. and there are fewer animal-based foods or meat products that are of local origin. This, therefore, created a need for the development of ready-to-eat/ready-to-cook animal products that can serve consumers living the urban-paced lifestyle.

As a major source of protein, fats, minerals and vitamins, meat is a significant part of the human diet. Meat processing improves the storage time of meat giving it an added value by presenting it in different ways that humans perceive as acceptable. The purchase and consumption of these meat and meat products are highly determined by the perception of such products by consumers. Consumption of processed meat can be perceived from two points of view: beneficial or harmful, according to Van Wezemael *et al*. (2010). Meat is beneficial is based on the nutrients that can be obtained when it is consumed while being harmful is related to the effects the added ingredients or the production process has on human health.

Meat curing is the process of using a mixture of salt, sugar and sodium nitrite to preserve and add the typical cured flavour and colour to meat (Aberle *et al.,* 2001). Ingredients for the cure could be rubbed on the meat's surface (dry curing), or dissolved in liquid (brine, wet, or pickle curing) (Heinz and Hautzinger, 2007). The most common use of sodium nitrite is to

ensure food protection in the supply chain. It prevents rancidity of meat, as well as formation of unwanted meat taste and odour during storage. It is a very effective inhibitor of the growth of *Clostridium botulinum*, the bacteria that causes botulism, in addition to inhibiting other pathogens and spoilage bacteria during meat processing (Honikel, 1998). However, there are considerable concerns as nitrite can react with amines and amino acids to form N-nitrosamines, which are considered to be carcinogens and mutagens (Byun *et al*., 2004). Natural ingredients, on the other hand, can enhance food product acceptability, palatability, safety, and shelf life for consumers. Therefore, there has been an increasing trend in using natural preservatives to replace synthetic preservatives in foods and most especially meat products. This has resulted in a noticeable increase in the search for natural additives, especially those derived from plants (Naveena *et al*., 2008).

Plant extracts have been shown to contain phenolic compounds that may function as antimicrobial and antioxidant agents. Plant polyphenols and their antioxidant, antimicrobial, antiviral, and anti-carcinogenic effects, are mainly due to bioactive materials' intrinsic ability to function as defensive agents (Aytuk, 2010). These are favoured to synthetic chemicals as protecting ingredients in the medicinal, dairy, and cosmetics sectors, as well as food additives, preservatives, and dietary supplements.

Red peppers are high in phenolic compounds, carotenoids, ascorbic acid, and vitamin A, although the levels vary based on their maturity and genotype. The quality of meat products can be improved using the carotenoids, antioxidants and antimicrobial compounds of red peppers (Jiménez *et al*., 2003). The red colouration and the anti-oxidative effect in red peppers are the responsibility of the carotenoids. Capsanthin accounts for 30-60% of the total carotenoids, while capsorubin, alpha-carotene, zeaxanthin and alpha-cryptoxanthin make up the remainder. Studies have shown that the inclusion of pepper extracts in the meat curing phase assists in colour stabilization by nitrosomyoglobin formation during chilling storage (Wojciak *et al.,* 2011). *Capsicum* peppers (sweet, red, and spicy cayenne) and piper peppers (black and white) added to fresh pork sausages have also enhanced their shelf life (Martinez *et al.,* 2006).

Another major ingredient used in meat product development with direct implications on the health of consumers is sodium chloride. Sodium chloride plays several purposes, including preservation, flavour, and texture enhancement (Weiss *et al*., 2010). It also has an effect on the functional properties of meat products, such as their ability to retain water, texture, and

the growth of bacteria. The World Health Organisation (WHO) suggests as little as 5 g of salt (sodium chloride) a day, which is equal to 2 g of sodium a day (WHO, 2012). Meat and meat products contribute between 16 and 25 percent of total daily sodium chloride intake, ranking second only to bread in terms of salt amounts (WHO, 2012). Excessive salt use, on the other hand, has been linked to a variety of health-related conditions and illnesses, including coronary disease, arthritis, neurological diseases, osteoporosis, gastric cancer, kidney disease, asthma, and obesity (McGregor, 2007). As a result, the World Health Organization (WHO) advises lowering sodium intake to promote health (World Health Organization, 2012). Ongoing global efforts are therefore being made to produce low-sodium foods, especially meat products. However, market acceptance of such foods may be influenced because salt plays a major role in the technological and sensory aspects of meat products, so qualities such as taste and appearance may be influenced when the formulation is greatly decreased (Desmond, 2006). To reduce salt levels in meat products, three options have been proposed: full replacement, partial substitution, or the use of a combination of other salts other than sodium chloride (Desmond, 2006). Many experiments have demonstrated the use of chloride salt mixes or formulations such as potassium chloride, calcium chloride, magnesium chloride, and other lactate and ascorbate salts. According to Carvalho *et al*. (2013), replacing NaCl with 50% KCl in marinated beef and chicken meat did not affect their physical and chemical properties, microbiological safety, or sensory quality. Choi *et al*. (2014) explored the combined effect of sodium chloride (NaCl) with potassium lactate (K-lactate) and calcium ascorbate (Ca-ascorbate) on the physicochemical and sensory properties of low salt sausages (1.2 percent NaCl), sausages made with 40% NaCl replaced by 30% K-lactate and 10% Ca-ascorbate had the same water retaining capacity, texture properties, and sensory characteristics as the control.

Therefore, the quality characteristics and consumer acceptability of cured smoked chicken fillets prepared using *Capsicum* extract and chloride salts was assessed in this study.

1.2 Justification

Consumption of processed meat/meat products containing high amounts of additives such as salt and nitrite has been linked to serious health risks. As a result, there is a greater global commitment by food manufacturers and experts to reduce or substitute these ingredients in refined meat products.

The consumption of ready-to-cook/ready-to-eat food products has increased significantly in the region, as evidenced by the rapid expansion of fast food restaurants and supermarkets selling packaged meat products, among other things. This has created a need to make available locally produced wholesome (less additives/fat/salt) meat products to enhance healthy consumption trend in the populace and to meet up with the prospects in the Nigerian meat products market.

Consumers are now more aware of the health implication of the foods they consume. They, therefore, are more inclined to purchase/consume food products with natural additives.

There is scanty information on the utilisation of improved breeds of indigenous chickens in meat product development, hence their use for this research.

1.3 Objectives of the study

General objective:

- To produce a shelf-stable, ready-to-eat chicken meat product with natural additive and reduced sodium level

Specific objectives:

The specific objectives of the study are to:

- assess the antioxidant, antimicrobial and pigmentation levels of extracts of selected red peppers (*Capsicum* sp.)
- assess the effect of extracts of red pepper (*Capsicum* sp.) on the quality of cured-smoked chicken fillets
- determine the effect of partial sodium chloride replacement with other chloride salts (Potassium chloride, Magnesium chloride and Calcium chloride) on the quality of cured-smoked chicken fillets
- assess the impact of *Capsicum* extract and lower sodium levels on quality and consumer acceptability of cured-smoked chicken fillets

1.4 Scope of the study

Numerous studies have shown that plant materials such as roots, leaves, fruits etc., have bioactive compounds that can positively influence human health. Also, consumption of foods, especially meat products with high sodium content has been identified to pose health risk to consumers. The aim of this study therefore was to assess the bioactive compounds in methanolic extracts of selected red peppers and evaluate its effect on quality of cured-smoked chicken fillets. The effect of partial sodium chloride replacement, as a means to reduce in-going sodium content in production of chicken fillets was also assessed. The prepared chicken fillets were also assessed by a representative of intended consumers for acceptability.

The scope of this study was however limited in the demography of the respondents, which were only members of the Faculty of Agriculture of the University of Ibadan.

Chapter 2 CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Meat Curing

Meat curing is the process of adding salt to meat to prevent or reduce microbial degradation and extend its shelf life. Fresh cuts of meat may be covered with a salt solution or with the addition of dry salt (Aberle *et al*., 2001). The nitrite used in meat curing ensures the production or "fixing" of the characteristic colour and flavour production associated with cured meats, as well as the extension of the product's shelf life through antioxidant action and the inhibition of *Clostridium botulinum* growth.

2.1.1 Curing Methods

2.1.1.1 Dry Curing

This involves systematic and quantified additions of ingredients such as salt, sugar, spices, and sodium nitrite to meat cuts. In dry form, the curing agents are rubbed on the surface of the beef, which is then allowed to cure in a cool environment. Since no water is used, the curing agents are dissolved in the moisture of the muscle tissue. The incremental incorporation of the cure into the meat over time, due to diffusion and microbial activity on the reduction of nitrate to nitrite, gives the food the distinctive colour and taste of cured meat (Fox, 1974). When curing is complete, the excess cure is rinsed out, and the meat is refrigerated (2 - 4 °C) for 20 to 40 days to allow for thorough salt distribution.

2.1.1.2 Brine Curing

Brine is a water-based formulation made up of Sodium chloride, Sodium nitrite, and seasonings

(spices and herbs). The meat samples are then immersed in brine for a predetermined amount of time. Hams and shoulders, for example, are normally brine-cured for 2 to 2.5 days per pound in 70° brine. Because of the high water activity, microbial growth and spoilage can occur during pickling. This phenomenon, however, may be limited by cooling (Aberle *et al*., 2001).

2.1.2 Sodium Nitrite and Formation of Nitrosamines

Despite its beneficial effects, the use of nitrite in cured meat products is a cause for concern due to its function in the production of nitrosamines in minute amounts under certain circumstances. Nitrosamines have been demonstrated in experiments to be carcinogenic, mutagenic, and teratogenic in laboratory organisms, with epidemiologic data suggesting this according to Tricker and Preussmann (1991). Any heat processing conditions, such as the interaction of nitrite and free amino acids and amines in meat and meat products, result in the formation of nitrosamines. Since the amount of amino acids, amines, and other endogenous influences is difficult to control, it may be necessary to reduce the amount of nitrite used as well as the reaction conditions. As a result, the permitted dose of nitrite in cured meats has been reduced to a maximum range of 150-200 mg/kg in most meat products, with a lower maximum volume of 120 mg/kg in bacon (Food Safety Authority of Ireland, 2010).

2.1.3 Advantages and Disadvantages of Nitrite Use in Curing

2.1.3.1 Colour Characteristics

A meat product's colour plays a significant role in affecting the consumer’s intent and decision to buy. The opinion that some colours affect the acceptance of food has been confirmed by numerous studies. For meat, the amount of haemoproteins, especially myoglobin, and their relationship with the immediate environment decide the colour of the meat (Ledward, 1992). The nitrite addition to meat and subsequent heat treatment produce a relatively permanent pink pigment, without which the substance will be beige, brownish, or tan in appearance. There have been records of the use of a variety of nitrite substitute colourants such as nicotinic acid, erythrosine, radish and beet root extracts, betalane pigments, red peppers etc. (Shahidi and Pegg, 1991). However, their use in the production of meat products has been inhibited because of problems with colour fixation, toxicology, oxidation, or thermal stability.

2.1.3.2 Antioxidant Properties

Nitrite serves in cured meats as an antioxidant, delaying the degradation of lipids. It however cannot play a complete antioxidant role. It needs to be used for efficient antioxidant activity in conjunction with other antioxidants (Shahidi *et al*., 1987). The addition of antioxidants to meat products causes an intermission in the development of autoxidation and rancidity, as well as decolourisation and nutrient depletion. The antioxidant's inhibitory action is due to its ability to donate a hydrogen atom or an electron to a free radical lipid and to form a complex between the antioxidants and the lipid molecule (Dziezak, 1986). Due to increasing awareness of consumers to butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and other synthetic additives used in meat product production, there has been an increasing interest in the use of natural ingredients. Many experiments have shown the components of aromatic plants may act as natural antioxidants, preventing or delaying food lipid rancidity, boosting sensory scores, and increasing food product market acceptability (Nakatani, 1997). The tendency of polyphenolics in raw extracts of plant parts such as spices and herbs to retard oxidative lipid degradation improves the nutritional content of meat and meat products (Amarowicz *et al*., 2004). While certain spices and herbs, or fractions thereof, may have significant antioxidant activity, their practical use in meat and meat products may be restricted due to the pungent or distinctive flavour imparted in the food, as well as their thermal stability. In pork systems, the antioxidant role of selected spices and their oleoresins has been studied. The lipid oxidation levels, as calculated by the amount of thiobarbituric acid reactive substance in extract-treated samples, were lower than in control samples, meaning that these spices shield the meat from lipid oxidation. The defence, however, was concentration-dependent, and after a certain amount of spice was added, a saturation point was reached. The addition of cloves at 500 mg/kg, for example, inhibited lipid oxidation by 96 percent, but this protection remained unchanged even though higher doses were used (Shahidi *et al*., 1995). Clove, rosemary, sage and oregano proved to be very effective in delaying lipid oxidation in pork systems, as thiobarbituric acid reactive substance values were less than 1 g per gram of sample over a 21-day refrigerated storage period.

2.1.3.3 Flavour Characteristics

It is assumed that the use of nitrite in meat curing imparts a distinct cured meat flavour to the meat product. However, the National Academy of Sciences (NAS, 1982) reported that the generation of cured meat taste was most likely attributed to a composite sensation caused by

the input of many odorous compounds. Although no experiments have been performed to ascertain a positive chemical contribution of nitrite to taste, the NAS has reported that nitrite is likely to affect the flavour of cured meat due to its anti-oxidative properties. Since the mechanism of developing the traditional cured-meat flavour is unclear, there is no known nitrite substitute that can reproduce this taste. The amount of salt used in the curing process can also play an important role in determining the overall taste of the product. The role of nitrite to prevent oxidative effects merely retards the breakup of unsaturated fatty acids and the production of secondary lipid oxidation products. It is the key mechanism that could be involved in reducing the formation of oxidation products, which will alter the volatile profile of the cooked cured meats and reveal the unique flavour of the cured products.

2.1.3.4 Antimicrobial Properties

Nitrite has a concentration-dependent antimicrobial activity in cured meat products, including the inhibition of the growth of spores of putrefactive and pathogenic bacteria such as *C. botulinum* (NAS, 1982). Alternatives that guarantee protection from botulinum hazards in abused animals must be supplemented by eliminating or reducing nitrite from meat products. At the same time, the traditional and proven individuality of cured meat products must be preserved. According to Sofos and Busta (1980), any material considered to be a replacement for nitrite should be suitable for use of all cured meat products and should control other microorganisms essential to public health protection, postpone spoilage and degeneration of the product, and not limit the growth of beneficial microorganisms required in the production of fermented meat products, such as lactic acid-producing cultures. Furthermore, the compound of choice must be clean, heat-stable, flavourless, and preferably effective at low concentrations in order to be at least as effective as nitrite.

2.2 Sodium chloride (NaCl)

Sodium chloride, also known as salt, is a chemical compound that is used as the primary flavouring agent in meat and meat products. It also acts as a preservative by inhibiting the development of spoilage microorganisms (Puolanne *et al*., 2001). Sodium chloride is an important source of sodium in human diets. Meat and meat products have up to 20% of sodium intake, equating to about half a gram of sodium or 1.38 g of salt a day (WHO, 2012).

2.2.1 Sodium chloride and health

Sodium is a mineral ingredient that is needed in normal human body functions, and the body requires 184-230 mg of sodium per day to maintain these capabilities. Sodium is the most abundant extracellular fluid cation, and it regulates cell length, water balance, and membrane potential (He and MacGregor, 2007). However, prolonged sodium intake has been related to fluid retention in humans. Many human trials have shown that an elevated sodium consumption is associated with the occurrence of cardiovascular disorders caused by high blood pressure. Sodium chloride use has also been related to an elevated risk of other diseases such as stomach cancer, bone mineral density, and kidney stones (He and MacGregor, 2007). As a result, health governing agencies such as the World Health Organization (WHO) have recommended an amount of regular salt consumption. These recommendations suggest that sodium consumption be limited to an average of 2/2.4 g of sodium per person per day, or 5/6 g of sodium chloride per individual per day (WHO, 2012).

2.2.2 Sodium chloride reduction strategies

For human consumption, the reduction of sodium chloride (NaCl) in food products, in particular meat products, will entail adding less salt to the formulations during processing. To achieve the recommended 5 g of sodium chloride intake per person per day, this reduction can be up to 50 percent of the formula (WHO, 2012). This decrease could result in a decrease in the sensory quality of meat products as well as a decrease in the physicochemical, textural, and safety characteristics of meat products. To maintain the degree of consistency conferred by the addition of sodium chloride to meat products, partial replacement with potential salt replacers or ingredients has been suggested (Desmond, 2006).

Salt replacers are additives used in a product to make up for salt (NaCl) reduction. There are three categories of salt replacers: ingredients with some salty taste (examples of such are mineral salts are potassium chloride, magnesium chloride, magnesium sulphate etc.); ingredients that improve the salty taste but do not have a salty feel (such as glutamate, lysine and yeast extract); and ingredients that prevent harsh or unwanted tastes in products (such as sucrose, yeast extract etc.) (Greiff, 2015). It is also possible to distinguish salt replacers on the basis of i) their effect on the functional properties of the product - mineral salts, including sea salt; ii). preservative effects - lactate and lactate salts; and iii). the taste of the product - sodium glutamate, enzymes, spices and herbs etc. (Greiff, 2015).

2.2.3 Sodium replacement with Potassium Chloride (KCl)

Partially substituting potassium chloride for sodium chloride is a popular method for lowering the sodium content of meat products (Aliño *et al*., 2011). This is attributed to the fact that potassium chloride, among other factors, has been found to have the same antimicrobial effect on disease-causing microorganisms as sodium chloride. The replacement of sodium chloride with more than 50% potassium chloride, on the other hand, would have a detrimental effect on the flavour of the products due to the production of metallic and bitter taste (Desmond, 2006), limiting the use of this salt. A higher potassium intake has health advantages as a result of replacing NaCl with KCl, such as a reduction in the development of hypertension and the incidence of stroke (He and MacGregor, 2001).

2.2.4 Sodium replacement with Magnesium Chloride ($MgCl_2$)

As a sodium ion replacement, magnesium ion can be a safe option in terms of health (Barat *et al*., 2013). However, a major substitution of sodium ion for magnesium ion in meat products may be impractical since $MgCl_2$ may develop an off-flavour. Furthermore, authors have reported that the partial replacement of sodium chloride with magnesium chloride can affect the enzyme function, protein matrix, and texture of the substance (Barat *et al*., 2013; Andreetta-Gorelkina *et al*., 2016). The inclusion of magnesium in brines prevents chloride from penetrating the beef, reducing its water holding capacity and extractable proteins (Barat *et al*., 2013). Currently, magnesium salts are used in reduced amounts in commercial 'low-sodium' salts (Barat *et al*., 2013), and further information on their application in food products is being encouraged.

2.2.5 Sodium substitution with Calcium Chloride ($CaCl_2$)

Other salt alternatives, such as calcium chloride, can also be used to reduce the sodium content of meat products (Aliño *et al*., 2010). When used as a sodium supplement, it has been shown to have a significant impact on the pH, yield, and stability of meat products. Calcium chloride is now being used more often to reduce the hardness of beef. Calcium chloride's tenderizing activity has been due to the activation of calpains (Koohmaraie, 1994) and the rise in protein solubilisation, which causes an increase in intracellular ionic pressure. However, it has been established that the use of calcium chloride modifies certain properties including colour and flavour. Although, this depends on concentration (Lansdell *et al*., 1995).

Similarly, Pérez *et al*. (1998) discovered that using a high calcium salt concentration resulted in a product with altered taste and bitter flavour.

2.2.6 Sodium reduction effect on taste

Sodium chloride enhances the salty taste and general flavour as well as eliminating bitterness (Breslin and Beauchamp, 1995). The ions Na^+ stimulate the buds, while the ions Cl^- give the salty taste. The release of flavour when eating food, according to Mattes (1997), also depends heavily on the nature of the food matrix. However, a step-by-step reduction in salt can result in increased familiarity and acceptance of less salty food.

2.2.7 Sodium reduction effect on microbial food safety

Sodium chloride, because of its food preservation properties, has been particularly essential for humans for thousands of years. Microbial growth causes spoilage or food poisoning during the storage of fresh foods and particularly high water-content foods. In order to reduce salt in foodstuffs, it is very important to maintain product shelf life and avoid developing undesired microorganisms, a task also played by salt (Kilcast and Angus, 2007). The preservative effect of sodium chloride is related to its ability to reduce water activity (a_w) in the product. Increased sodium chloride addition reduces a_w and the probability of microorganism development while decreasing the sodium chloride content would increase the pathogenic and spoilage micro-organisms' growth potential. The preservative effect of sodium chloride through reduction of the a_w of meat products is just one of many factors affecting the shelf life of the products. Other preservative factors are pH decrease, the addition of preservatives, the application of modified atmospheres and the heating and chilled storage to control microorganisms' development (Kilcast and Angus, 2007).

2.3 Red Peppers

Pepper (*Capsicum spp*) comes from South and Central America and is an important economic crop (Pickersgill, 1997). *Capsicum annuum, Capsicum frutescens, Capsicum chinense, Capsicum pubescens, and Capsicum baccatum* are the five domesticated species of the genus *Capsicum*. It is a small perennial shrub with a white or greenish-white corolla, one or two pedicels at each node, and fruit in different sizes and shapes (Doku, 2015). The crop is often characterized by its pungency, which differs by cultivar but is usually higher in smaller fruit types than larger thick-fleshed forms. Pepper matures rapidly, with a maturation period of 3-4

months. In Nigeria, it is cultivated in home gardens and suitable locations near villages, sometimes as an intercrop. It is often cultivated as a monocrop on a wide scale by both small-scale and industrial farmers. Pepper is an important commercial crop grown for processed vegetables, spices, and value-added goods (Kumar *et al*., 2006). It is an essential component of many foods, contributing flavour, colour, vitamins A and C, and pungence. It may be used to relieve fevers, colds, indigestion, constipation, and discomfort (Sokona *et al*., 2013).

2.3.1 Carotenoids in red peppers

Red peppers are known for their pharmacologically active pungent capsaicinoids and carotenoids which serve to boost their dietary appeal. Pepper carotenoids, which include β-carotene, β-cryptoxanthin, lutein, zeaxanthin, antheraxanthin, and violaxanthin, are primarily composed of the unique, potent, and highly stable capsanthin and capsoroubin. These carotenoids, biosynthetically linked to the stages of fruit maturity, are present at various profiles and levels. In addition to phenolics and flavonoids, carotenoids are among red pepper's most essential antioxidants, working synergistically as powerful free radical scavengers (Mercy, 2018). Carotenoids' ability to scavenge free radicals is due to their expanded linear series of conjugated double bonds, which allows resonance to occur while maintaining structural stability (Abbeddou *et al*., 2013). Carotenoid terminated free radicals are generated by exchanging electrons, creating an adduct (attached to the free radical), or contributing hydrogen to produce comparatively stable carotenoid radicals. The number of conjugated double bonds in carotenoids greatly contributes to their function as lipophilic antioxidants. The existence and number of functional groups, such as carbonyl and hydroxyl groups, also influence lipophilicity (González-Ponce *et al*., 2018). With advanced maturation phases, the antioxidant activity of pepper increases as more carotenoids are synthesized (Cervantes-Paz *et al*., 2012).

2.3.2 Plant Polyphenols

Polyphenols are a class of organic compounds with an aromatic ring arrangement and one or more hydroxyl groups. Simple molecules such as phenolic acids are distinguished from heavily polymerized compounds such as condensed tannins. Phenolic acids, flavanoids, and tannins are also essential types of phenolic compounds. Lignans, quinons, coumarins, and stilbenes are examples of subgroups (Pietta, 2000).

2.3.2.1 Extraction of plant polyphenols

Extraction is the use of conventional extraction methods, using selective solvents, to remove bioactive material from plants or animal tissues. Because of the different antioxidant ability of compounds of different polarities, the extraction yield and bioactivity of extracts are highly dependent on the solvent (Moure *et al.,* 2001). The solvent is added to the sample during the extraction processes, and the sample is then collected using a number of appropriate methods. Other approaches include vacuum drying, ultra-filtration, and evaporation.

The degradation of phenolic compounds in the plant matrix and their diffusion into the external solvent medium are two critical steps in the solvent extraction mechanism of phenolic material from its solid hosts (Shi *et al*., 2005).

i. Initial phase

The sample is immersed in the solvent. The solvent flows through the sample's cavities and capillaries due to osmotic forces, filling the plant matrix and dissolving phenolic compounds such that internal concentration rises with time and a gradient of concentration is created (Urquiaga and Leighton, 2000). Polyphenols that have been exposed or degraded during the grinding process are often washed away in this stage.

ii. Diffusion phase

Polyphenols spread to the solution media from the plant matrix. The accumulation of outer phenolics in external media is beginning to grow. This is similar to the first step of a microencapsulated drug's distribution (Urquiaga and Leighton, 2000).

Extraction methods can be enhanced by specifying the required solvent type, temperature, solvent to solid ratio, sample particle size, and solvent viscosity. The method's usefulness can be increased by changing certain parameters to find optimum conditions (Moure *et al*., 2001).

2.4 Genetics and Meat Quality

Meat quality is defined by those traits the consumer perceives as desirable which includes both visual and sensory traits. Also included are credence traits of safety and health and more intangible traits such as ‘clean' and ‘green’ or welfare status of the production system (Becker, 2000). Important visual traits include colour and texture of the meat, fat colour, amount and distribution of fat as well as the absence of excess water (purge) in the tray

(Glitsch, 2000). Once cooked, consumer satisfaction is largely determined by how tender the meat is as well as its flavour/odour and juiciness (Glitsch, 2000). Genetic influence on meat quality is interrelated with environmental factors. While genetics may play a role in meat quality, the phenotypic expression of meat quality traits is dependent upon the environment, both pre- and post-mortem (Froning, 1995). The environmental effects on meat quality are best defined as those not attributable to genetics, and include on-farm, pre-slaughter, and post-slaughter processing factors. Variation in a meat quality trait of interest can also be influenced by genetic-environment (G×E) interactions where the expression of a meat quality genetic trait, or genotype, changes in response to the environment (Warner *et al*., 2010).

Factors affecting meat quality are: visual identification which is based on colour, marbling and water holding capacity, firmness, juiciness, tenderness and flavour (Becker, 2000).

2.4.1 African Chicken Genetic Gain Project

African Chicken Genetic Benefit (ACGG) is a research project carried out in Ethiopia, Nigeria and Tanzania for a development collaboration (Wondmeneh *et al*., 2015). The goal was to establish public-private partnerships to help increase the productivity of chicken for the benefit of smallholders. The project evaluated and disseminated improved chicken breeds that are likely to meet farmers' needs in low-input systems (Wondmeneh *et al*., 2015). In order to show changes in chicken production, growth of household income and consumption and chicken preferences, ACGG along with more than 7,500 smallholder farmers conducted on-farm and on-station germplasm research. Community-level innovation networks have called on women to join hands in finding solutions to their problems, which include feasible models of delivery of service, gaining access to favoured strains of chicken and solutions relating to marketing of the chickens and products. At the same time, the national innovation platform targeted and encouraged the establishment of partnerships between the public and private sector in encouraging and delivering affordable hatching, vaccination, brooding and selling to farmers of accepted chicks (Wondmeneh *et al*., 2015). The ACGG goals, according to Dessie (2016), were:

- Identifying, characterizing and analysing tropically-adapted chicken germplasm to assess its performance under different agro-ecological management conditions
- Defining the preferences of farmers
- Build stable multiplication lines of germplasm preferred by farmers

- Creating templates to facilitate access to germplasm in the private and public sectors through a long-term genetic benefits initiative focused on quality improvement
- Creating and fostering an innovation forum at multiple levels to promote private sector participation and the development of business models aimed at assisting poor smallholder farmers, particularly women in the chicken value chain, in improving their livelihoods

Dessie (2016) reported the identification of ten active and tropically adapted strains, as well as the establishment of a relationship with suppliers. Productivity (egg number), body weight gains and egg weight, adaptability (survival), breed selection/improvement selection, and crossbreeding for improved productivity/adaptability (increased egg production, weight gain, and survival) are all economically significant characteristics of interest in the ACGG.

2.4.2 Funaab Alpha Breeds of Chicken

2.4.2.1 History and Origin

As the need to create more indigenous bird breeds adapted to the climate and immune to endemic tropical diseases, a team at the Federal University of Agriculture, Abeokuta (FUNAAB), headed by Professor Oluwafunmilayo Adebambo created the Funnab Alpha breed of chickens. The Funaab poultry breeding project began in 1994 with the collection of 500 birds from typical feathered, frizzle-feathered, and bare neck indigenous birds in southwestern Nigeria (Adebambo, 2015). The methods of characterization used include biometric data collection and genetic screening for longevity, reproductive success and broodiness removal, with ten generations of selection aimed at the production of enhanced indigenous Nigerian chicken. The bird is referred to as the Funaab Alpha Chickens.

2.4.2.2 Performance of Funaab Alpha Breed of Chicken

In their research on the semen assessment involving the Alpha and other indigenous cocks, Peters *et al*. (2008) stated that genetic variance occurred in the quantity and consistency of the semen, and that the alpha and indigenous cock had comparable results with the exotics. They suggested that as a donor to unusual genes, it could be used in artificial insemination for genetic improvement. Studies by Akanni *et al.* (2009) reported that the Funaab Alpha performed better than the local chicken in terms of egg production and linear measurements

than the local chicken and also that in terms of egg production characteristics and linear body parameters, the Alpha strain had comparable results to exotic ISA Brown chickens. Sonaiya (2015) suggested that the first-lay egg weight of the Funaab Alpha was 46 g.

There are also other breeds developed by the Funaab researchers, namely Funaab Alpha broiler, Funaab Alpha layer white, Funaab Alpha black, female and male; Alpha gold cock, Alpha brown females, Alpha blue females, Alpha blue cock, Alpha naked neck cock, Alpha naked neck females, Alpha white cocks, Alpha white females, Alpha barred cock, Alpha barred females, Alpha frizzle females (Adebambo, 2015).

One of the chicken genotypes adopted by the African Chicken Genetic Gains (ACGG) Project is the Funaab Alpha race of chickens. On-station testing of currently available lines began in June 2015 at Nigeria's Federal University of Agriculture, Abeokuta and Obafemi Awolowo University. On-farm study involved 2,700 rural households from five agro-ecological zones distributed across ten states (Adebambo, 2015). Farmers preferred that updated, productive chickens be selected for marketing in order to increase women's economic participation and boost nutrition and health (Adebambo, 2015).

Chapter 3 CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location of study

The research was conducted at the Animal Products and Processing Laboratory of the Department of Animal Science, Faculty of Agriculture, University of Ibadan.

3.2 Experiment One: Chemical Analysis of Red Pepper (*Capsicum* sp.) Extracts

3.2.1 Collection of Test Ingredients

Matured fresh red pepper (*Capsicum* sp.) varieties were collected randomly from Bodija market in Ibadan, Oyo State. Red pepper samples were carefully washed in purified water before being cut and dried in a thermostat oven at 60^{o}C until crispy with constant weight. Dried samples were thereafter finely ground and extracted using methanol at ratio 1:5 (1 gram dried pepper in 5 mL methanol) as described by Nkambule (2008).

3.2.1.1 Varieties of red peppers used

- *Capsicum annuum* (English name: Bell pepper; Local name: Tatase) (Plate 3.1)
- *Capsicum frutescens* (English name: Bird pepper; Local name: Ata wewe) (Plate 3.2)
- *Capsicum annuum* (English name: Cayenne pepper; Local name: Bawa) (Plate 3.3)
- *Capsicum chinense* (English name: Scotch bonnet; Local name: Ata rodo) (Plate 3.4)

Plate 3.1: Bell pepper (*Capsicum annuum*) fruits

Plate 3.2: Bird pepper (*Capsicum frutescens*) fruits

Plate 3.3: Cayenne pepper (*Capsicum annuum*) fruits

Plate 3.4: Scotch bonnet (*Capsicum chinense*) fruits

3.2.2 Assays of Bioactive Properties of Red Pepper Extracts

3.2.2.1 Antioxidant Assay of Red Pepper Extracts

Determination of Total Phenol Content

The procedure reported by Makkar *et al*. (1993) was used. One (1) mL of pepper extract was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and 2 mL of Na_2CO_3 (75 percent w/v) (0.5 mL). The resulting mixture was vortexed for 15 seconds before incubation at 40°C for 30 minutes to develop colour. A spectrophotometer (Model: Jenway 6305 Spectrophotometer, UK) set at 765 nm wavelength was used to measure the sample absorbance. Using the equation: $Y = 0.1216x$, $R^2 = 0.9365$, total phenolic content was calculated as mg/g tannic acid equivalent from the calibration curve. Where 'x' represented absorbance and 'Y' represented tannic acid equivalent (mg/g). The procedure was done three times.

Determination of Total Flavonoids

The total flavonoid content of the solution was estimated using the Ordoñez *et al*. (2006) method, which is based on the formation of a flavonoid-aluminium complex. A 0.5 mL amount of 2 percent $AlCl_3$ ethanol solution was added to the 0.5 mL extract solution. A UV-VIS spectrophotometer was used to measure absorbance at 420 nm after 1 hour of incubation at room temperature. All measurements were taken in triplicate, and the values were calculated using the equation: $Y = 0.0255x$, $R^2 = 0.9812$ from the quercetin calibration curve. Where 'x' was the absorbance and 'Y' was the quercetin counterpart (mg/g).

Determination of Total Flavonols

The total flavonol content was estimated using the method defined by Ordoñez *et al*. (2006). In the reacting mixture, 2 mL of the sample, 2 mL of $AlCl_3$ prepared in ethanol, and 3 mL of sodium acetate solution (50 g/L) were used. The absorption at 440 nm was measured after 2.5 hours at room temperature. Using the equation: $Y= 0.0255x$, $R_2 = 0.9812$, the total flavonoid content of the calibration curve was measured as equal to quercetin (mg/g). Where 'x' represented absorbance and 'Y' represented the equivalent of quercetin (mg/g).

Determination of 1,1-diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity

The extracts' free radical scavenging activity was determined using the approach defined by Braca *et al*. (2003), based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The red pepper extract (0.1 mL) was thoroughly ground with 3 mL of DPPH 0.004 percent MeOH solution. After 30 minutes, absorbance at 517 nm was measured with a spectrophotometer and the percentage of inhibition activity was estimated by multiplying $[(A_0-A_1)/A_0]$ by 100.

If A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/standard.

3.2.2.2 Antimicrobial Assay of Red Pepper Extracts

Determination of Zone of Inhibition of Extracts

The antimicrobial activity of red pepper extracts was evaluated using Riazi *et al*. (2015) agar diffusion process. *Escherichia coli, Staphylococcus aureus, Bacillus subtilis,* and *Pseudomonas aeruginosa* were the four bacterial cultures used. These bacteria cultures were used to make slants, which were then incubated at 32°C for 24 to 48 hours. To prepare the suspension for each culture, loops from each slant culture were transferred individually into a 5 mL sterile saline solution tube. The suspensions were then moved separately into 150 mL of sterile SCDA after cooling to 40-45°C. Every conical flask containing SCDA and culture suspension was shaken to ensure a uniform distribution of microbial cells in the medium. After shaking, each SCDA medium with culture suspension was poured onto four plates, labelled, and allowed to solidify. Following the solidification of the medium in the vessels, the wells were cut into each plate with a sterile borer. 0.1 mL of red pepper extracts to be tested were poured into each well, and the plates were incubated at 32 °C for 24 hours. After incubation, the plates were examined for the existence of an inhibition region (mm). If an inhibition region was present, the diameter was measured with a Vernier Calliper.

3.2.2.3 Estimation of Pigmentation of Red Pepper Extracts

Red Pigment, Yellow Pigment and Total Carotenoids Determination

Samples (1 g) of red pepper powders were repeatedly extracted with cool acetone (1:2 w/v) until the samples lost their colour. All extracts were thoroughly mixed and an aliquot (3 mL) was taken to determine the pigmentation level using a spectrophotometer. Red and yellow pigments were determined at 473nm and 423nm respectively while total carotenoid was determined at 450 nm. Total pigment yield was calculated according to the following:

$$TC\,(mg/100g) = \frac{A \times y(mL) \times 10^6}{A^{1\%}_{1cm} \times 1000 \times w}$$

Where A was the absorbance value of extract, y was the volume of extract, % 1*cm A* = 2,500 was the extinction coefficient of carotenoids, and w was the weight of red peeper powder (g) (Wang and Liu, 2009).

3.2.3 Experimental design and statistical analysis

The experimental design was a completely randomised design. Data were subjected to analysis of variance using SAS (2012) package and means separated using Duncan Multiple Range Test.

3.3 Experiment Two: Effects of *Capsicum* sp. Extract on Quality of Cured-Smoked Chicken Fillets

3.3.1 Source of Meat

Fresh chicken meat was obtained from matured (20 weeks old) *Funaab Alpha II* birds, an improved breed of indigenous chickens. The birds were purchased from the Teaching and Research Farm of the Federal University of Agriculture, Abeokuta, Nigeria. The birds were slaughtered, dressed and manually deboned to obtain the meat used for the study.

3.3.2 Production of Cured Smoked Chicken Fillets

Batches of chicken meat were cured for 48 h at 4 ^{o}C in a curing solution containing increasing levels of bell pepper extract (Table 3.1) which had the most acceptable antioxidant, antimicrobial and colour imparting properties from study one. The cured meat was thereafter slightly rinsed with distilled water, drained and hot-smoked at 60-80^{o}C for 1-2 h. The smoked chicken meat was allowed to cool at room temperature, sliced and vacuum-packed using a Vacuum sealer and vacuum-grade nylon (Figure 3.1). These were thereafter stored on shelf, at room temperature for 60 days. Quality parameters such as pH, cooking loss, water holding capacity etc. were measured on stored cured-smoked chicken fillets on day 0, 15, 30, 45 and 60 of storage.

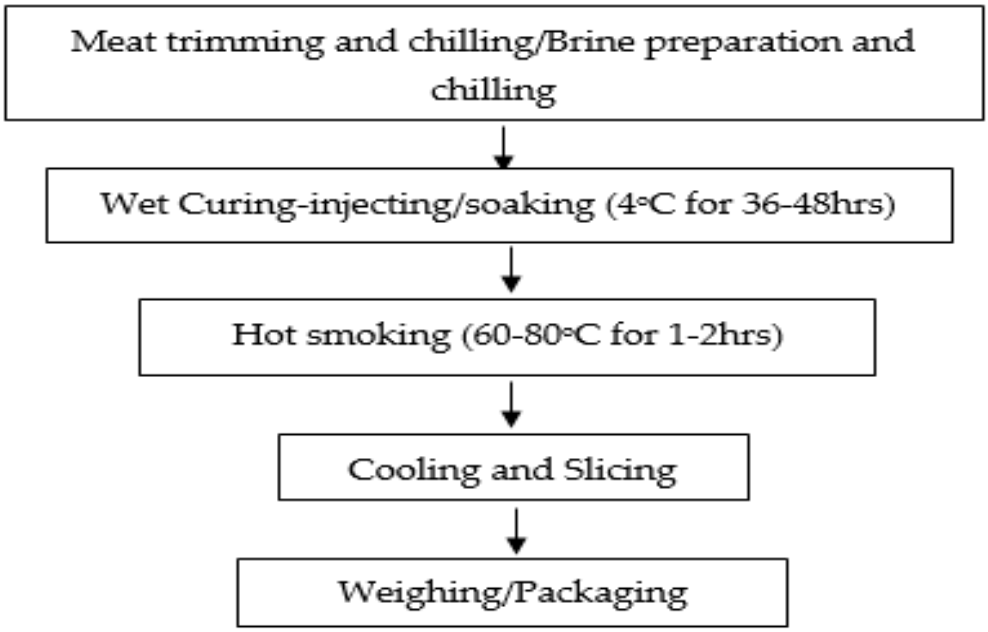

Figure 3.1: Flow chart for the production of cured-smoked chicken fillets (Heinz and Hautzinger, 2007)

Table 3.1: Composition (%) of curing mixture for chicken fillets with varied *Capsicum* extract levels

	Treatments						
Ingredients	**A**	**B**	**C**	**D**	**E**	**F**	**G**
Meat (g)	67.00	67.00	67.00	67.00	67.00	67.00	67.00
Water (mL)	24.985	25.00	24.85	24.70	24.55	24.40	24.25
Salt (g)	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Sugar (g)	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Spices*(g)	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Nitrite (g)	0.015	____	____	____	____	____	____
Cap. Extract (mL)	____	____	0.15	0.30	0.45	0.60	0.75
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Cap. Extract: Capsicum Extract

***Spices: Thyme (15%), Nutmeg (7.5%), Clove (15%), Garlic (10%), Monosodium glutamate (12.5%), Onion (20% wet basis), Ginger (20% wet basis)**

Table 3.2: Temperature-humidity readings during 60-day storage of smoked chicken fillets cured with increasing levels of *Capsicum* extract

Parameters	Mean ± SD	Minimum	Maximum
Temp (Morning- ºC)	29.26±1.29	25.00	31.50
Temp (Evening- ºC)	29.84±1.07	27.00	32.20
RH (Morning- %)	49.78±9.01	24.00	72.00
RH (Evening- %)	50.77±9.45	24.00	72.00

Temp- Temperature; RH: Relative humidity; SD: Standard Deviation

3.3.3 Parameters measured

3.3.3.1 pH

The pH was measured with a spear probe pH meter calibrated with buffers at pH 4.0 and pH 7.0. Measurements were made in triplicates for each treatment.

3.3.3.2 Cooking loss

Cooking loss was determined according to the procedure described by Mahendrakar *et al*. (1988). Cured chicken meat samples from each batch were weighed before smoking for 1-2 h and thereafter weighed after smoking and cooling at room temperature. Cooking loss was calculated using the formula:

$$\text{Cooking loss \%} = \frac{\text{weight of sample before cooking - weight of sample after cooking}}{\text{weight of sample before cooking}} \times 100$$

3.3.3.3 Product yield

Product yield was calculated using the following formula:

$$\text{Yield \%} = \frac{\text{Weight of Product}}{\text{Initial Weight of Sample}} \times 100$$

3.3.3.4 Water holding capacity

The water-holding capacity (WHC) was determined by the method of Zayas (1997) and was calculated as follows:

$$\text{WHC \%} = \frac{\text{Meat film area}}{\text{Area of spread juice}} \times 100$$

Briefly, using a table device, one (1) gram chicken fillet sample was pressed between two filter papers with a plexi glass for more than 1 minute. The quantity of juice emitted from the sample was determined indirectly by calculating the area of wetted filter paper compared to the area of the sample being pressed.

3.3.3.5 Warner Bratzler Shear Force determination

Parallel to the muscle fibre orientation, three (3) 1.27-cm-diameter cores were cut, and each core was sheared once with a Warner-Bratzler Shear Force (WBSF) system equipped with a 50 kg tension/compression load cell. Readings per treatment, determined in triplicates, were reported accordingly.

3.3.3.6 Lipid oxidation

This was carried out with modifications according to the method described by Deuri *et al*. (2016). Ten (10) g of the sample was thoroughly ground, followed by the addition of 25 mL of 20% trichloroacetic acid (TCA) and 20 mL of distilled water. For 2 minutes, the mixture was thoroughly homogenized and then filtered using Whatman filter paper (No 1). The filtrate was blended with 0.02 M thiobarbituric acid (TBA) at an equivalent volume and incubated for 35 minutes at 100 ^{o}C. It was then cooled for 10 minutes under running tap water. Solution absorbance was measured using a UV-VIS Spectrophotometer at 532 nm.

3.3.3.7 Analytical colour analysis

This was carried out as defined by Skiepko *et al.* (2016). In brief, colour readings (CIELAB L*-Lightness, a*-redness and b*-yellowness) were taken using a colorimeter in triplicates with an aperture size of 40 mm, illuminant D_{65} and a regular observer of 10^{0}. Using the given standard white ceramic guide, the colorimeter was calibrated. During the tests, all samples were at room temperature.

3.3.3.8 Volatile basic nitrogen

A sample (10 g) was minced with 100 mL of distilled water, washed with 100 mL of distilled water, and then 2 g of magnesium oxide and an antifoaming agent were applied to the distillation flask. The blend was distilled with the aid of a micro-Kjeldahl distillation apparatus. 4% boric acid and 5 drops of Tashero indicator were collected into a 25 mL distillate after distillation for 25 minutes. The solution was titrated with (0.1 M) HCl to determine the total volatile basic nitrogen in the sample (mg VBN/100g sample) (Pearson, 1976).

Tashero indicator is a pH indicator (pH: 4.4-6.2) consisting of 0.1% methylene blue and 0.03% methylene red solution in ethanol or methanol.

3.3.3.9 Microbial examination of stored cured-smoked chicken fillets

Culture media: Fastidious Anaerobe Agar and De Man Rogosa Sharpe (MRS) agar

Total anaerobic plate count and Lactic acid bacteria counts on Fastidious Anaerobe Agar and De Man Rogosa Sharpe (MRS) agar, respectively, were determined as recommended by the American Public Health Associations for Foodstuff Review (APHA, 1992).

Medium Preparation: Sufficient quantities of culture media were weighed into 1000 mL conical flasks as indicated by the manufacturers' guide. To dissolve the media, distilled water was added and the mixture was put in a hot water bath and continuously stirred until a homogenized solution was obtained. It was then firmly corked and autoclaved for 15 minutes at 121^{o}C. At room temperature, it was then allowed to cool.

The medium was then poured and gently swirled into the petri dishes containing drops of serial dilution samples and then allowed to gel. The anaerobic incubation was at 37^{0}C for 72 hours.

Serial dilution: One (1) gram of sample fillet was applied to 9 mL of distilled sterile water. This is the original dilution. Then 1 mL was moved to a second tube from the first dilution (10^1) (the tube contains 9 mL of sterile distilled water), then the 2nd dilution (10^2) till the 5th dilution (10^5). One (1) mL was then pipetted from dilution 10^3 and 10^5 test tubes to inoculate the sample and transferred drop-wise to the sterile petri-dishes. According to the pour plate process, the inoculation was performed where the sample was first placed into the petri dishes and then 15 mL of agar was poured into the plate. By gentle rotation of the petri-dishes, agar and sample were thoroughly mixed.

Plate counting: Plate counting was carried out by counting the number of developed discrete colonies. The following formula was used to measure the quantitative enumeration of the species as colony-forming units per gram:

$$\frac{\Sigma c}{\text{Inoculum size}} \times \text{Dilution factor}$$

Where: Σc= number of colonies

Dilution factor = reciprocal of total dilution

3.3.3.10 Sensory analysis

A ten-member trained panellists evaluated the prepared cured-smoked chicken fillet samples for different sensory quality attributes using a 3-point descriptive scale (Baston and Barna, 2010), vis external quality attributes - slime formation (3 = without slime; 1 = slime on all surfaces) and microbial growth (3 = not visible; 1 = visible on all surfaces), odour (3 = normal/characteristic; 1 = foreign/rancid/putrid), colour (3 = pink to light red; 1 = dark brown), muscular elasticity (3 = fast return; 1 = no return) and overall quality (3 = excellent; 1 = unacceptable) on day 0, 15, 30, 45 and 60 of storage.

3.4 Experiment Three: Effects of Sodium Chloride Replacement on Quality of Cured-Smoked Chicken Fillets

3.4.1 Source of Meat

Same as experiment two

3.4.2 Production of Cured-Smoked Chicken Fillets

This was carried out as in experiment two following the production flow chart (Figure 3.1). However, in this study, batches of chicken meat were cured for 48 h at 4 ^{o}C in curing solution containing different combinations of chloride salts as presented in Table 3.3. The best inclusion level for pepper extract **(0.45% - treatment 5)** from experiment two was used in this and further experiments. Other production processes remained the same as in experiment two and quality parameters were measured on stored cured-smoked chicken fillets on day 0, 15, 30, 45 and 60 of storage.

3.4.3 Parameters measured

Quality parameters such as pH, cooking loss, water holding capacity etc. were measured as stated in experiment two. However, fatty acid determination and mineral analysis were further carried out on fillets in experiment three.

3.4.3.1 Determination of Fatty acid profile

This was achieved using techniques defined by Sahi *et al*. (2019). Two (2) g of the sample were weighed into a 100 mL conical flask and added 20 mL of benzene, while shaking thoroughly to remove the fatty acids. In a 250 mL separating funnel, the mixture was transferred and 2 mL of 10 percent copper acetate solution was applied to isolate the benzene extract. In the range of 0-10ppm, the standard solution of each fatty acid was prepared and the absorption concentration of the various standard solutions of each particular fatty acid and the sample benzene extracts were read on a spectrophotometer at a wavelength specified for each fatty acid as follows: Lauric Acid (672nm), Stearic Acid (650nm), Palmitic Acid (630nm), Oleic Acid (670nm), Linoleic Acid (660nm), Linolenic Acid (680nm), Arachidonic

Acid (690nm), Behemic Acid (615nm), Palmitoleic Acid (625nm), Myristic Acid (635nm), Caprylic Acid (645nm), Margaric (674nm)

The % fatty acid was obtained using the formula:

$$\% \text{ Fatty acid} = \frac{\text{Absorbance of sample x gradient factor x dilution factor}}{\text{Weight of sample taken X } 10000}$$

3.4.3.2 Determination of Mineral Composition

The mineral composition of the fillet samples (Sodium - Na, Magnesium - Mg, Calcium - Ca and Potassium - K) was determined using the AOAC (2012) method. Two (2) g of the sample were weighed in a crucible and ashed for 6 hours in a muffle furnace at 550^0C. The ash was cooled and 5 ml of 30 percent HCl was added and boiled for 10 minutes, while a glass watch protected the crucible. The sample was cooled and filtered into a 100-ml volumetric flask after boiling. The crucible was washed with purified water and the washing added to the ash filtrate. Distilled water was then added to the ash filtrate to make up 50 mL. An aliquot of the ash filtrate was aspirated into the atomic absorption spectrophotometer-AAS which was used to determine the absorption values corresponding to the various minerals. Also, standard solutions of the minerals were prepared and aspirated into the AAS and their absorption values reported. The percentage of elements in the samples was recorded from the sample absorption and standard solution values.

Table 3.3: Inclusion levels of chloride salts in curing media for cured-smoked chicken fillets

INGREDIENTS	A	B	C	D	E	F	G	H
Sodium chloride (NaCl)	2.50	1.25	1.25	1.25	1.25	1.25	1.25	0.675
Calcium chloride ($CaCl_2$)	__	1.25	__	__	0.675	0.675	__	0.675
Potassium chloride (KCl)	__	__	1.25	__	0.675	__	0.675	0.675
Magnesium chloride ($MgCl_2$)	__	__	__	1.25	__	0.675	0.675	0.675

A- 100%NaCl

B- 50%NaCl + 50%$CaCl_2$

C- 50%NaCl + 50%KCl

D- 50%NaCl + 50%$MgCl_2$

E- 50%NaCl + 25%$CaCl_2$ + 25%KCl

F- 50%NaCl + 25%$CaCl_2$ + 25%$MgCl_2$

G- 50%NaCl + 25%KCl + 25%$MgCl_2$

H- 25%NaCl + 25%$CaCl_2$ + 25%KCl + 25%$MgCl_2$

Table 3.4: Inclusion levels of chloride salts in curing media for cured-smoked chicken fillets

Parameters	Mean ± SD	Minimum	Maximum
Temp (Morning- ºC)	29.22±0.17	25.40	32.10
Temp (Evening- ºC)	30.01±0.17	25.40	32.20
RH (Morning- %)	49.30±1.25	24.00	65.00
RH (Evening- %)	49.35±1.14	24.00	65.00

Temp- Temperature; RH: Relative humidity

3.5 Experiment Four: Consumer Acceptability and Keeping Quality of Cured Smoked Chicken Fillets Developed with *Capsicum* spp. Extract and Reduced Sodium Level

3.5.1 Source of Meat

Same as previous experiments

3.5.2 Production of Cured Smoked Chicken Fillets

This followed the production flow chart used in previous experiments. Chicken fillets was prepared with the curing formulation of the preferred treatment selected from experiment three, that is curing solution containing **50%NaCl, 25%KCl and 25%$MgCl_2$ (treatment 7).**

3.5.3 Experiment One: Keeping quality of cured-smoked chicken fillets developed with Capsicum sp. extract and reduced sodium level

Prepared chicken fillets were vacuumed-packed and stored under two storage conditions to determine its keeping quality over an extended storage period of 90 days (Aaslyng *et al.*, 2014). Quality parameters were assessed on day 0, 15, 30, 45, 60, 75 and 90 of storage.

3.5.3.1 Experimental layout

Treatment 1: Product stored at refrigerated temperature

Treatment 2: Product stored at room temperature

3.5.3.2 Parameters measured

Quality parameters such as pH, cooking loss, water holding capacity, colour analysis etc. were measured on stored chicken fillets as stated in experiment two.

3.5.4 Experiment Two: Consumer acceptability of cured-smoked chicken fillets developed with *Capsicum* spp. extract and reduced sodium level

A total of eighty-four (84) respondents consisting of students and faculty members in the Faculty of Agriculture, University of Ibadan were recruited to participate in the consumer acceptability assessment study. Evaluation of the chicken fillets was carried out using a 9-point hedonic scale (Colour: 1 = extremely dark/red; 9 = extremely light/pale; flavour-smoky: 1 = not perceptible 9 = extremely intense; hotness: 1 = extremely hot; 9 = extremely mild; tenderness: 1 = extremely tough; 9 = extremely tender; juiciness: 1 = extremely dry; 9 = extremely juicy and overall acceptability: 1 = dislike extremely; 9 = like extremely) and a structured questionnaire (Person *et al.,* 2005).

Respondents were required to give information on their demography, general perception and consumption of meat products, sensory evaluation of freshly prepared chicken fillets, willingness to buy/pay for the fillets etc.

3.6 Experimental design and statistical analysis

The experimental design was completely randomised design with treatments effect assessed on each storage day using a one-way variance analysis (ANOVA) of SAS (2012) package. Duncan Multiple Range Test of the same software was used to determine significance between treatment means.

Data were further subjected to PROC MIXED Procedure of SAS (2012) package with specified treatment and storage day effects to determine interaction effect of the independent variables (treatment and storage day). A Microsoft Excel Data Analysis Package was thereafter used for a Regression Analysis and a second-degree polynomial function was fitted to the storage day independent variable to determine its relationship to the dependent variables measured.

Data for consumer acceptability study were subjected to descriptive analysis and one-way ANOVA using SAS (2012) package and means separated using Duncan Multiple Range Test of the same software.

Chapter 4 CHAPTER FOUR

4.0 RESULTS

4.1 Experiment One: Chemical Analysis of Red Pepper (*Capsicum* sp.) Extracts

4.1.1 Antioxidant activities of extracts of selected red peppers

Total phenol, flavonoid and flavonol contents of red pepper extracts is shown in Figure 4.1. Significant ($p<0.05$) effect of treatments (pepper types) were observed for only the total phenol content of the red pepper extracts while total flavonoid and total flavonol contents were not significantly ($p>0.05$) affected. Highest total phenol was observed in Bird pepper (0.48 mg/g) followed by Cayenne pepper (0.42 mg/g) while least value was obtained in Scotch bonnet (0.29 mg/g). Cayenne pepper had highest total flavonoid content of 0.054 mg/g, followed by Scotch bonnet (0.047 mg/g) and least in Bell pepper (0.044 mg/g). Total flavonol was highest in Cayenne pepper (0.05 mg/g) and least in Scotch bonnet (0.025 mg/g). Significant effect ($p<0.05$) of treatments (pepper types) was observed for the 1, 1-diphenyl 2-picrylhyrazyl (DPPH) radical scavenging activity of the pepper extracts (Figure 4.2). Bell pepper had highest value of 85.17% followed by Cayenne pepper (52.03%) and least value was observed in bird pepper which had a value of 34.90%.

4.1.2 Antimicrobial activity of extracts of selected red peppers

Antimicrobial activity of red pepper extracts (Figure 4.3) against selected bacteria species showed that Scotch bonnet had the highest zone of inhibition of 25.00 mm against *Escherichia coli (E. coli)*, followed by Bird pepper (19.50 mm) and Bell pepper (15.50 mm). Cayenne pepper however had no inhibition against *E. coli*. For *Bacillus subtilis* (*B. subtilis*), Bell pepper had the highest inhibition value (23.50 mm) followed by Scotch bonnet (21.50 mm) and Cayenne pepper (19.50 mm) while Bird pepper had the least zone of inhibition. Highest zone of inhibition was observed for *Staphylococcus aureus* by Scotch bonnet (27.50 mm), closely followed by Bird pepper (27.00 mm). Bell pepper had a value of 23.50 mm

while Cayenne pepper however did not inhibit its growth. Lastly, highest zone of inhibition was observed against *Pseudomonas aeruginosa* by Bird pepper (22.50 mm), followed by Scotch bonnet (21.50 mm) and Bell pepper (20.00 mm). Also, Cayenne pepper failed to inhibit the growth of *P. aeruginosa.*

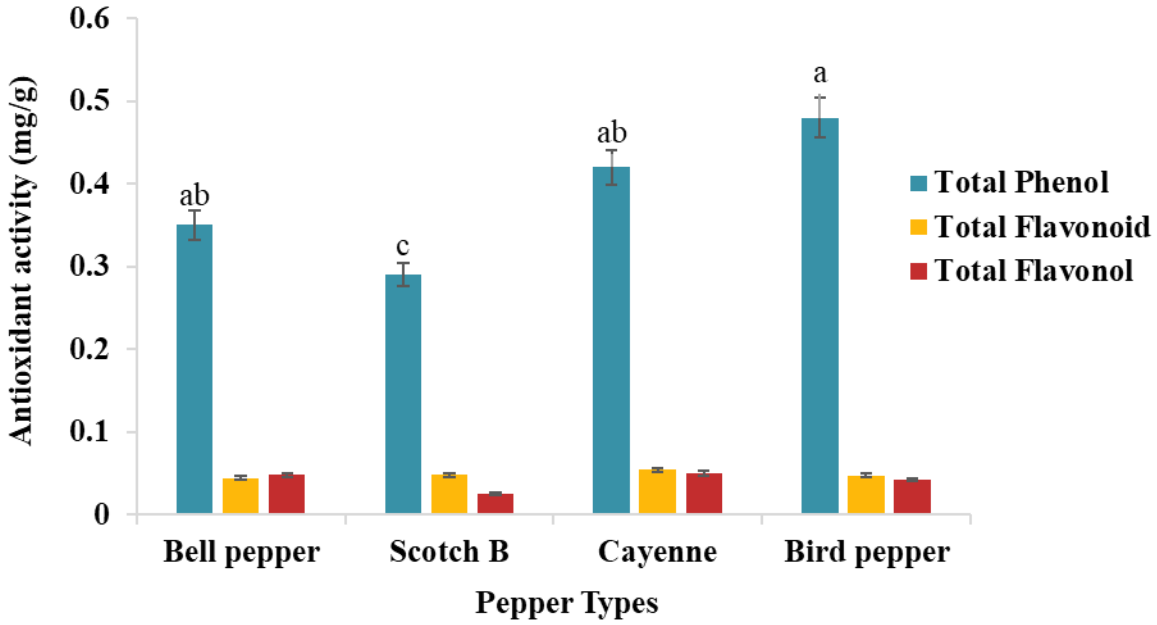

Figure 4.1: Total phenol, flavonoid and flavonol contents of red pepper extracts

Scotch B: Scotch Bonnet pepper; Cayenne: Cayenne pepper

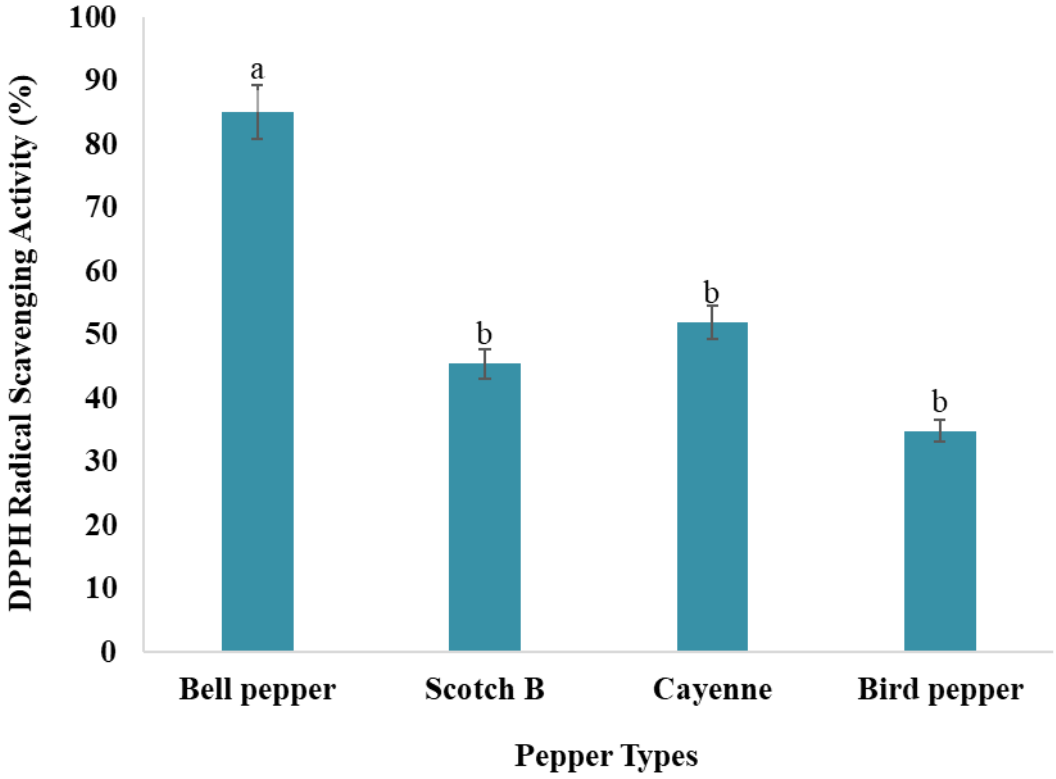

Figure 4.2: DPPH radical scavenging activity of red pepper extracts

Scotch B: Scotch Bonnet pepper; Cayenne: Cayenne pepper; DPPH: 1, 1-diphenyl 2-picrylhyrazyl

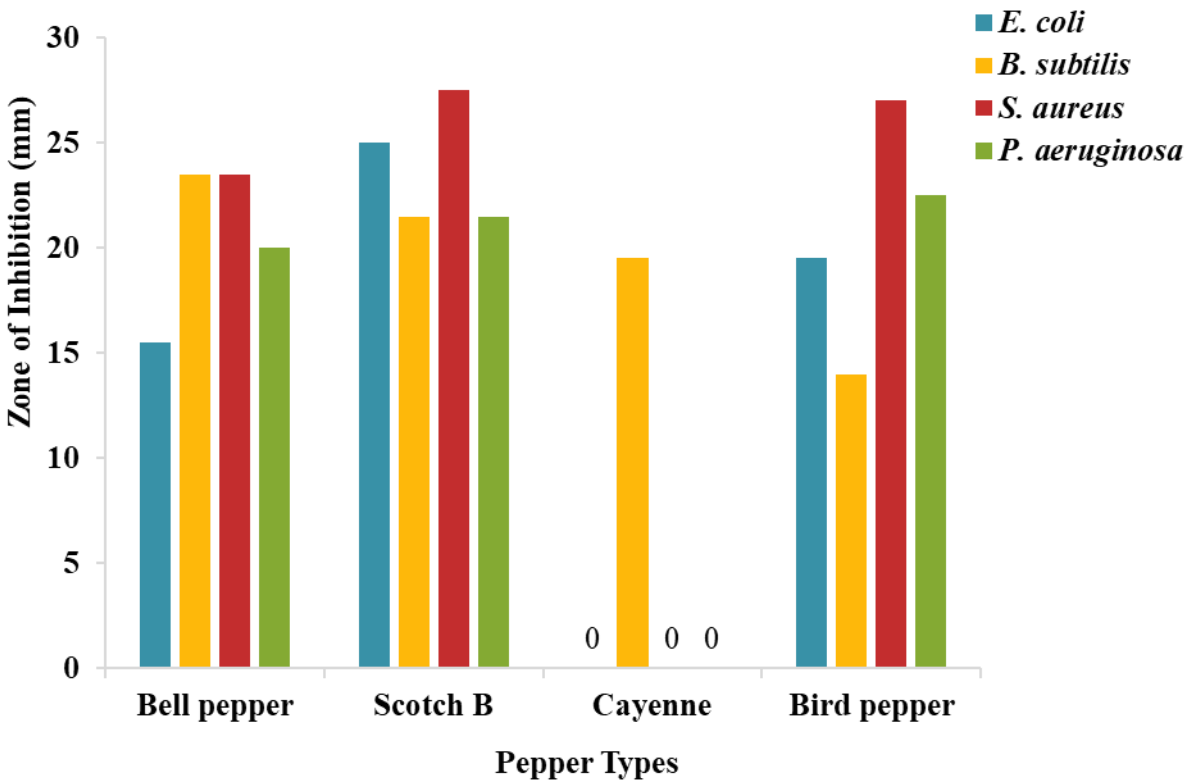

Figure 4.3: Antimicrobial activity of red pepper extracts

Scotch B: Scotch Bonnet pepper; Cayenne: Cayenne pepper

E. coli: Escherichia coli; B. subtilis: Bacillus subtilis; S. aureus: Staphylococcus aureus; P. aeruginosa: Pseudomonas aeruginosa

4.1.3 Pigmentation level of selected red pepper extracts

Figure 4.4 shows the pigmentation level of red pepper extracts. Significant effect ($p<0.05$) of treatments (pepper types) was observed for all parameters measured. Total carotenoid was highest ($p<0.05$) in Cayenne pepper (43.63 mg/100g) and least in Bird pepper (22.32 mg/100g). Bell pepper had a value of 37.85 mg/100g while Scotch bonnet had 33.02 mg/100g. Yellow pigment was highest ($p<0.05$) in Cayenne pepper (44.07 mg/100g), followed by Bell pepper (40.36 mg/100g) and Scotch bonnet (39.81 mg/100g). Least value was observed in Bird pepper (30.00 mg/100g). Red pigment was observed to be highest ($p<0.05$) in Cayenne pepper (41.64 mg/100g) and least in Bird pepper (17.88 mg/100g). Bell pepper had a value of 36.15 mg/100g while Scotch bonnet had 26.53 mg/100g.

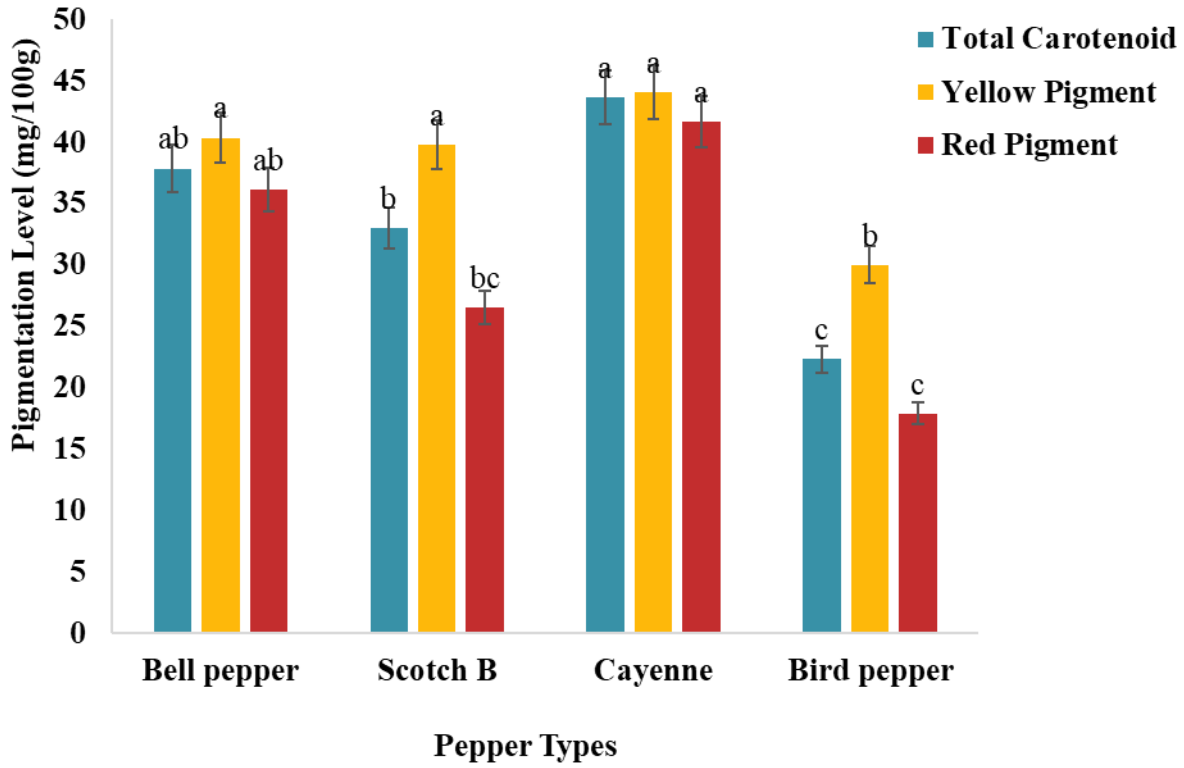

Figure 4.4: Pigmentation level of red pepper extracts

Scotch B: Scotch Bonnet pepper; Cayenne: Cayenne pepper

4.2 Experiment Two: Effects of Bell Pepper Extract on Quality of Ready-To-Eat Cured-Smoked Chicken Fillets

4.2.1 Physical properties of freshly prepared cured-smoked chicken fillets

Physical properties of cured-smoked chicken fillets freshly prepared with increasing levels of bell pepper extract is presented in Table 4.1. Significant effect of treatment was observed for cooking loss and yield of the products. Treatment 5 (0.45% extract) had highest ($p<0.05$) cooking loss value of 55.71% while treatments 1 (nitrite), 4 (0.30% extract) and 7 (0.75% extract) had ($p<0.05$) least percentage values of 51.37, 51.55 and 51.01 respectively. Product yield (Table 4.1) was also significantly affected by the curing treatments and highest ($p<0.05$) percentage yields were observed in treatments 1 (48.63), 4 (48.45) and 7 (48.99). Least percentage value of 44.29 was obtained in treatment 5.

4.2.2 Physicochemical properties of stored smoked chicken fillets cured with bell pepper extract

Physicochemical properties of stored cured-smoked chicken fillets (Table 4.2) showed a gradual significant decrease in pH and shear force values over storage, while water holding capacity increased ($p<0.05$) gradually up to day 45 after which a gradual ($p<0.05$) drop was observed till day 60 of storage. The pH reduced from 6.06 in treatment 6 on day 0 to 5.12 in treatment 1 by day 60 of storage. Water holding capacity of stored fillets increased from 26.30 on day 0 to 66.06 on day 45, then a reduction occurred for all treatments by day 60. Shear force of fillets was significantly highest (2.48) on day 0 in treatment 1 and it reduced ($p<0.05$) to 0.69 on day 60 in treatment 7. A significant ($p<0.05$) treatment and storage day interaction was observed for physicochemical properties of stored cured smoked fillets. A positive correlation of storage period with pH (Figure 4.5) and shear force (Figure 4.7) of fillets was also observed for all treatments. Regression coefficients ranged from 0.57 in treatment 3 to 0.95 in treatment 6 for pH while least and highest regression coefficients were observed for treatment 7 (0.32) and treatment 1 (0.77), respectively. Regression coefficients for water holding capacity (Figure 4.6) of the fillets was also positively correlated, however only a slight correlation of storage duration and water holding capacity was observed for treatment 2 (0.07) while treatment 6 has the highest coefficient of 0.67.

Table 4.1: Physical properties of freshly prepared cured-smoked chicken fillets

Parameters (%)	1	2	3	4	5	6	7	SEM
Cooking loss	51.37^{c}	53.98^{b}	54.58^{b}	51.55^{c}	55.71^{a}	54.29^{b}	51.01^{c}	0.39
Product yield	48.63^{a}	46.02^{b}	45.42^{b}	48.45^{a}	44.29^{c}	45.71^{b}	48.99^{a}	0.39

$^{a,b,c\cdots}$- Rows with different superscripts indicate significant ($p<0.05$) variations in means

SEM - Standard Error of Mean

1 - Nitrite

2 - 0% nitrite/Bell pepper extract

3 - 0.15% Bell pepper extract

4 - 0.30% Bell pepper extract

5 - 0.45% Bell pepper extract

6 - 0.60% Bell pepper extract

7 - 0.75% Bell pepper extract

Table 4.2: Physicochemical properties of smoked chicken fillets cured with bell pepper extract during storage

Parameters	Storage day	Treatments						
		1	**2**	**3**	**4**	**5**	**6**	**7**
pH	**0**	6.01^{a}	5.91^{a}	5.88^{a}	6.01^{a}	5.97^{a}	6.06^{a}	5.92^{a}
	15	5.68^{b}	5.62^{bc}	5.82^{ab}	5.27^{cd}	5.76^{b}	5.72^{b}	5.70^{b}
	30	5.47^{c}	5.55^{c}	5.45^{c}	5.46^{b}	5.51^{c}	5.52^{c}	5.37^{c}
	45	5.56^{bc}	5.78^{ab}	5.15^{d}	5.13^{d}	5.32^{d}	5.38^{d}	5.24^{d}
	60	5.12^{d}	5.91^{a}	5.60^{bc}	5.43^{bc}	5.45^{c}	5.55^{c}	5.20^{d}
	SEM	0.08	0.04	0.08	0.08	0.06	0.06	0.08
WHC (%)	**0**	38.92^{b}	41.11^{ab}	31.11^{b}	39.29	40.45^{b}	27.30^{b}	36.94^{b}
	15	41.16^{b}	36.35^{b}	37.45^{ab}	52.17	45.98^{b}	55.28^{a}	56.85^{a}
	30	57.65^{a}	57.05^{a}	49.66^{ab}	45.01	39.32^{b}	56.64^{a}	56.70^{a}
	45	47.73^{ab}	27.30^{b}	55.53^{a}	43.22	66.06^{a}	63.59^{a}	59.93^{a}
	60	42.79^{b}	41.03^{ab}	31.88^{b}	34.61	44.21^{b}	55.08^{a}	42.50^{ab}
	SEM	2.37	3.38	3.48	2.96	3.08	3.78	3.23
Shear force (kg/m^{3})	**0**	2.48^{a}	2.01^{a}	2.03^{a}	1.70^{a}	1.82^{a}	1.80^{a}	1.27^{ab}
	15	1.83^{b}	1.76^{a}	1.67^{ab}	1.47^{a}	1.60^{b}	1.48^{ab}	1.07^{bc}
	30	1.37^{c}	1.33^{bc}	0.76^{c}	1.55^{a}	1.50^{ab}	0.98^{c}	1.55^{a}
	45	1.08^{c}	1.18^{c}	1.19^{cd}	1.07^{b}	1.20^{bc}	1.13^{bc}	1.37^{ab}
	60	1.19^{c}	1.72^{ab}	1.44^{bc}	0.94^{b}	0.92^{c}	1.48^{ab}	0.69^{c}
	SEM	0.12	0.08	0.11	0.08	0.08	0.08	0.08

^{a,b,c}: Columns having varying superscripts indicate significant ($p<0.05$) variations in means

WHC: Water Holding Capacity

1- Nitrite

2 - 0% nitrite/Bell pepper extract

3 - 0.15% Bell pepper extract

4 - 0.30% Bell pepper extract

5 - 0.45% Bell pepper extract

6 - 0.60% Bell pepper extract

7 - 0.75% Bell pepper extract

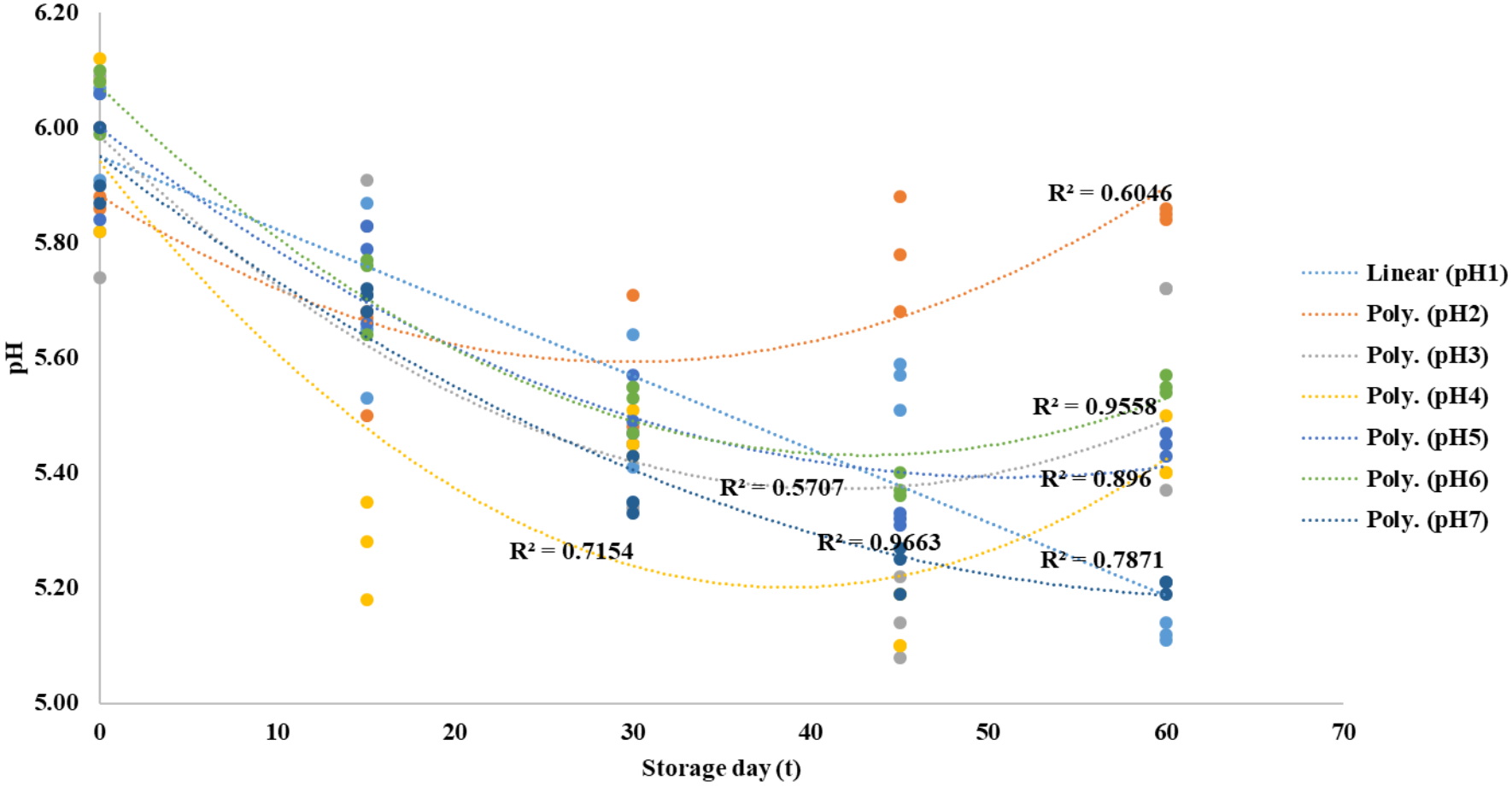

Figure 4.5: Relationship between storage duration and pH of smoked chicken fillets cured with bell pepper extract

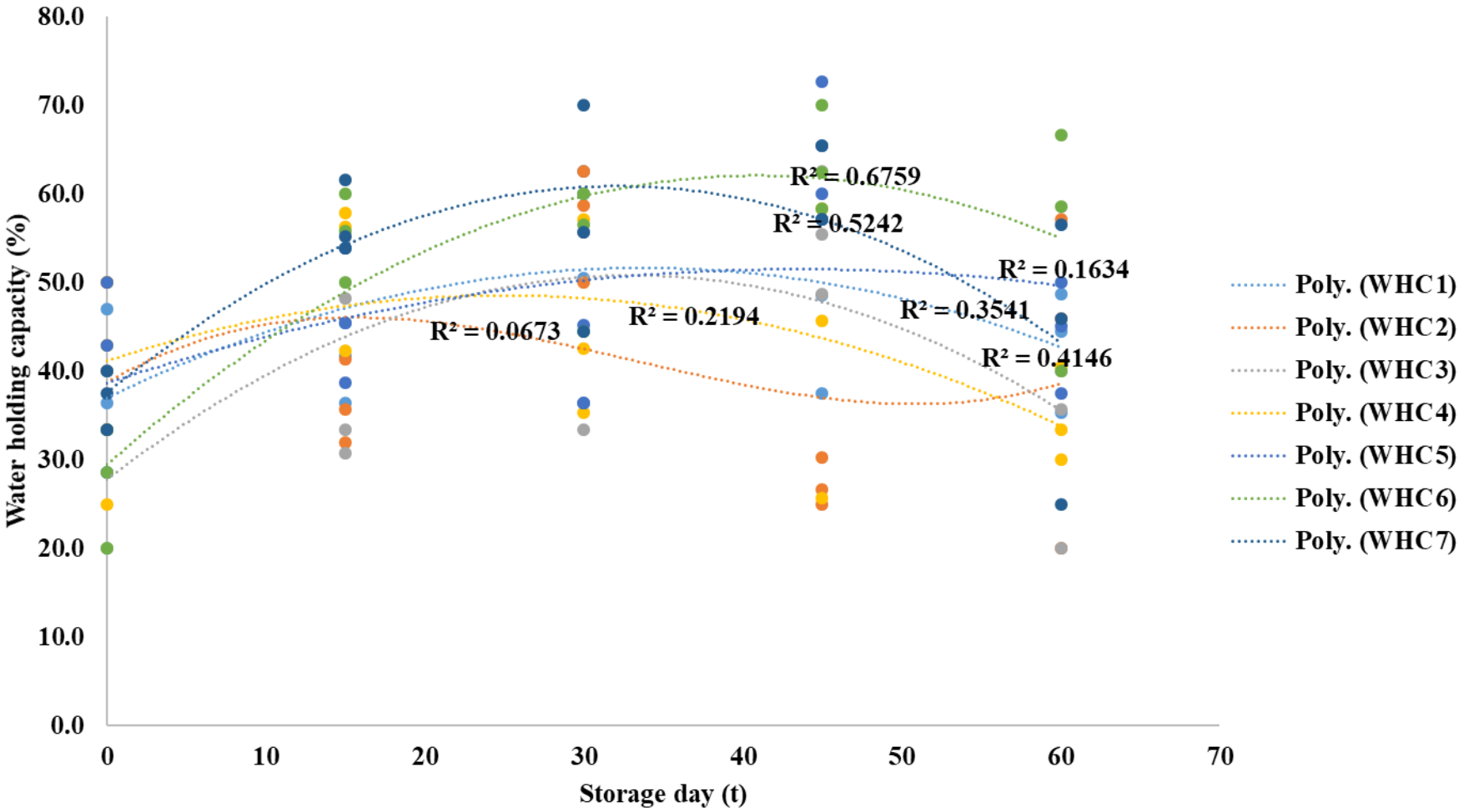

Figure 4.6: Relationship between storage duration and water holding capacity of smoked chicken fillets cured with bell pepper extract

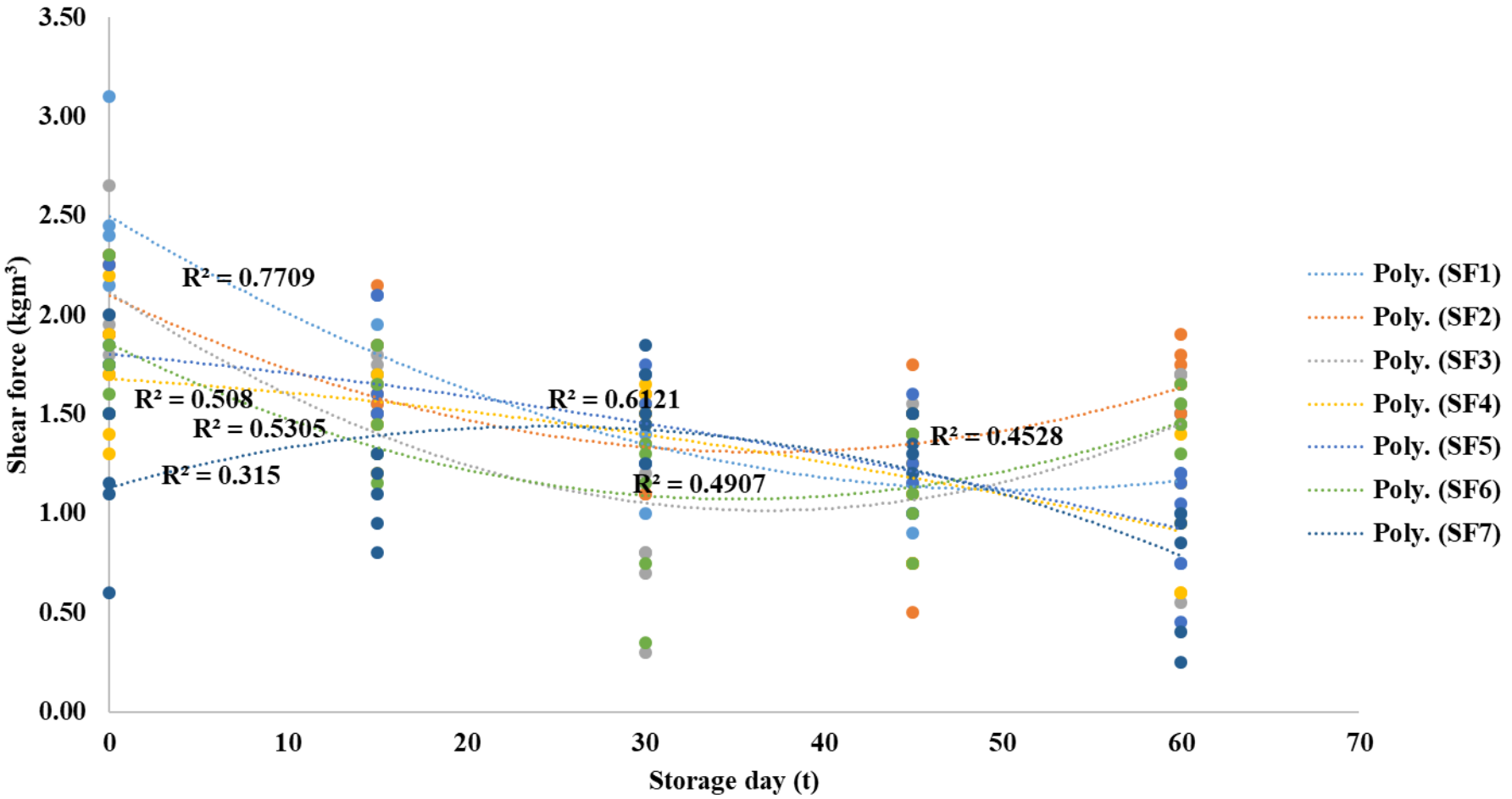

Figure 4.7: Relationship between storage duration and shear force of smoked chicken fillets cured with bell pepper extract

4.2.3 Lipid oxidation and protein deterioration of stored smoked chicken fillets cured with bell pepper extract

The rate of lipid oxidation during storage of cured smoked fillets (Table 4.3) was significantly highest on day 0, after which a sharp reduction was observed on day 15 of storage for all treatments. Lipid oxidation values of fillets thereafter gradually increased ($p<0.05$) till day 60. Volatile basic nitrogen values of fillets (Table 4.3), which depicts level of protein deterioration, did not follow a particular pattern during storage for all treatments. It was however lowest in treatment 7 (23.60) on day 0 and highest in treatment 3 (89.08) on day 15 of storage.

The interaction of extract treatment and duration of storage was observed to be significant ($p<0.05$) for both lipid oxidation and volatile basic nitrogen of fillets. Lipid oxidation of stored chicken fillets (Figure 4.8) showed positive correlation with storage duration, with regression coefficients ranging from 0.32 in treatment 4 to 0.64 in treatment 5. Volatile basic nitrogen values of chicken fillets also showed a positive correlation (Figure 4.9) with the storage period with least and highest regression coefficient of 0.18 and 0.90 in treatments 3 and 2, respectively.

Table 4.3: Lipid oxidation and volatile basic nitrogen of smoked chicken fillets cured with bell pepper extract during storage

		Treatments						
Parameters	**Storage day**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Lipid oxidation (mgMDA/100g)	**0**	1.36^{a}	1.15^{ab}	1.04^{a}	1.15^{a}	1.23^{a}	0.91^{a}	1.28^{a}
	15	0.50^{b}	1.30^{a}	0.52^{bc}	0.49^{b}	0.57^{b}	0.52^{ab}	0.65^{bc}
	30	0.67^{b}	0.65^{c}	0.48^{bc}	0.64^{ab}	0.76^{c}	0.49^{ab}	0.89^{ab}
	45	0.29^{b}	0.26^{c}	0.23^{c}	0.10^{a}	0.12^{c}	0.26^{b}	0.36^{c}
	60	0.71^{b}	0.71^{bc}	0.68^{ab}	1.06^{a}	0.56^{b}	0.77^{a}	1.26^{a}
	SEM	0.11	0.11	0.09	0.09	0.10	0.08	0.11
Volatile basic nitrogen (mg/100g)	**0**	57.40^{c}	75.47^{a}	28.57^{e}	37.20^{d}	29.30^{d}	29.90^{c}	23.60^{e}
	15	86.13_{a}	68.10^{b}	89.08^{a}	47.50^{c}	54.27^{b}	85.73^{a}	82.93^{a}
	30	73.13^{b}	52.13^{c}	34.47^{d}	45.40^{c}	44.53^{c}	55.73^{b}	41.57^{d}
	45	73.46^{b}	54.67^{c}	78.87^{b}	62.80^{a}	76.40^{a}	84.20^{a}	63.33^{b}
	60	46.27^{d}	41.87^{d}	56.73^{c}	56.53^{b}	55.33^{b}	51.07^{b}	49.73^{c}
	SEM	3.52	3.21	6.35	2.40	4.12	5.70	5.35

^{a,b,c}: Columns having varying superscripts indicate significant ($p<0.05$) variations in means

1- Nitrite

2 - 0% nitrite/Bell pepper extract

3 - 0.15% Bell pepper extract

4 - 0.30% Bell pepper extract

5 - 0.45% Bell pepper extract

6 - 0.60% Bell pepper extract

7 - 0.75% Bell pepper extract

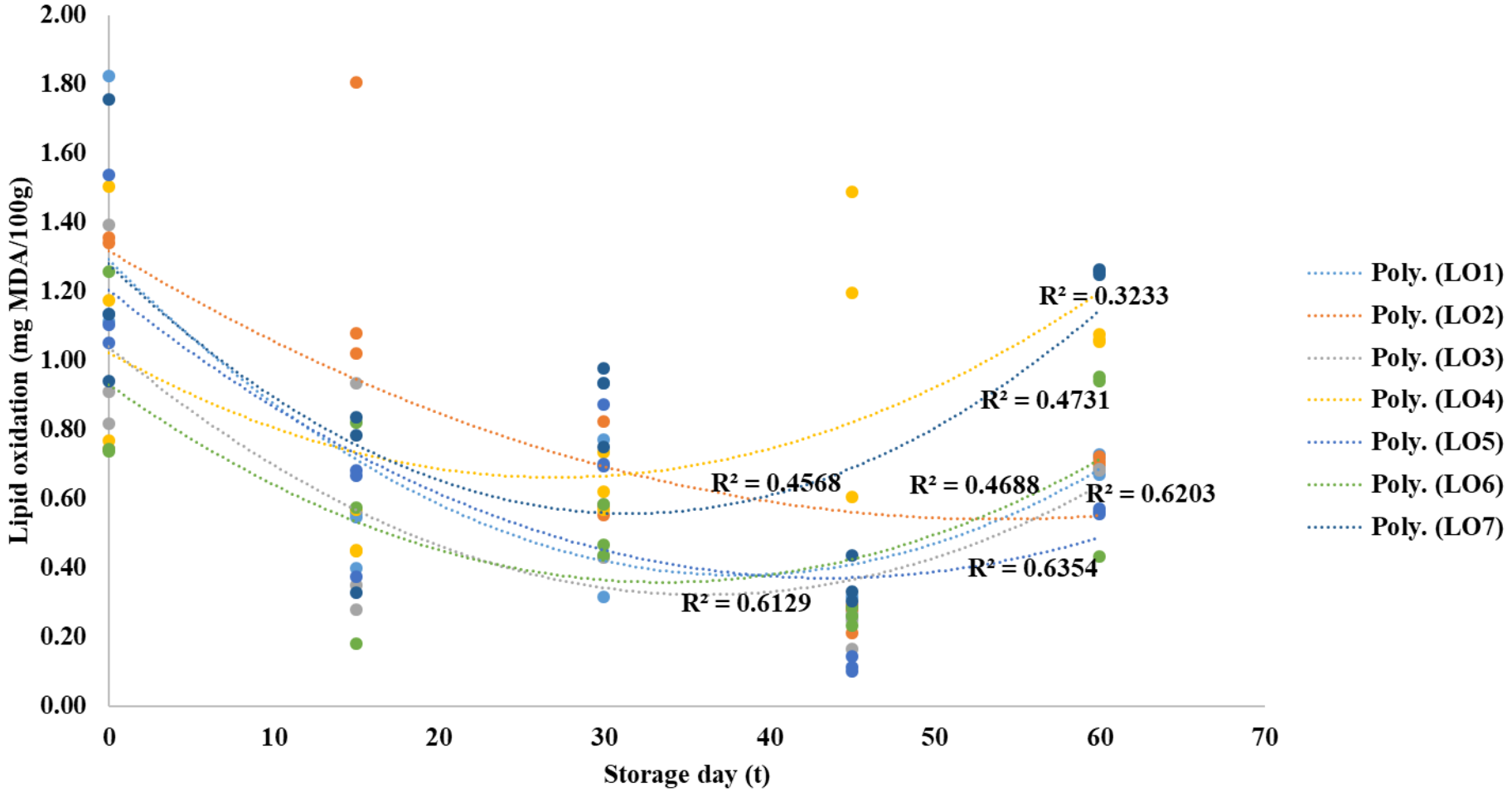

Figure 4.8: Relationship between storage duration and lipid oxidation of smoked chicken fillets cured with bell pepper extract

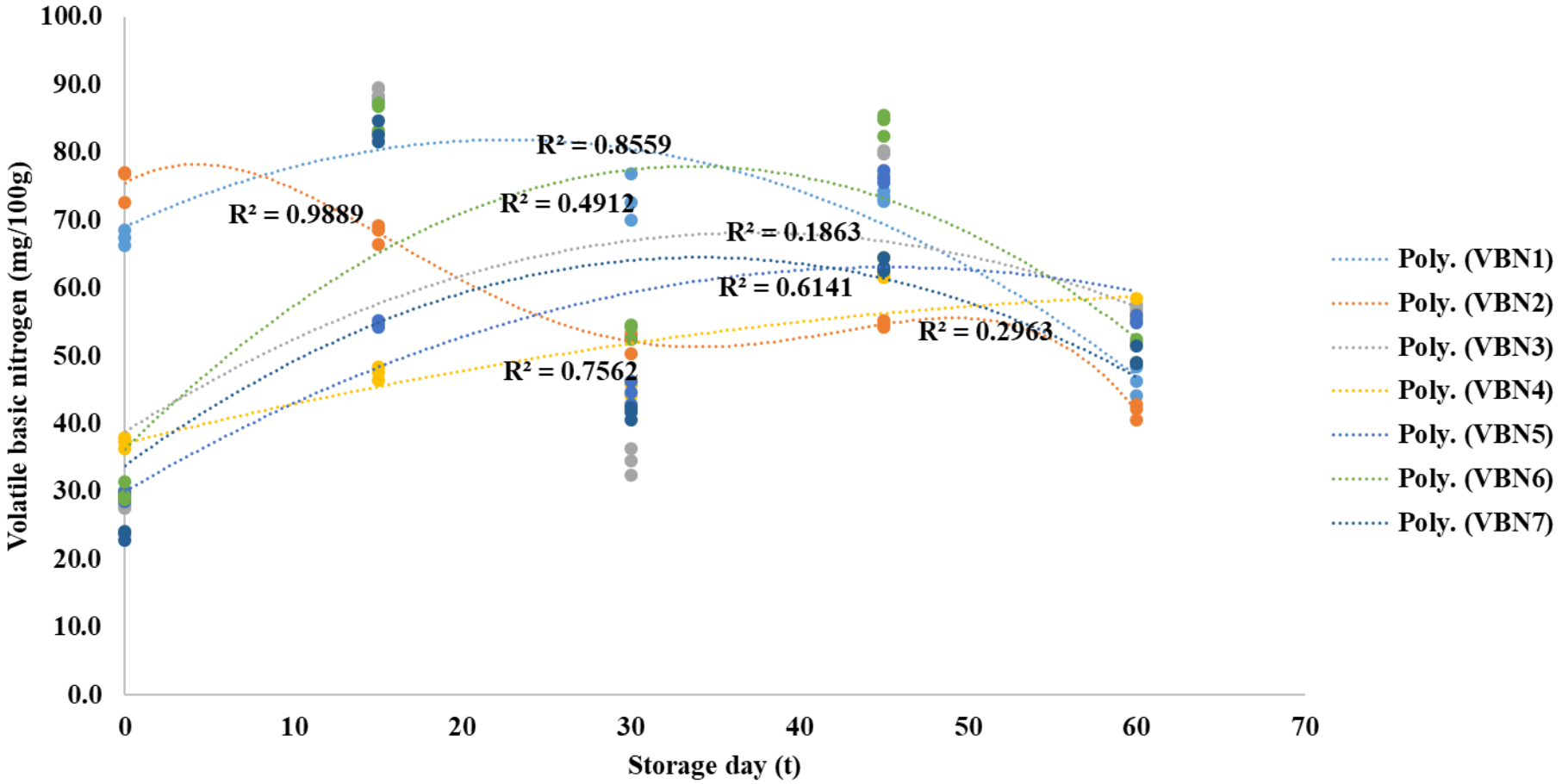

Figure 4.9: Relationship between storage duration and volatile basic nitrogen of smoked chicken fillets cured with bell pepper extract

4.2.4 Colour properties of stored smoked chicken fillets cured with bell pepper extract

Significant effect of storage duration was observed for colour properties of cured smoked chicken fillets (Table 4.4) for all treatments during storage. Lightness and redness values of fillets reduced during storage while yellowness increased. Lightness gradually ($p<0.05$) reduced over storage from 43.17 in treatment 7 on day 0 to 14.60 in treatment 2 by day 60, while redness reduced ($p<0.05$) from 23.20 in treatment 1 on day 0 to 13.82 in treatment 5 on day 60 of storage. Yellowness increased ($p<0.05$) from 17.68 on day 0 in treatment 2 to 37.21 on day 60 in treatment 6.

Significant effect of interaction of treatment and storage day was observed for colour properties of fillets. High correlation of colour properties to storage duration was also observed. Lightness had least and highest regression coefficients (Figure 4.10) of 0.36 in treatment 6 and 0.85 in treatment 5. Regression coefficients of redness of fillets ranged from 0.41 to 0.77 in treatments 5 and 1, respectively while yellowness ranged from 0.48 in treatment 1 to 0.72 in treatment 6.

Table 4.4: Colour properties of smoked chicken fillets cured with bell pepper extract during storage

		Treatments						
Parameters	**Storage day**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Lightness	**0**	17.56^{bc}	27.27^{a}	36.24^{a}	27.64^{b}	43.02^{a}	36.33^{a}	43.17^{a}
	15	31.52^{a}	20.76^{a}	27.87^{b}	35.02^{a}	21.17^{c}	23.56^{b}	27.64^{b}
	30	17.83^{bc}	12.26^{b}	29.53^{b}	23.72^{b}	20.60^{c}	31.03^{a}	28.85^{b}
	45	10.41^{c}	10.08^{c}	25.72^{b}	24.79^{b}	22.26^{c}	20.69^{b}	23.30^{b}
	60	27.97^{ab}	14.60^{b}	23.16^{b}	25.92^{b}	31.52^{b}	31.21^{a}	29.77^{b}
	SEM	2.38	2.86	1.38	1.18	2.41	1.72	1.94
Redness	**0**	23.20^{a}	18.35^{ab}	11.76^{b}	15.47^{a}	11.28^{b}	12.18^{c}	10.27^{b}
	15	13.01^{b}	10.01^{c}	10.96^{b}	9.68^{c}	11.44^{b}	13.09^{bc}	11.33^{b}
	30	17.55^{b}	17.64^{b}	12.76^{b}	15.28^{a}	14.31^{a}	14.66^{ab}	10.77^{b}
	45	23.69^{a}	20.31^{ab}	12.32^{b}	13.46^{b}	14.45^{a}	16.55^{a}	15.81^{a}
	60	14.45^{b}	24.08^{a}	17.00^{a}	14.01^{ab}	13.82^{a}	16.39^{a}	14.76^{a}
	SEM	1.34	1.42	0.64	0.59	0.44	0.52	0.63
Yellowness	**0**	23.24^{bc}	17.68^{bc}	20.62^{bc}	21.80^{c}	18.95^{b}	18.70^{b}	18.18^{d}
	15	21.07^{c}	16.54^{bc}	19.50^{c}	17.27^{c}	12.90^{b}	17.92^{b}	24.42^{c}
	30	26.34^{c}	20.81^{b}	28.72^{ab}	29.43^{b}	28.63^{a}	31.06^{a}	27.27^{c}
	45	20.61^{c}	14.32^{c}	32.21^{a}	35.09^{a}	35.13^{a}	32.14^{a}	45.68^{a}
	60	31.33^{a}	30.63^{a}	36.46^{a}	33.22^{ab}	34.37^{a}	37.21^{a}	36.63^{b}
	SEM	1.19	1.65	2.02	1.89	2.48	2.28	2.67

^{a,b,c}: Columns having varying superscripts indicate significant ($p<0.05$) variations in means

1- Nitrite

2 - 0% nitrite/Bell pepper extract

3 - 0.15% Bell pepper extract

4 - 0.30% Bell pepper extract

5 - 0.45% Bell pepper extract

6 - 0.60% Bell pepper extract

7 - 0.75% Bell pepper extract

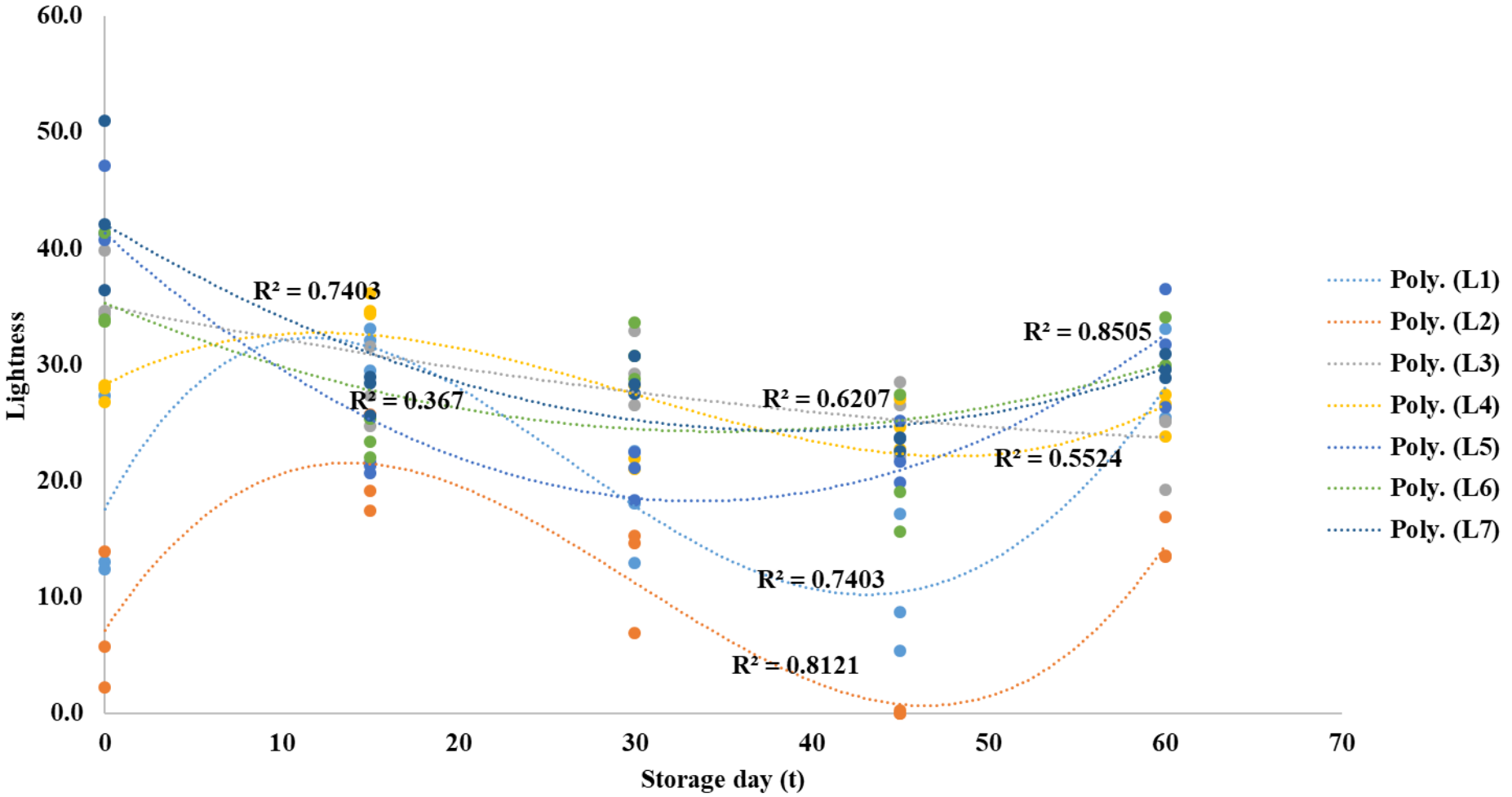

Figure 4.10: Relationship between storage duration and lightness of smoked chicken fillets cured with bell pepper extract

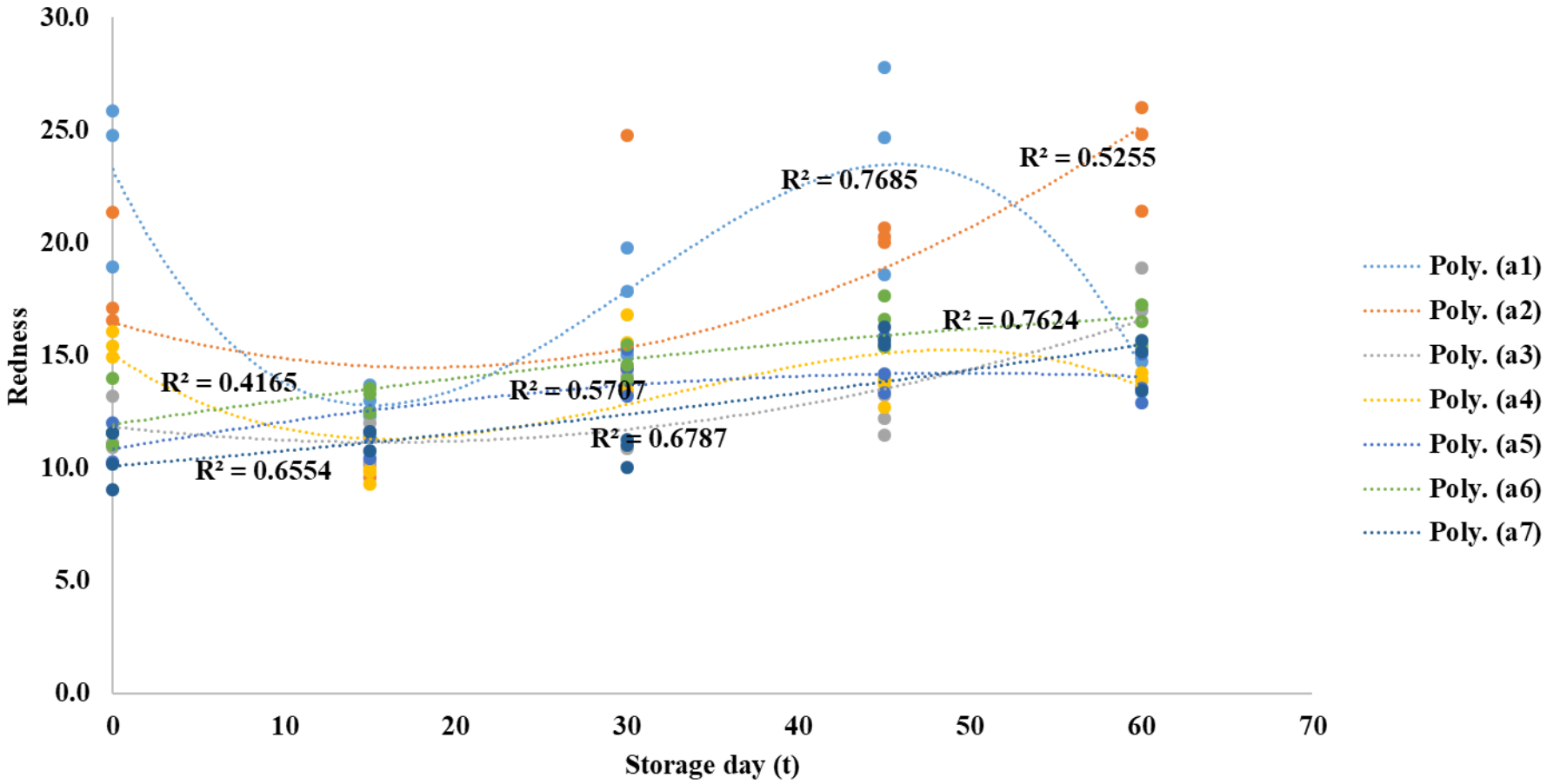

Figure 4.11: Relationship between storage duration and redness of smoked chicken fillets cured with bell pepper extract

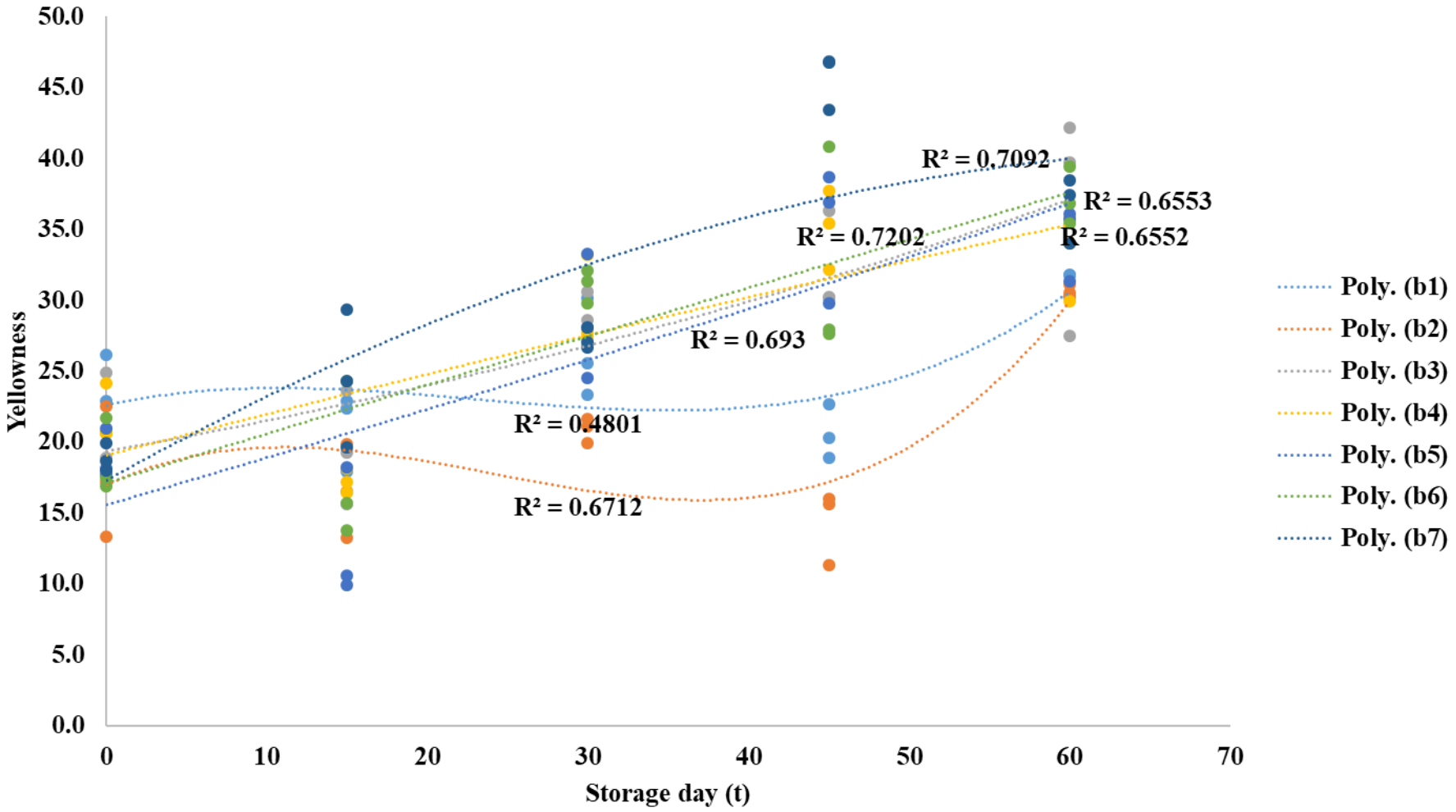

Figure 4.12: Relationship between storage duration and yellowness of smoked chicken fillets cured with bell pepper extract

4.2.5 Microbial load of stored smoked chicken fillets cured with bell pepper extract

Storage duration significantly affected treatments 3, 5, 6 and 7 for total anaerobic bacteria counts of fillets during storage (Table 4.5), while treatments 1, 2 and 4 were not significantly affected. A slight increase over storage was however observed during storage for significantly affected treatments. Lactic acid bacteria count of fillets (Table 4.5) were all significantly affected by the storage duration. The count increased form 1.49 in treatments 1 and 2 on day 0 to 3.80 in treatment 7 by day 60 of storage.

A non-significant ($p>0.05$) interaction of extract treatment and storage day was observed for microbial load of chicken fillets. This results in a single regression coefficient value which represents the relationship of the storage duration to microbial load of the fillets, irrespective of the treatments. Total anaerobic bacteria count had a regression coefficient of 0.12 while lactic acid bacteria count was 0.59.

Table 4.5: Microbial counts (log_{10}cfu/g) of smoked chicken fillets cured with bell pepper extract during storage

		Treatments						
Parameters	**Storage day**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
TAB counts	**0**	2.72	2.49	2.59^{b}	2.74	2.30^{c}	2.46^{b}	2.16^{d}
	15	2.80	2.83	2.80^{ab}	3.15	2.58^{bc}	3.35^{a}	3.44^{a}
	30	3.25	3.23	3.24^{a}	3.22	3.24^{a}	3.27^{a}	3.28^{ab}
	45	2.85	2.76	2.85^{ab}	2.84	2.87^{abc}	2.38^{b}	2.88^{bc}
	60	3.11	2.94	2.93^{ab}	3.04	2.96^{ab}	2.90^{ab}	2.75^{c}
	SEM	0.10	0.11	0.09	0.09	0.11	0.12	1.13
LAB counts	**0**	1.49^{b}	1.49^{b}	2.26^{b}	2.32^{c}	1.59^{b}	2.42^{c}	2.26^{d}
	15	3.55^{a}	3.27^{a}	2.99^{a}	3.48^{ab}	3.20^{a}	3.02^{b}	3.36^{c}
	30	3.55^{a}	3.37^{a}	3.39^{a}	3.38^{b}	3.50^{a}	3.48^{a}	3.50^{bc}
	45	3.32^{a}	3.45^{a}	3.38^{a}	3.81^{a}	3.54^{a}	3.45^{a}	3.62^{ab}
	60	3.49^{a}	3.24^{a}	3.18^{a}	3.25^{b}	3.09^{a}	3.06^{b}	3.80^{a}
	SEM	0.25	0.24	0.18	0.14	0.24	0.10	1.15

^{a,b,c}: Columns having varying superscripts indicate significant ($p<0.05$) variations in means

TAB counts: Total Anaerobic Bacteria Counts; LAB Counts: Lactic Acid Bacteria Counts

1- Nitrite

2 - 0% nitrite/Bell pepper extract

3 - 0.15% Bell pepper extract

4 - 0.30% Bell pepper extract

5 - 0.45% Bell pepper extract

6 - 0.60% Bell pepper extract

7 - 0.75% Bell pepper extract

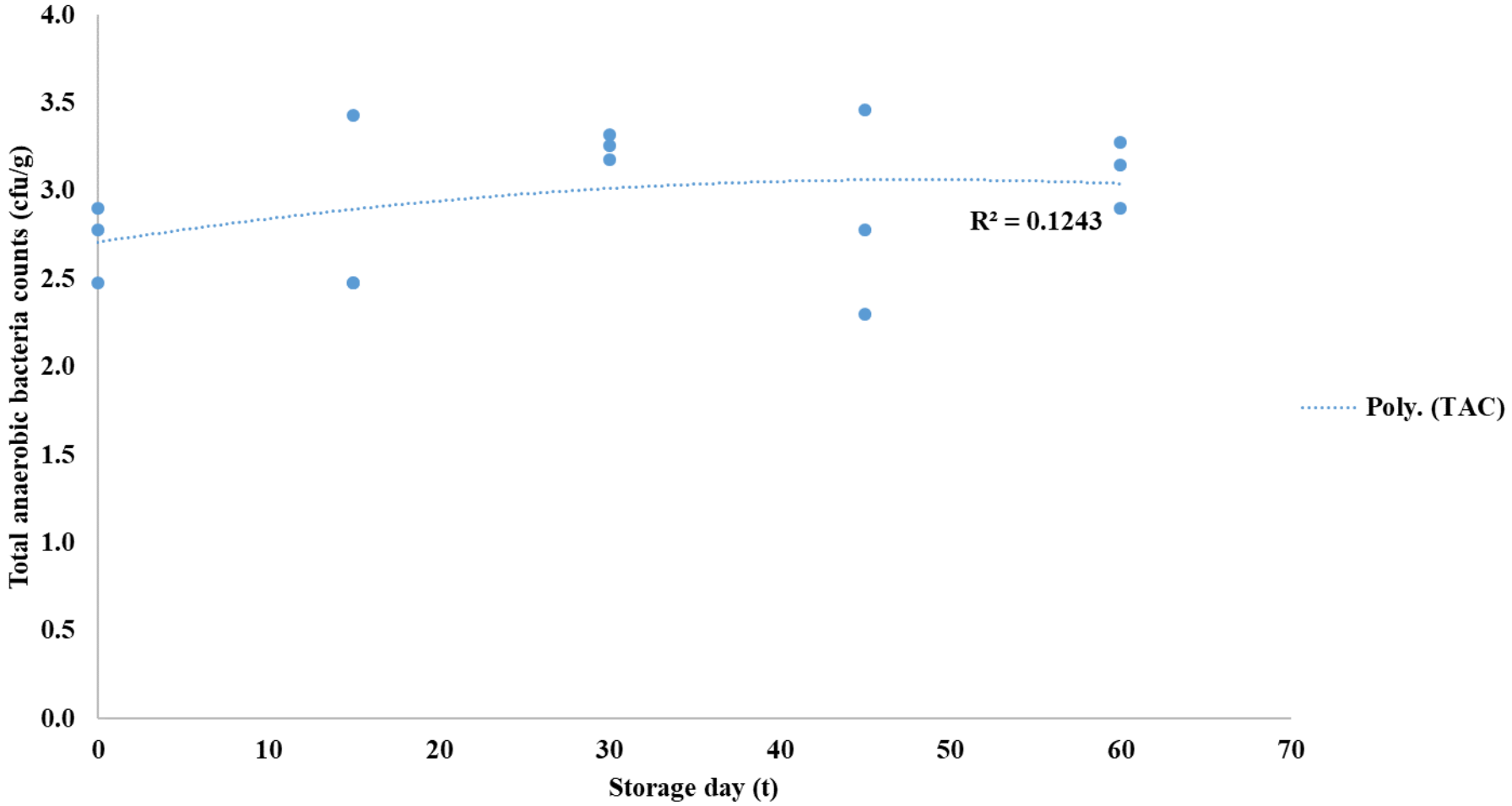

Figure 4.13: Relationship between storage duration and total anaerobic bacteria counts of smoked chicken fillets cured with bell pepper extract

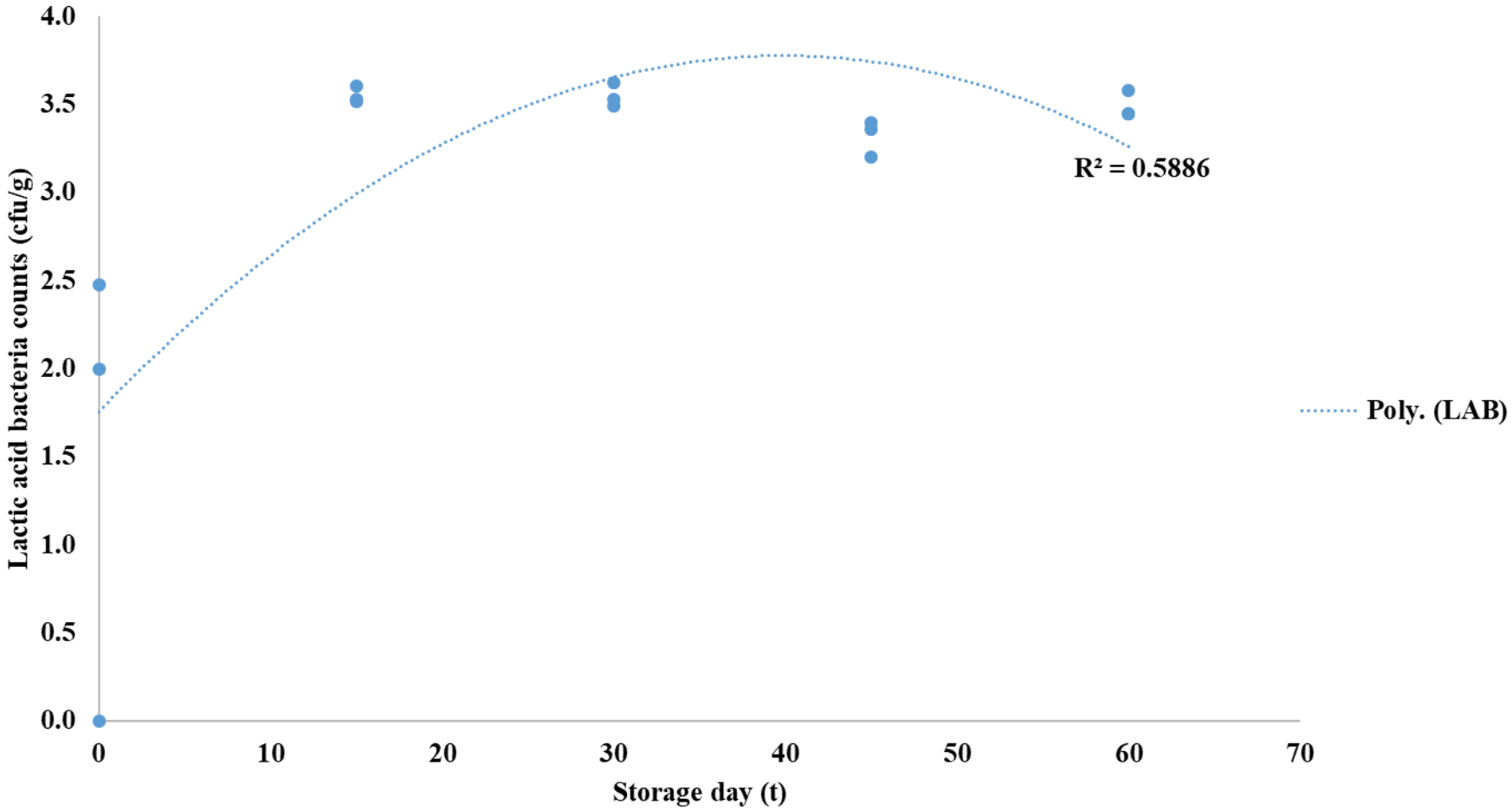

Figure 4.14: Relationship between storage duration and lactic acid bacteria counts of smoked chicken fillets cured with bell pepper extract

4.2.4 Sensory quality of stored smoked chicken fillets cured with bell pepper extract

Sensory quality of fillets decreased significantly over storage for all treatments (Table 4.6). Microbial growth score was highest on day 0 (3.00) for all treatments depicting a no microbial growth on some/all surface of the fillets in storage while it was least (2.40) in treatment 5 by day 60 of storage, which depicts microbial growth on some surfaces. Odour score of fillets were also observed to significantly decrease over storage for all treatments from 3.00 showing a fillets with normal characteristic cured smoked odour, to 1.60 on day 60 of storage in treatment 1, which depicts fillets with off odour or slightly rancid. Overall quality scores of fillets also decreased during the storage period. Excellent (3.00) quality was observed for all treatments on day of storage. A decrease ($p<0.05$) to slightly unacceptable (1.30) quality was observed for fillets by day 60 of storage.

There was no significant ($p>0.05$) interaction effect of storage day and extract treatment on the sensory quality of stored fillets during storage. This therefore results in a single regression coefficient value which represents the relationship of the storage duration and the sensory quality parameters of the fillets, irrespective of the treatments. Regression coefficients of microbial growth, odour and overall quality were 0.32, 0.28 and 0.43, respectively.

Table 4.6: Sensory properties of smoked chicken fillets cured with bell pepper extract during storage

		Treatments						
Parameters	**Storage day**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Microbial growth	**0**	3.00^{a}	3.00	3.00	3.00^{a}	3.00^{a}	3.00^{a}	3.00
	15	3.00^{a}	2.90	3.00	2.90^{ab}	3.00^{a}	2.90^{ab}	3.00
	30	3.00^{a}	3.00	3.00	2.90^{ab}	2.70^{ab}	2.70^{ab}	2.70
	45	3.00^{a}	2.80	2.80	2.50^{b}	2.70^{ab}	3.00^{a}	2.90
	60	2.30^{a}	2.90	2.90	2.60^{ab}	2.40^{b}	2.60^{b}	2.70
	SEM	0.07	0.04	0.03	0.07	0.08	0.05	0.05
Odour	**0**	3.00^{a}	3.00^{a}	3.00^{a}	3.00^{a}	3.00^{a}	3.00^{a}	3.00^{a}
	15	2.30^{b}	2.40^{b}	2.60^{ab}	2.10^{c}	2.80^{ab}	2.20^{b}	2.70^{ab}
	30	2.60^{ab}	2.60^{ab}	2.40^{b}	2.70^{ab}	2.30^{bc}	2.50^{ab}	2.30^{b}
	45	2.60^{ab}	2.30^{b}	2.40^{b}	2.10^{c}	2.40^{bc}	2.30^{b}	2.60^{ab}
	60	1.60^{c}	2.70^{ab}	2.40^{b}	2.30^{bc}	2.00^{c}	2.10^{b}	2.30^{b}
	SEM	0.10	0.09	0.09	0.10	0.09	0.10	0.10
Overall quality	**0**	3.00^{a}	3.00^{a}	3.00^{a}	3.00^{a}	3.00^{a}	3.00^{a}	3.00^{a}
	15	2.00^{b}	1.70^{b}	2.20^{bc}	1.80^{c}	2.40^{b}	2.10^{bc}	2.30^{b}
	30	2.30^{b}	2.20^{b}	2.40^{b}	2.30^{b}	2.20^{b}	2.30^{b}	2.30^{b}
	45	1.90^{b}	1.80^{b}	1.70^{c}	1.60^{c}	1.70^{c}	2.10^{bc}	2.10^{b}
	60	1.30^{c}	2.10^{b}	2.00^{bc}	1.60^{c}	1.50^{c}	1.80^{c}	1.60^{c}
	SEM	0.11	0.10	0.10	0.10	0.10	0.08	0.09

^{a,b,c}: Columns having varying superscripts indicate significant ($p<0.05$) variations in means

1- Nitrite

2 - 0% nitrite/Bell pepper extract

3 - 0.15% Bell pepper extract

4 - 0.30% Bell pepper extract

5 - 0.45% Bell pepper extract

6 - 0.60% Bell pepper extract

7 - 0.75% Bell pepper extract

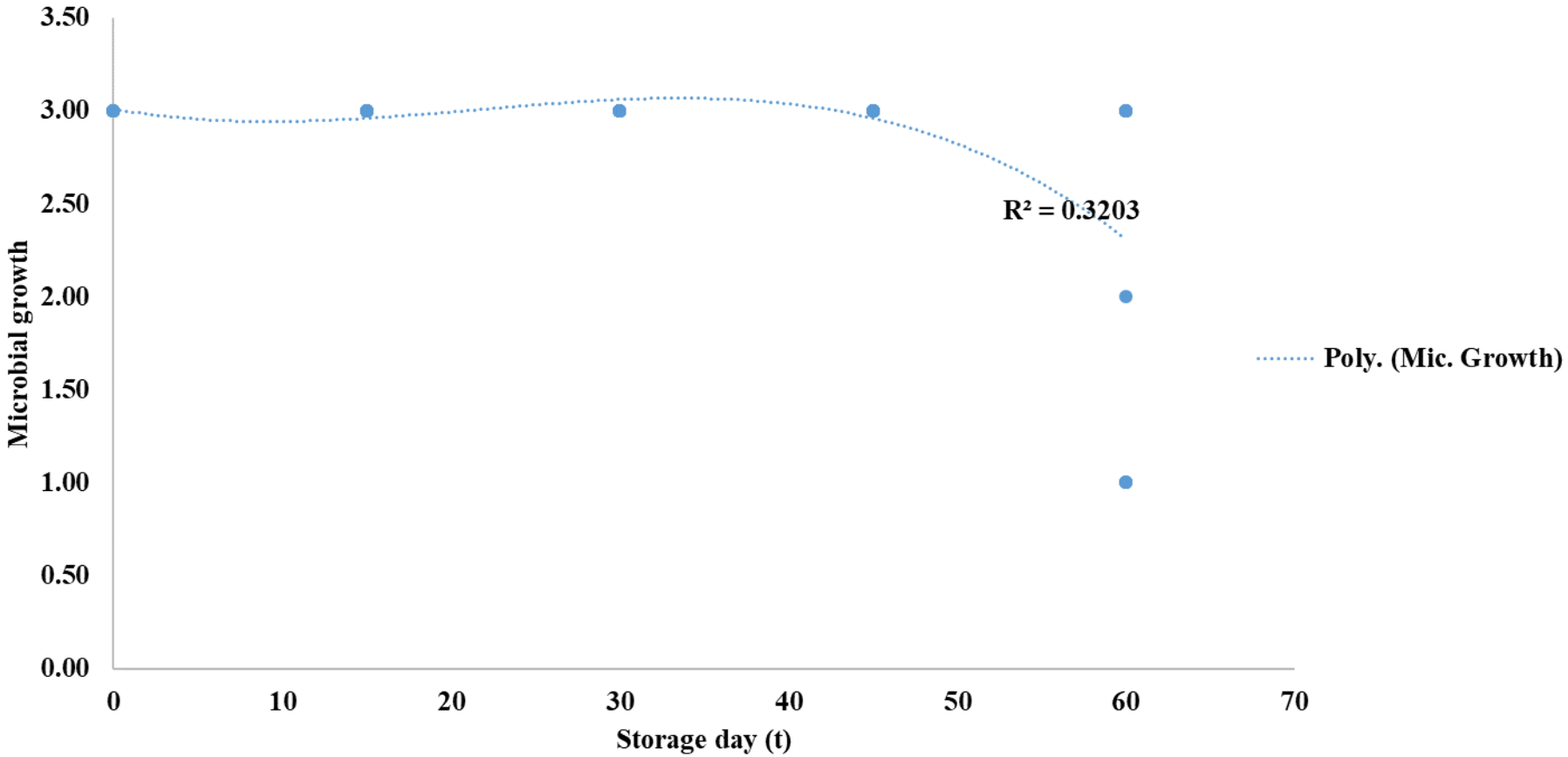

Figure 4.15: Relationship between storage duration and microbial growth of smoked chicken fillets cured with bell pepper extract

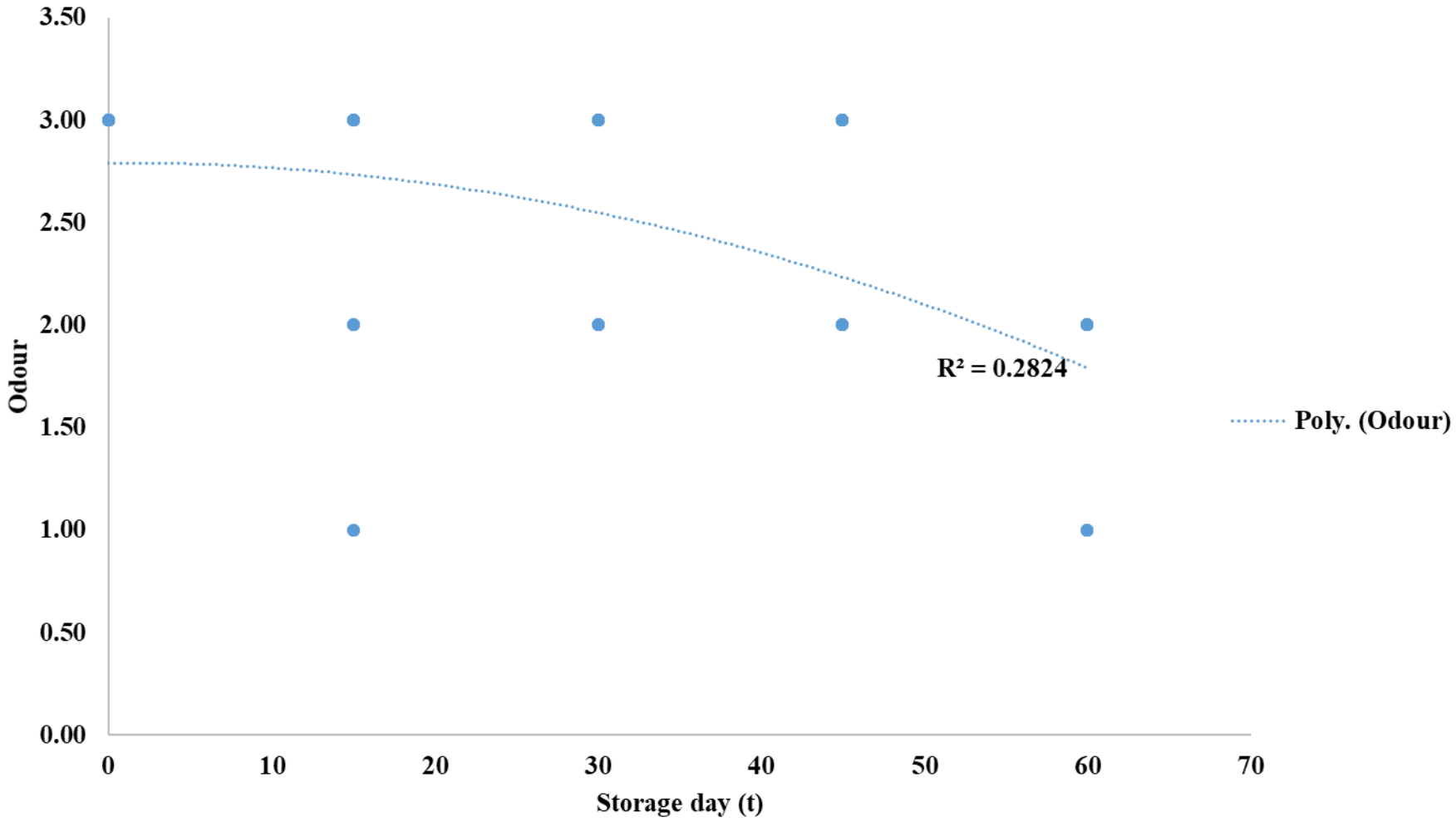

Figure 4.16: Relationship between storage duration and odour of smoked chicken fillets cured with bell pepper extract

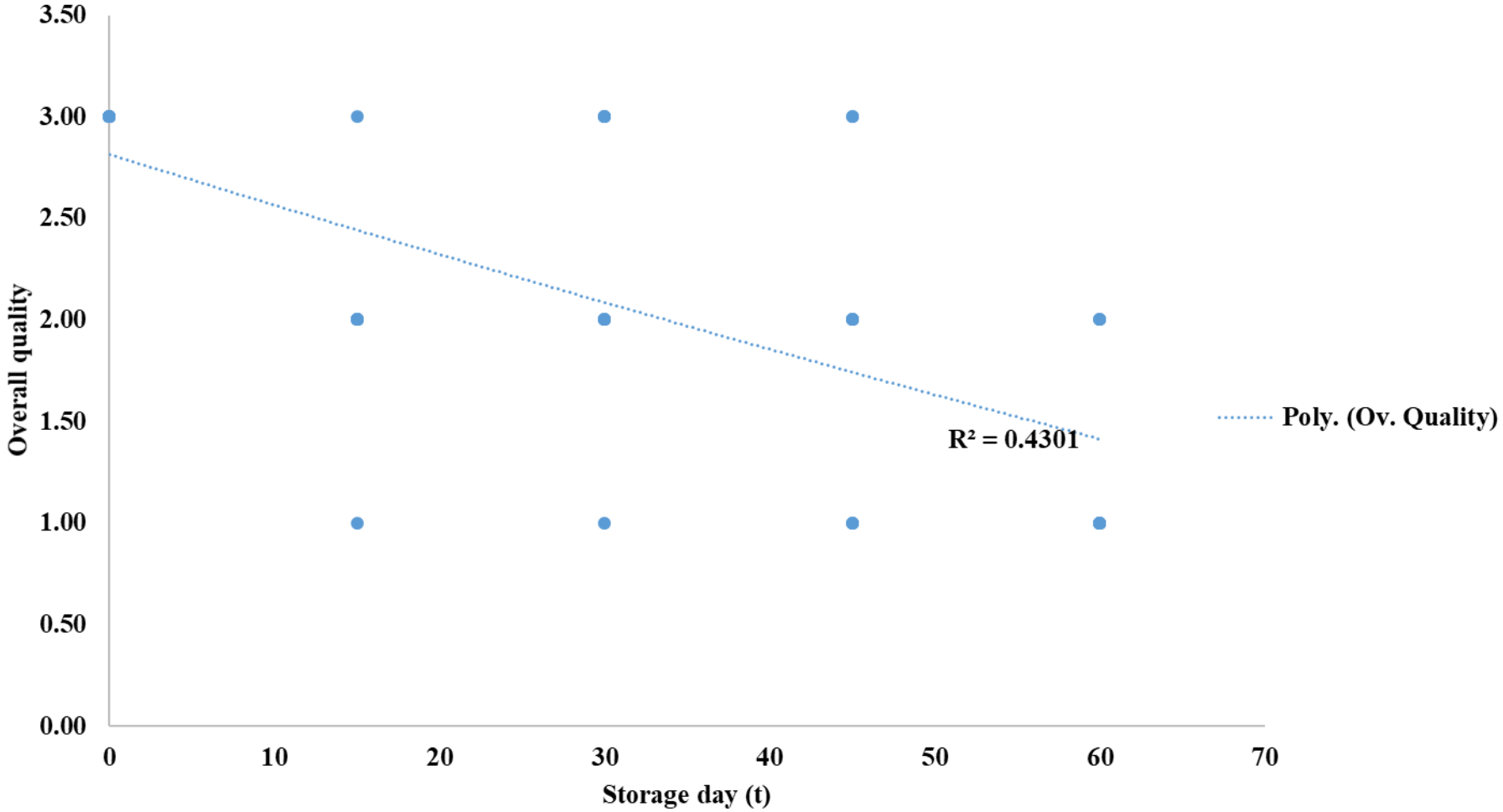

Figure 4.17: Relationship between storage duration and overall quality of smoked chicken fillets cured with bell pepper extract

4.3 Experiment Three: Effects of Sodium Chloride Replacement on Quality of Ready-To-Eat Cured Smoked Chicken Fillets

4.3.1 Physical properties of freshly prepared smoked fillets cured with varying combinations of chloride salts

Physical properties of freshly smoked fillets cured with varying salt combinations (Table 4.7) was significantly ($p<0.05$) affected by salt combination treatments. Cooking loss of 54.80 was least ($p<0.05$) in treatment 3 and highest ($p<0.05$) in treatment 8 (65.64). Product yield was highest ($p<0.05$) in treatment 3 (45.20%) and least ($p<0.05$) in treatment 7 (34.36%).

4.3.2 Physicochemical properties of stored smoked chicken fillets cured with varying salt combinations

Significant effect of storage period was observed for physicochemical properties of stored smoked chicken fillets cured with varying salt combinations (Table 4.8). The pH was observed to gradually reduce ($p<0.05$) over storage with highest values observed on day 0 in treatment 7 (6.05) and least ($p<0.05$) in treatment 1 (4.93) on day 60. Water holding capacity was significantly affected by storage period for all treatments except treatments 2 and 7. A gradual decrease was observed over storage from 48.67 in treatment 3 on day 0 to 37.00 in treatment 8 on day 60. Storage day significantly affected shear force values of fillets in all treatments except treatments 5, 6 and 8. No regular pattern of the effect of storage period was observed, however least and highest shear force was obtained in treatment 1 (0.87) and 3 (1.64) on day 0 while least and highest values were obtained in treatments 4 (1.12) and 2 (1.82) on day 60 of storage.

Significant interaction effect of salt combination treatments and storage day was observed for physicochemical properties of stored fillets. The relationship of storage duration and pH (Figure 4.18) of fillets was highly correlated with regression coefficients ranging from 0.92 in treatment 5 to 0.56 in treatment 1. For water holding capacity (Figure 4.19), slight positive correlation was observed in its relationship with storage duration. Regression coefficient ranged from 0.09 in treatment 5 to 0.37 in treatment 7. A positive but slight correlation was also observed for shear force (Figure 4.20) of fillets with regression coefficients ranging from 0.08 in treatment 8 to 0.71 in treatment 7.

Table 4.7: Physical properties of freshly prepared smoked chicken fillets cured with varying chloride salts

	Salt inclusion								
Parameters (%)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**SEM**
Cooking loss	55.07^{b}	54.88^{b}	54.80^{b}	63.39^{a}	63.48^{a}	63.48^{a}	65.08^{a}	65.64^{a}	1.06
Product yield	44.93^{a}	45.12^{a}	45.20^{a}	36.61^{b}	36.52^{b}	36.52^{b}	34.92^{b}	34.36^{b}	1.06

$^{a,b,c\cdots}$- Rows with different superscripts indicate significant ($p<0.05$) variations in means

SEM- Standard Error of Mean

Salt inclusion:

1 - 100%NaCl;

2 - 50%NaCl + 50%$CaCl_2$;

3 - 50%NaCl + 50%KCl;

4 - 50%NaCl + 50%$MgCl_2$;

5 - 50%NaCl + 25%$CaCl_2$ + 25%KCl;

6 - 50%NaCl + 25%$CaCl_2$ + 25%$MgCl_2$;

7 - 50%NaCl + 25%KCl + 25%$MgCl_2$;

8 - 25%NaCl + 25%$CaCl_2$ + 25%KCl + 25%$MgCl_2$

Table 4.8: Physicochemical properties of smoked chicken fillets cured with varying salt combinations

Parameters	Storage day	Treatments 1	2	3	4	5	6	7	8
pH	**0**	5.74^{a}	5.74^{a}	5.91^{a}	5.83^{a}	5.65^{ab}	5.57^{a}	6.05^{a}	5.90^{a}
	15	5.53^{a}	5.67^{ab}	5.87^{a}	5.62^{b}	5.75^{a}	5.48^{b}	5.24^{b}	5.66^{b}
	30	5.29^{ab}	5.58^{bc}	5.42^{b}	5.38^{c}	5.77^{a}	5.48^{b}	5.19^{b}	5.66^{b}
	45	5.20^{ab}	5.52^{c}	5.22^{c}	5.21^{c}	5.59^{b}	5.11^{c}	5.02^{c}	5.53^{bc}
	60	4.93^{b}	5.45^{c}	5.26^{c}	5.25^{c}	5.68^{ab}	5.05^{c}	4.97^{c}	5.41^{c}
	SEM	0.10	0.03	0.08	0.07	0.03	0.06	0.10	0.05
WHC (%)	**0**	35.00^{b}	28.00	44.67^{ab}	48.67^{ab}	25.00^{b}	44.00^{ab}	49.33	43.67^{b}
	15	38.67^{b}	28.67	55.67^{a}	54.67^{a}	45.67^{a}	49.67^{ab}	58.00	55.67^{a}
	30	53.33^{a}	34.00	40.67^{b}	50.67^{ab}	37.00^{a}	54.67^{a}	55.00	41.67^{b}
	45	43.67^{ab}	31.00	39.33^{b}	43.33^{b}	27.00^{b}	47.33^{ab}	46.67	40.33^{b}
	60	48.67^{ab}	33.67	40.00^{b}	43.33^{b}	39.67^{a}	39.00^{b}	55.33	37.00^{b}
	SEM	2.34	1.25	2.23	1.45	2.32	2.09	1.81	2.03
Shear force (kg/m^3)	**0**	0.87^{b}	1.45^{ab}	1.64^{a}	1.57^{a}	1.62	1.38	1.43^{a}	1.78
	15	1.78^{a}	1.53^{ab}	1.70^{a}	0.90^{b}	1.89	1.32	1.50^{a}	1.57
	30	0.65^{b}	1.33^{b}	1.53^{a}	0.85^{b}	1.70	1.77	0.48^{b}	1.70
	45	0.40^{b}	1.41^{ab}	0.87^{b}	1.32^{ab}	1.86	1.46	0.65^{b}	1.53
	60	1.65^{a}	1.82^{a}	1.23^{ab}	1.12^{ab}	1.55	1.14	1.37^{a}	1.54
	SEM	0.13	0.06	0.09	0.10	0.08	0.09	0.10	0.07

^{a,b,c}: Columns having varying superscripts indicate significant ($p<0.05$) variations in means

WHC: Water Holding Capacity

Salt inclusion:
1 - 100%NaCl;
2 - 50%NaCl + 50%$CaCl_2$;
3 - 50%NaCl + 50%KCl;
4 - 50%NaCl + 50%$MgCl_2$;
5 - 50%NaCl + 25%$CaCl_2$ + 25%KCl;
6 - 50%NaCl + 25%$CaCl_2$ + 25%$MgCl_2$;
7 - 50%NaCl + 25%KCl + 25%$MgCl_2$;
8 - 25%NaCl + 25%$CaCl_2$ + 25%KCl + 25%$MgCl_2$

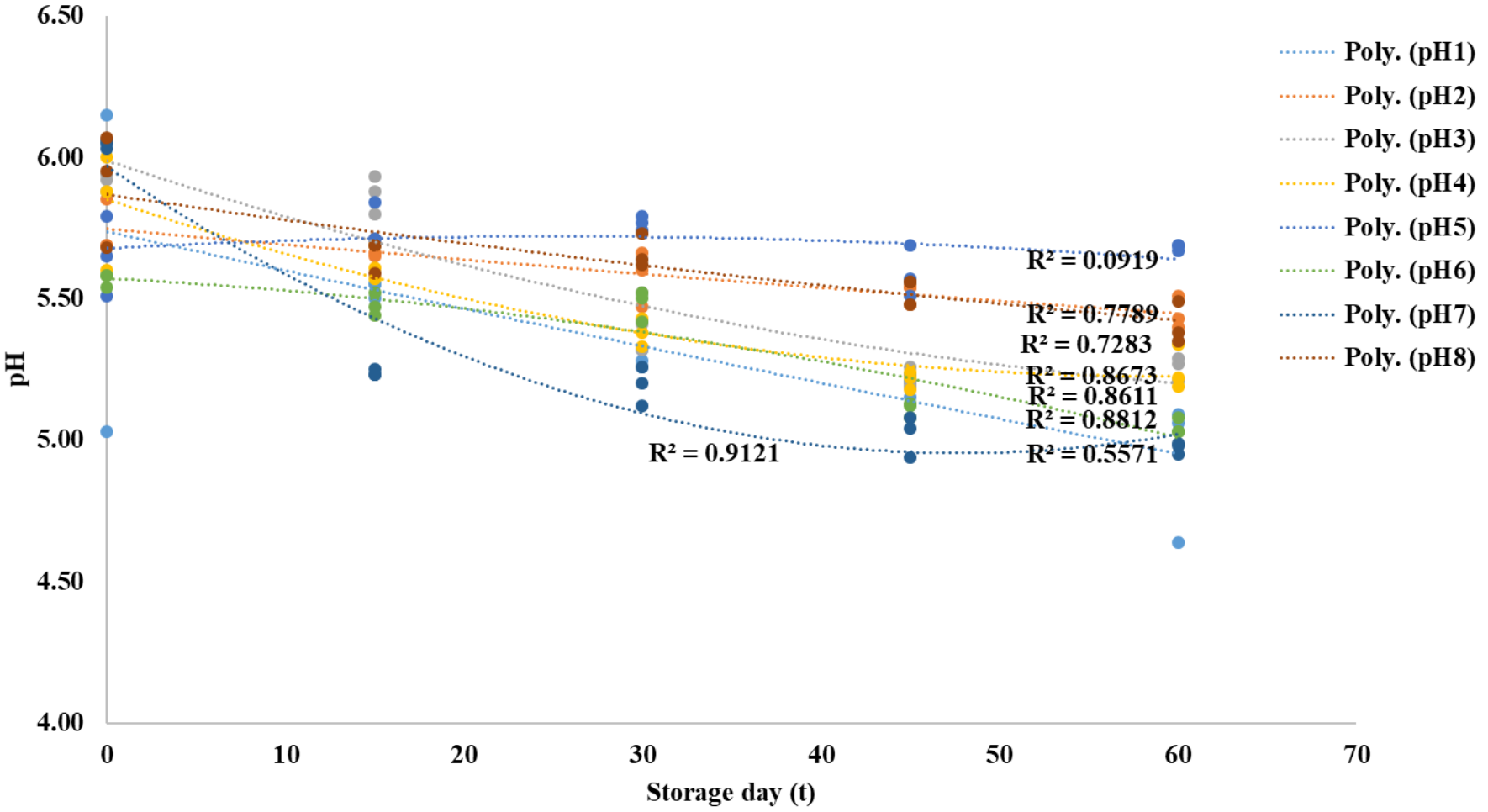

Figure 4.18: Relationship between storage duration and pH of smoked chicken fillets cured with varying salt combinations

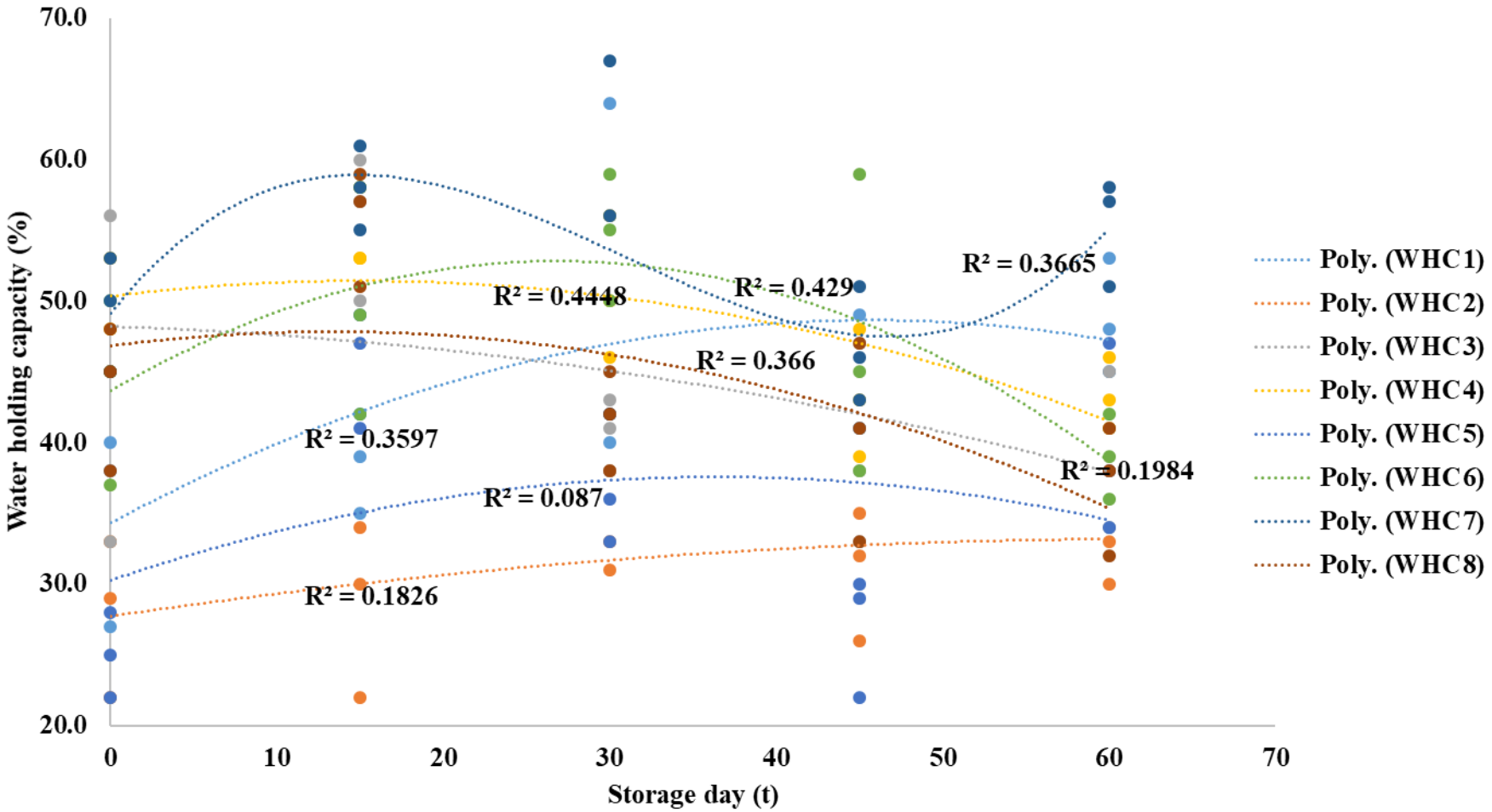

Figure 4.19: Relationship between storage duration and water holding capacity of smoked chicken fillets cured with varying salt combinations

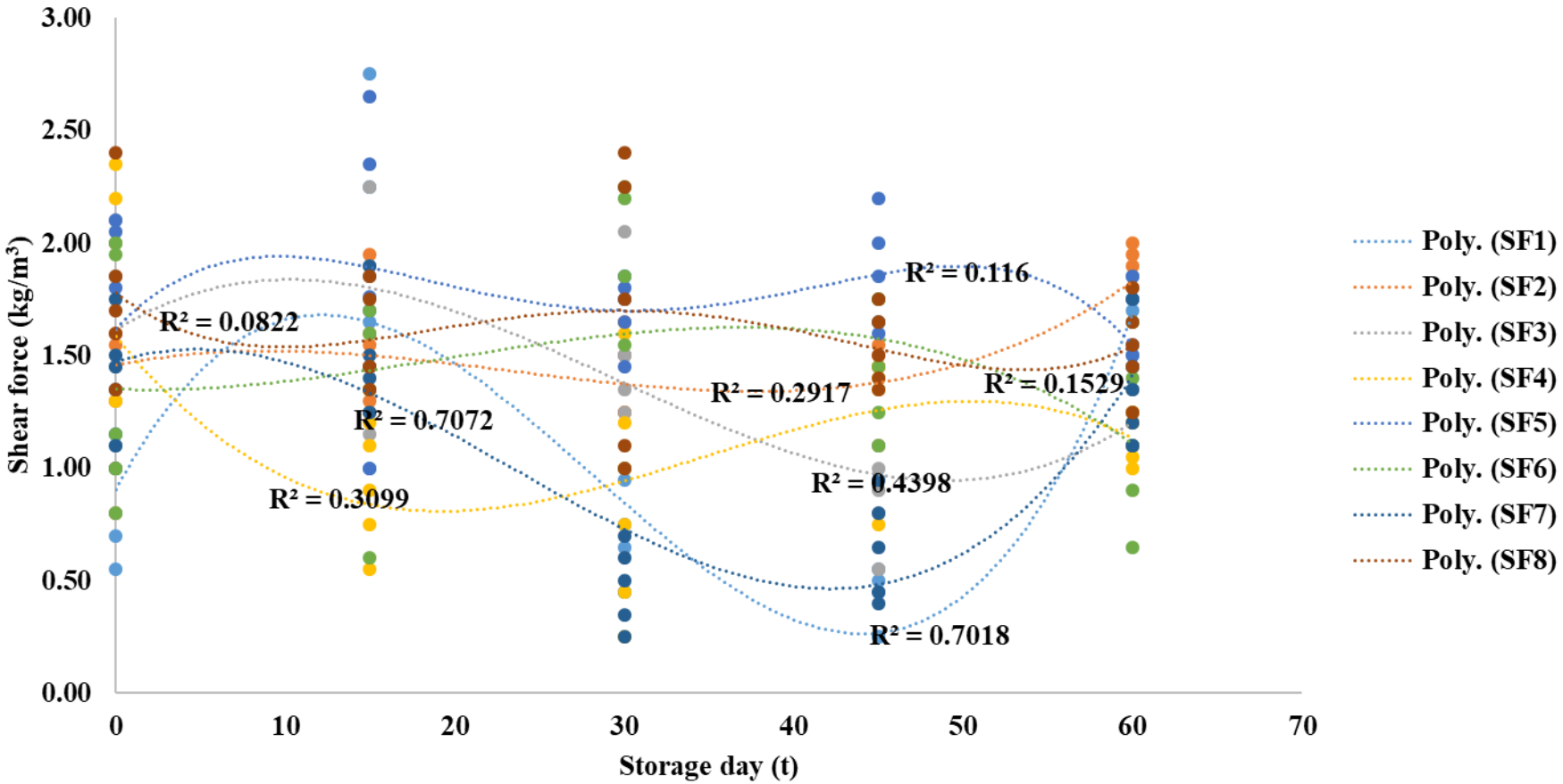

Figure 4.20: Relationship between storage duration and shear force of smoked chicken fillets cured with varying salt combinations

4.3.3 Lipid oxidation and protein deterioration of smoked chicken fillets cured with varying salt combinations

Significant effect of storage was observed for only treatments 1, 5, 6 and 7 for lipid oxidation of fillets (Table 4.9) with significant increase in lipid oxidation values from 0.07 on day 0 in treatment 7 to 0.40 on day 60 in treatment 6. Significant effect of storage period was also observed for all treatments for volatile basic nitrogen values of fillets (Table 4.9), however no defined pattern of effect was observed for the treatments. On day 0, treatments 8 (44.40) and 1(89.57) has least and highest values while by day 60 of storage, treatments 7 and 1 has least and highest values, respectively.

Significant ($p<0.05$) interaction effect was observed for volatile basic nitrogen (Figure 4.22) of chicken fillets while lipid oxidation (Figure 4.21) was not significantly ($p>0.05$) affected. A slight correlation was observed for lipid oxidation with regression coefficient of 0.30 while regression coefficient ranged from 0.06 in treatment 4 to 0.55 in treatment 7 for volatile basic nitrogen values of fillets.

Table 4.9: Lipid oxidation and volatile basic nitrogen of smoked chicken fillets cured with varying salt combinations

Parameters	Storage day	Treatments 1	2	3	4	5	6	7	8
Lipid oxidation (mgMDA/100g)	**0**	0.38^{a}	0.24	0.34	0.17	0.23^{a}	0.10^{b}	0.07^{b}	0.28
	15	0.14^{b}	0.17	0.17	0.17	0.21^{a}	0.24^{b}	0.35^{a}	0.29
	30	0.15^{b}	0.09	0.17	0.18	0.02^{b}	0.14^{b}	0.07^{b}	0.11
	45	0.30^{ab}	0.23	0.22	0.16	0.14^{ab}	0.20^{b}	0.32^{a}	0.34
	60	0.29^{ab}	0.24	0.36	0.32	0.30^{a}	0.40^{a}	0.37^{a}	0.29
	SEM	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.03
Volatile basic nitrogen (mg/100g)	**0**	89.57^{a}	69.33^{c}	71.67^{a}	59.13^{c}	71.27^{c}	63.07^{c}	53.27^{c}	44.40^{d}
	15	71.40^{c}	90.53^{a}	65.80^{a}	66.73^{b}	81.20^{b}	74.20^{b}	77.93^{a}	87.27^{a}
	30	55.53^{d}	76.07^{b}	49.47^{b}	49.47^{d}	49.00^{d}	63.00^{c}	63.00^{b}	66.73^{b}
	45	78.40^{b}	47.13^{e}	65.33^{a}	86.33^{a}	95.67^{a}	81.67^{a}	81.20^{a}	62.53^{b}
	60	69.53^{c}	55.07^{d}	53.67^{b}	54.13^{cd}	63.93^{c}	56.47^{c}	48.07^{c}	52.27^{c}
	SEM	3.09	4.17	2.36	3.54	4.30	2.55	3.57	3.98

^{a,b,c}: Columns having varying superscripts indicate significant ($p<0.05$) variations in means

Salt inclusion:

1 - 100%NaCl;

2 - 50%NaCl + 50%$CaCl_2$;

3 - 50%NaCl + 50%KCl;

4 - 50%NaCl + 50%$MgCl_2$;

5 - 50%NaCl + 25%$CaCl_2$ + 25%KCl;

6 - 50%NaCl + 25%$CaCl_2$ + 25%$MgCl_2$;

7 - 50%NaCl + 25%KCl + 25%$MgCl_2$;

8 - 25%NaCl + 25%$CaCl_2$ + 25%KCl + 25%$MgCl_2$

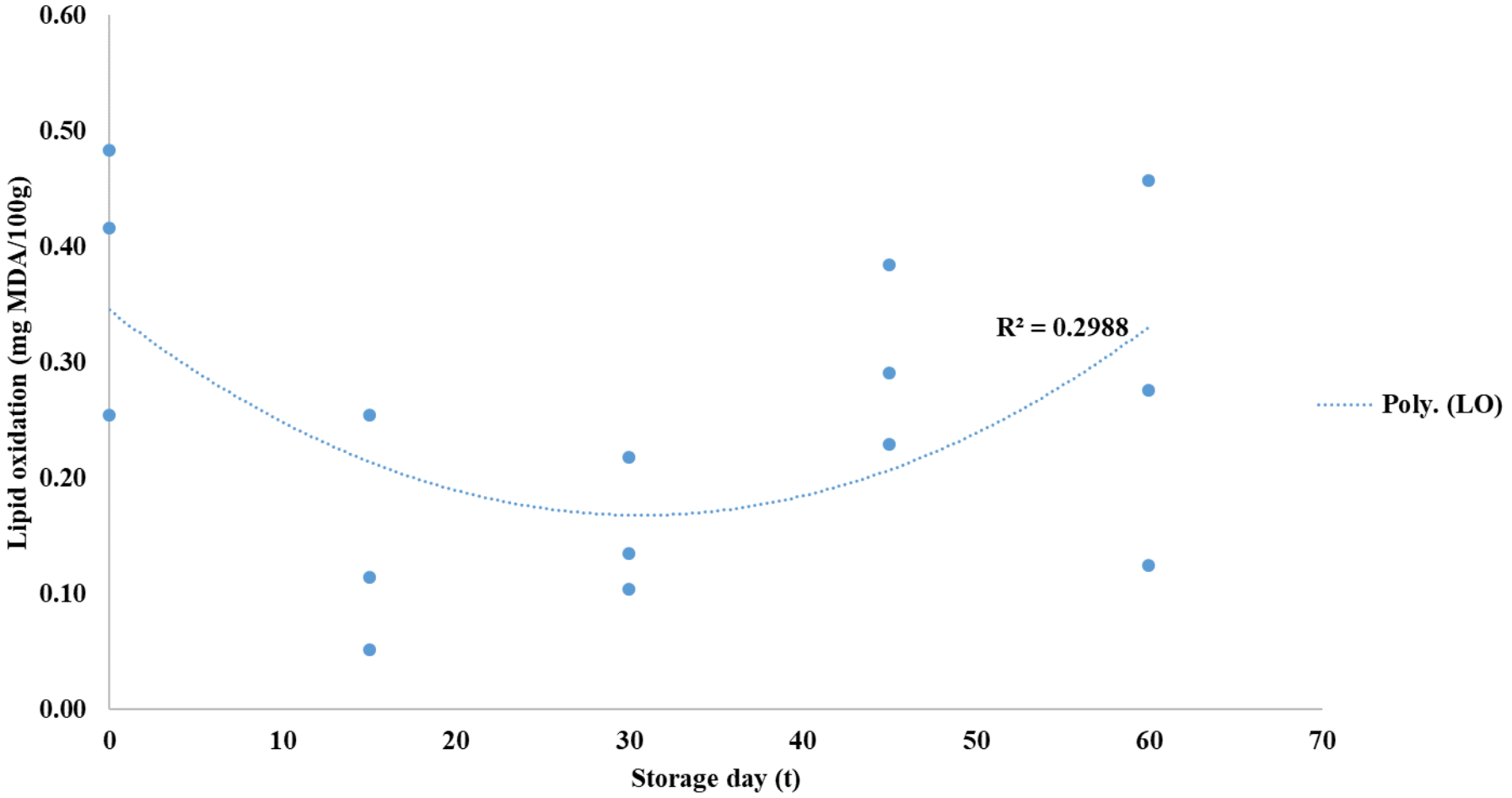

Figure 4.21: Relationship between storage duration and lipid oxidation of smoked chicken fillets cured with varying salt combinations

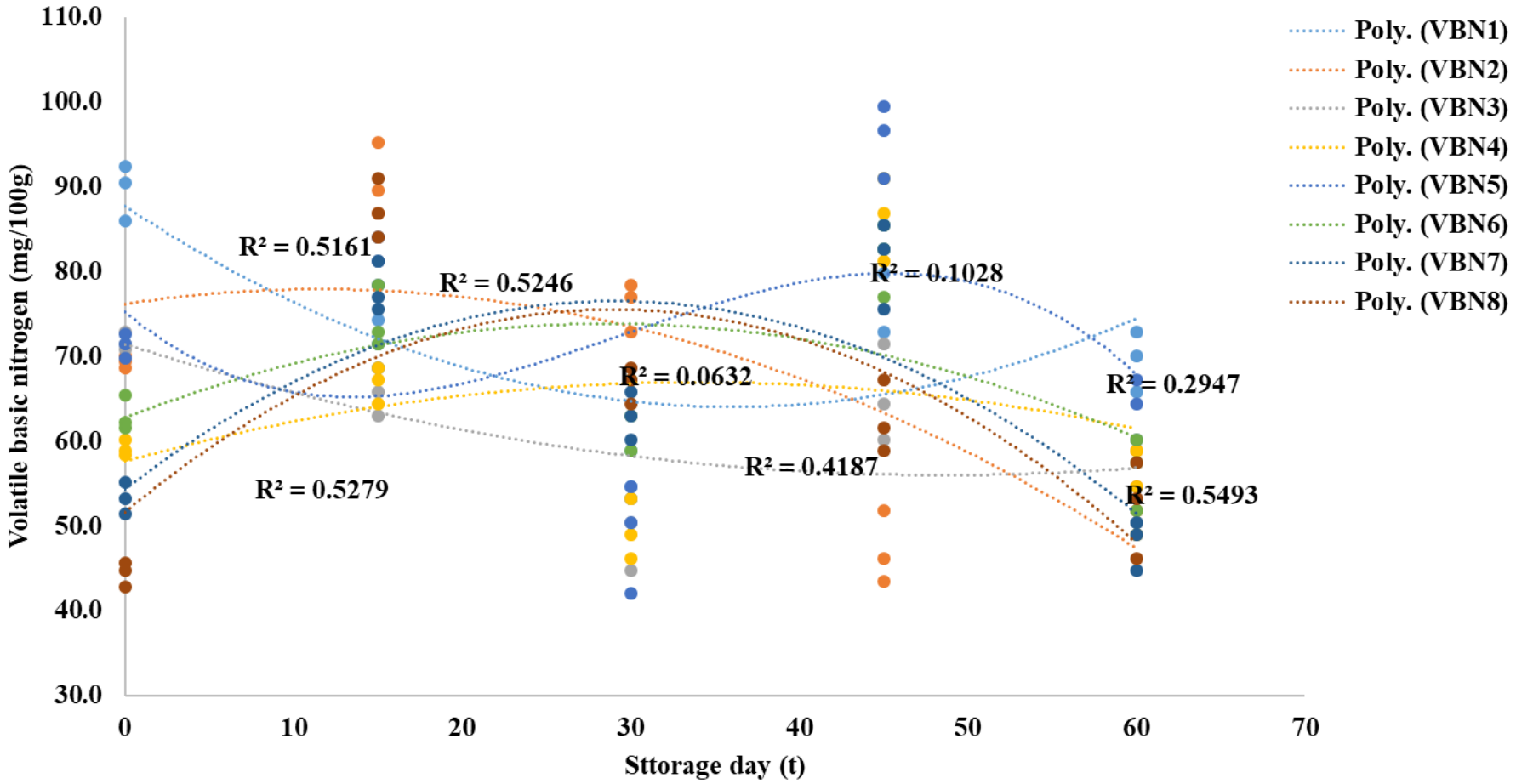

Figure 4.22: Relationship between storage duration and volatile basic nitrogen of smoked chicken fillets cured with varying salt combinations

4.3.4 Colour properties of smoked chicken fillets cured with varying salt combinations

Colour properties of chicken fillets were significantly ($p<0.05$) affected by storage duration (Table 4.10). A gradual decrease in lightness of fillets was observed for all treatments during storage with values ranging from 44.41 in treatment 7 on day 0 to 3.19 in treatment 5 on day 60 of storage. Redness was observed to slightly increased for the fillets for most treatments over storage, although a non-significant ($p>0.05$) effect of storage was observed for treatments 6 and 7. Yellowness increased significantly for all treatments with values ranging from 21.85 in treatment 7 on day 0 and 47.77 in treatment 1 on day 60.

Lightness and redness (Figures 4.23 and 4.24) of fillets was significantly ($p<0.05$) affected by the interaction of treatment and storage day while yellowness (Figure 4.25) was not significantly affected ($p>0.05$). A high positive correlation was observed for lightness in relationship with storage duration with regression coefficient ranging from 0.41 in treatment 2 to 0.80 in treatment 5. Redness of fillets was only slightly correlated with storage duration as least and highest coefficient were 0.08 in treatment 6 and 0.76 in treatment 5. Yellowness of fillets had regression coefficients of 0.38, representing the relationship between the storage duration and yellowness of fillets, irrespective of the treatments.

Table 4.10: Colour properties of smoked chicken fillets cured with varying salt combinations

Parameters	Storage day	Treatments 1	2	3	4	5	6	7	8
Lightness	**0**	12.49^{b}	19.88^{b}	13.65^{c}	17.16^{c}	16.18^{bc}	35.90^{a}	44.41^{a}	16.12^{b}
	15	32.05^{a}	30.80^{a}	33.84^{a}	33.33^{a}	28.43^{a}	37.80^{a}	40.25^{a}	30.88^{a}
	30	25.58^{ab}	21.51^{ab}	29.32^{ab}	30.46^{a}	22.12^{b}	29.33^{ab}	30.14^{b}	32.39^{a}
	45	24.04^{ab}	21.87^{ab}	25.34^{ab}	29.17^{ab}	10.75^{c}	28.83^{ab}	28.60^{b}	27.06^{a}
	60	16.37^{b}	14.46^{b}	19.95^{ab}	20.03^{ab}	3.19^{d}	18.99^{b}	28.17^{b}	13.68^{b}
	SEM	2.42	1.77	2.19	2.03	2.45	2.25	2.08	2.38
Redness	**0**	20.30^{a}	19.21^{ab}	22.15^{ab}	17.02^{ab}	17.94^{c}	16.88	11.27	20.15^{bc}
	15	13.57^{b}	17.05^{b}	18.50^{b}	13.56^{c}	14.53^{d}	16.16	13.91	15.55^{c}
	30	17.69^{ab}	24.56^{a}	19.14^{b}	16.54^{abc}	20.67^{bc}	16.77	13.37	23.73^{ab}
	45	13.60^{b}	19.50^{ab}	18.09^{b}	14.17^{bc}	21.91^{b}	15.99	13.13	20.28^{bc}
	60	18.94^{ab}	23.27^{ab}	25.33^{a}	19.58^{a}	26.57^{a}	17.58	11.18	27.36^{a}
	SEM	1.01	1.02	0.89	0.69	1.14	0.42	0.48	1.26
Yellowness	**0**	36.69^{ab}	35.03^{ab}	32.49^{b}	27.45^{b}	28.61^{ab}	36.27^{ab}	21.85^{d}	46.76^{a}
	15	24.28^{b}	23.81^{b}	26.64^{b}	23.94^{b}	22.66^{b}	25.36^{c}	23.60^{cd}	24.15^{b}
	30	39.00^{ab}	35.60^{ab}	38.26^{ab}	38.39^{a}	30.88^{a}	31.24^{bc}	29.16^{bc}	38.16^{ab}
	45	45.02^{a}	40.16^{a}	45.60^{a}	39.92^{a}	29.58^{ab}	38.10^{ab}	33.41^{b}	41.78^{a}
	60	47.77^{a}	40.46^{a}	46.52^{a}	46.58^{a}	31.71^{a}	46.41^{a}	48.49^{a}	45.42^{a}
	SEM	2.87	2.14	2.52	2.44	1.21	2.25	2.65	2.75

^{a,b,c}: Columns having varying superscripts indicate significant ($p<0.05$) variations in means

Salt inclusion:

1 - 100%NaCl;

2 - 50%NaCl + 50%$CaCl_2$;

3 - 50%NaCl + 50%KCl;

4 - 50%NaCl + 50%$MgCl_2$;

5 - 50%NaCl + 25%$CaCl_2$ + 25%KCl;

6 - 50%NaCl + 25%$CaCl_2$ + 25%$MgCl_2$;

7 - 50%NaCl + 25%KCl + 25%$MgCl_2$;

8 - 25%NaCl + 25%$CaCl_2$ + 25%KCl + 25%$MgCl_2$

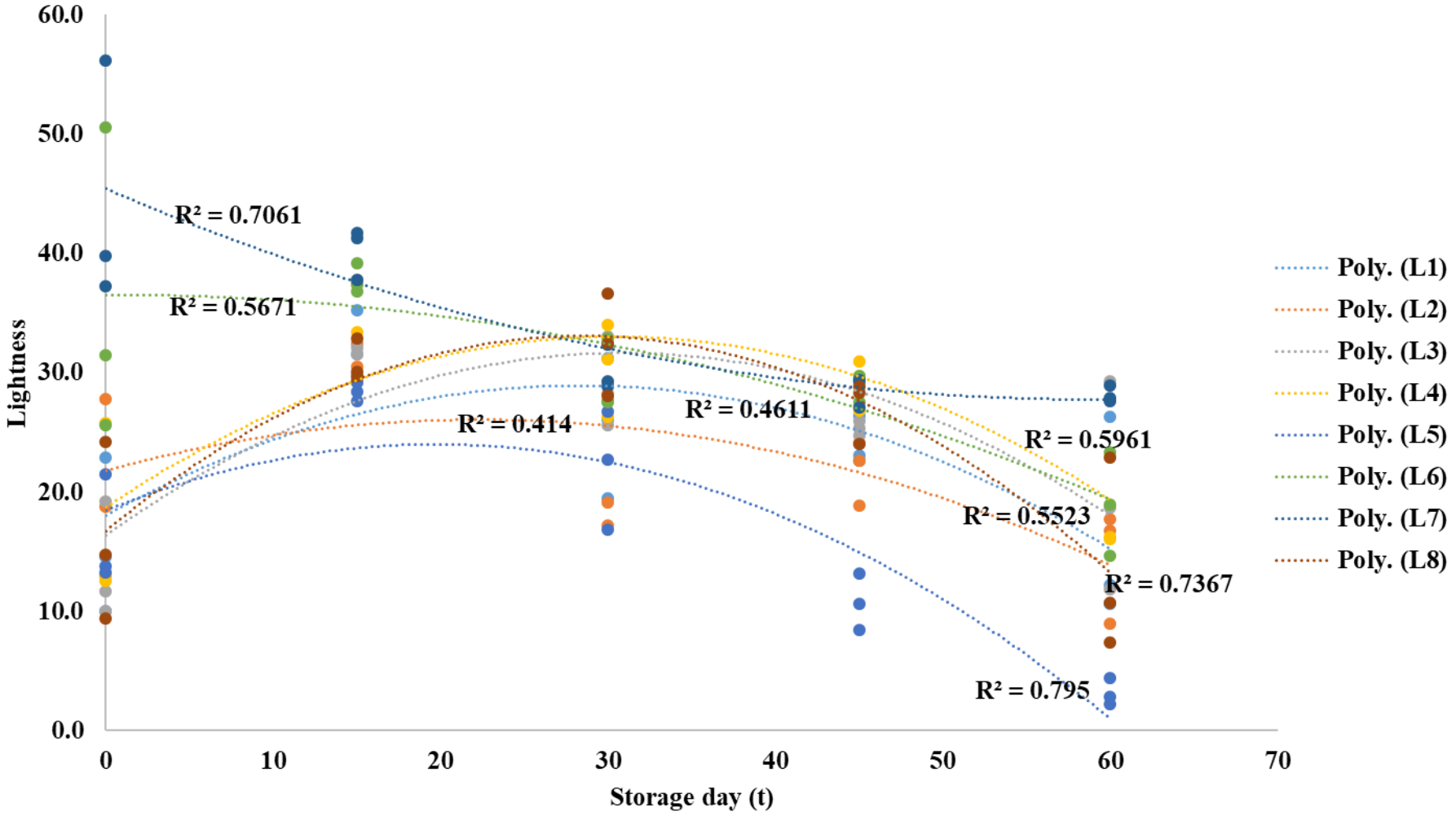

Figure 4.23: Relationship between storage duration and lightness of smoked chicken fillets cured with varying salt combinations

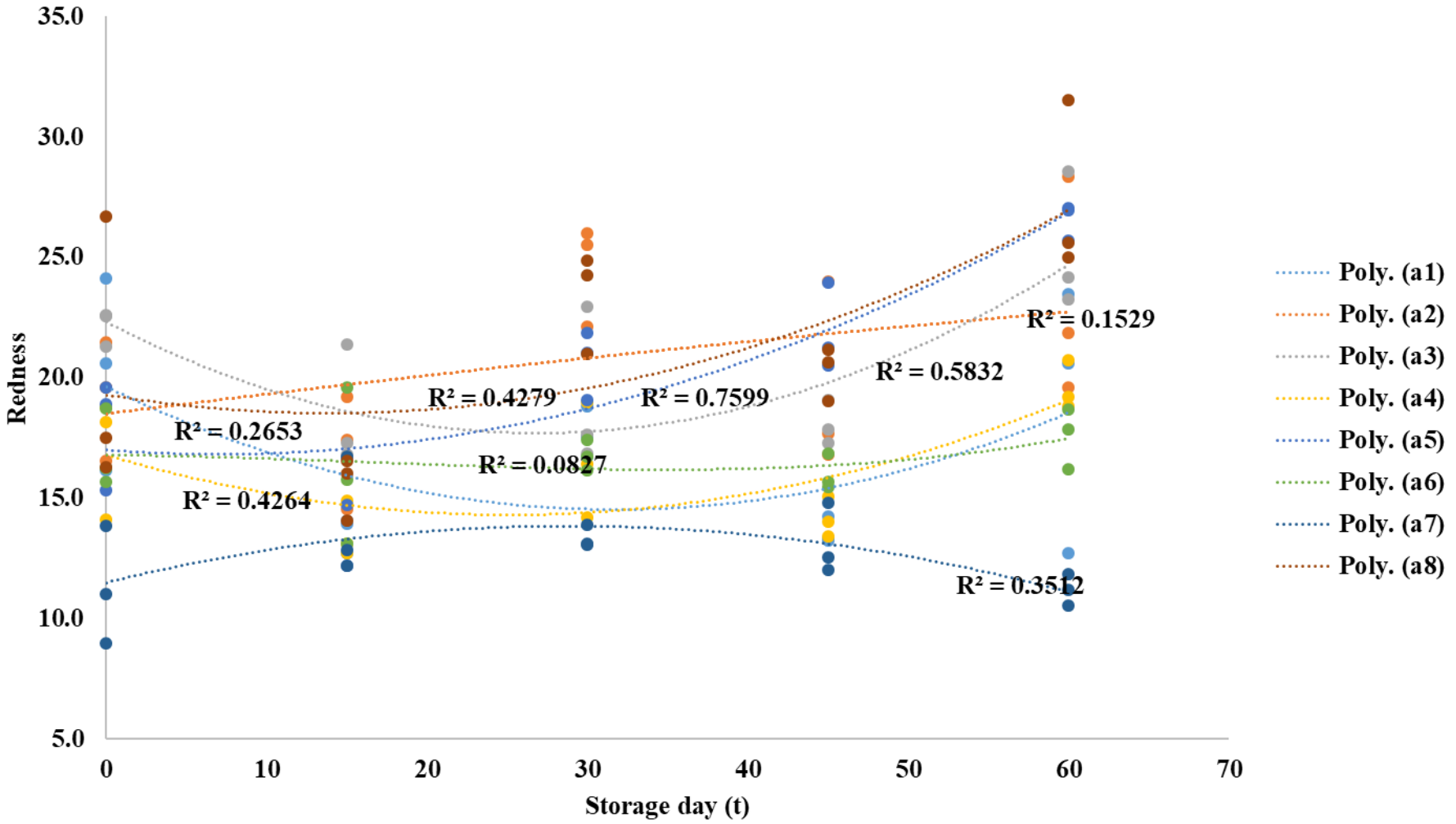

Figure 4.24: Relationship between storage duration and redness of smoked chicken fillets cured with varying salt combinations

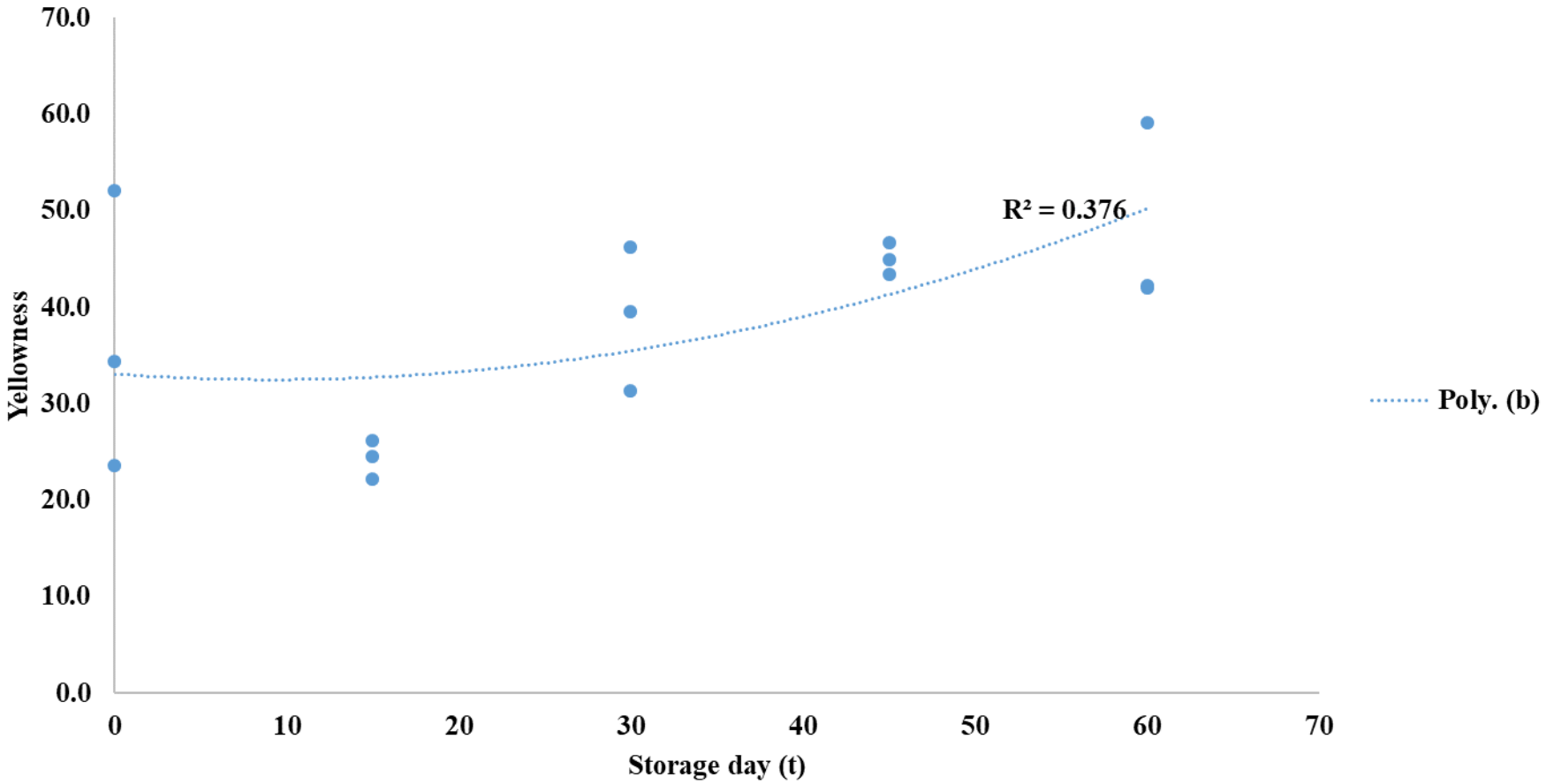

Figure 4.25: Relationship between storage duration and yellowness of smoked chicken fillets cured with varying salt combinations

4.3.5 Microbial load of smoked chicken fillets cured with varying salt combinations

Significant ($p<0.05$) effect of storage period was observed for microbial load (Table 4.11) of cured smoked chicken fillets. A slight but significant ($p<0.05$) increase was observed for all treatments over storage for both total anaerobic bacteria and lactic acid bacteria counts. Total anaerobic count was lowest on day 0 in treatment 6 (3.21) and highest in treatment 4 (4.01) on day 60. Lactic acid bacteria count increased from 2.58 in treatment 2 on day 0 to 3.82 in treatment 2 on day 60.

The interaction of storage day and salt treatment on total anaerobic bacteria count (Figure 4.26) was non-significant while a significant effect of their interaction was observed for lactic acid bacteria count (Figure 4.27) of the fillets. A high correlation with storage duration was observed for both parameters. Regression coefficient was 0.69 for total anaerobic bacteria count while least and highest coefficients were 0.42 in treatment 4 and 0.90 in treatment 2.

Table 4.11: Microbial load (cfu/g) of smoked chicken fillets cured with varying salt combinations

Parameters	Storage day	Treatments 1	2	3	4	5	6	7	8
TAB counts	**0**	3.40^{c}	3.26^{b}	3.51	3.33^{bc}	3.38^{b}	3.21^{ab}	3.32^{bc}	3.31^{b}
	15	3.39^{c}	3.03^{b}	3.29	2.99^{c}	2.94^{c}	2.76^{b}	3.11^{c}	2.89^{c}
	30	3.57^{b}	3.60^{ab}	3.15	3.64^{ab}	3.71^{a}	3.41^{ab}	3.50^{b}	3.54^{ab}
	45	3.95^{a}	3.94^{a}	3.92	3.79^{a}	3.87^{a}	3.79^{a}	3.77^{a}	3.81^{a}
	60	3.80^{a}	3.62^{ab}	3.77	4.01^{a}	3.90^{a}	3.83^{a}	3.74^{a}	3.66^{ab}
	SEM	0.06	0.11	0.13	0.11	0.10	0.13	0.07	0.10
LAB counts	**0**	2.78^{c}	2.58^{d}	3.32^{ab}	3.11^{c}	2.95^{c}	2.87^{b}	2.94^{c}	2.86^{b}
	15	3.21^{b}	3.26^{c}	3.12^{b}	3.27^{b}	3.25^{b}	3.14^{b}	3.13^{bc}	3.35^{a}
	30	3.65^{a}	3.49^{bc}	3.47^{a}	3.40^{b}	3.53^{a}	3.12^{b}	3.31^{b}	3.44^{a}
	45	3.56^{a}	3.70^{ab}	3.59^{a}	3.61^{a}	3.67^{a}	3.61^{a}	3.57^{a}	3.48^{a}
	60	3.70^{a}	3.82^{a}	3.61^{a}	3.59^{a}	3.63^{a}	3.62^{a}	3.60^{a}	3.61^{a}
	SEM	0.10	0.12	0.06	0.05	0.08	0.09	0.07	0.08

^{a,b,c}: Columns having varying superscripts indicate significant ($p<0.05$) variations in means

TAB counts: Total Anaerobic Bacteria Counts; LAB Counts: Lactic Acid Bacteria Counts

Salt inclusion:

1 - 100%NaCl;

2 - 50%NaCl + 50%$CaCl_2$;

3 - 50%NaCl + 50%KCl;

4 - 50%NaCl + 50%$MgCl_2$;

5 - 50%NaCl + 25%$CaCl_2$ + 25%KCl;

6 - 50%NaCl + 25%$CaCl_2$ + 25%$MgCl_2$;

7 - 50%NaCl + 25%KCl + 25%$MgCl_2$;

8 - 25%NaCl + 25%$CaCl_2$ + 25%KCl + 25%$MgCl_2$

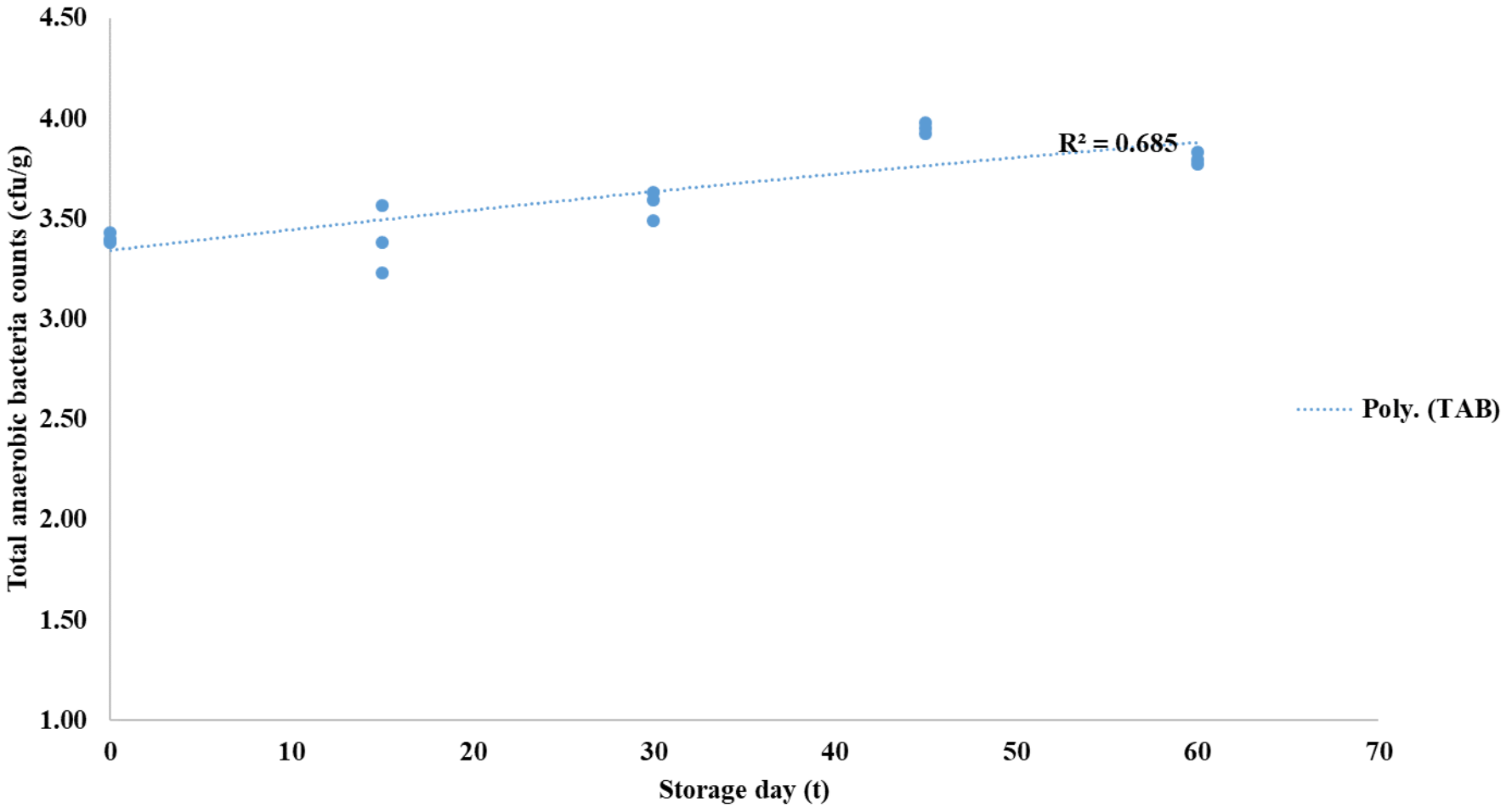

Figure 4.26: Relationship between storage duration and total anaerobic bacteria counts of smoked chicken fillets cured with varying salt combinations

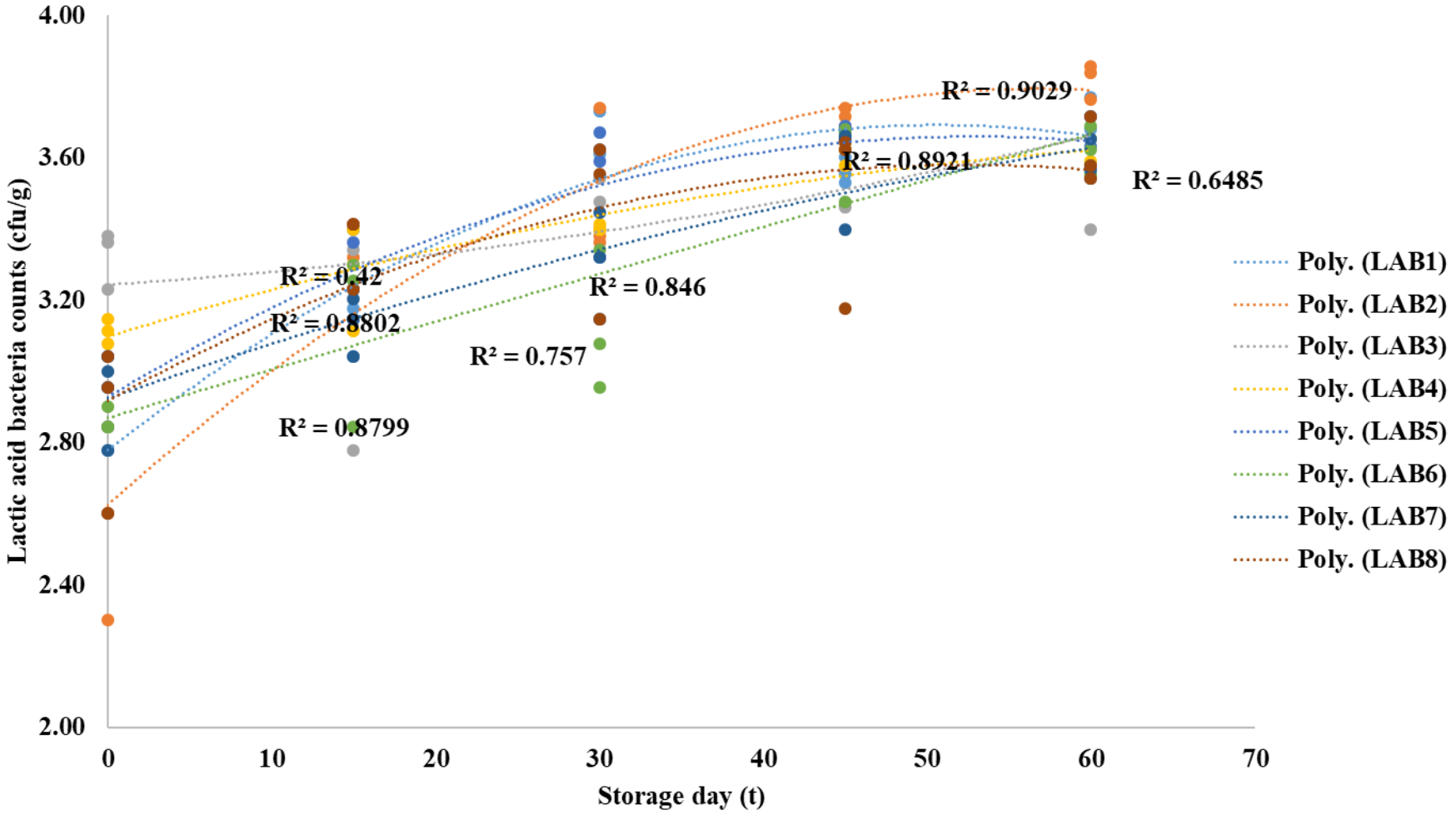

Figure 4.27: Relationship between storage duration and lactic acid bacteria count of smoked chicken fillets cured with varying salt combinations

4.3.6 Sensory quality of smoked chicken fillets cured with varying salt combinations

Sensory quality of cured smoked fillets was significantly ($p<0.05$) affected by storage period (Table 4.12). Presence of visible microbial growth during storage of fillets was only significant for treatments 1, 3, 4 and 7. A decrease occurred during storage from 3.00 (no visible microbial growth on surfaces) in all treatments on day 0 to a range of 2.40 to 2.90 (visible on some surfaces) on day 60 of storage. Odour of fillets decreased for all treatments from 3.00 (normal characteristic cured smoked odour) on day 0 of storage to 1.80 (off or rancid odour) in treatment 4 on day 60 of storage. Overall quality was also observed to reduce significantly from 3.00 (excellent) to a range of 1.30 to 2.50 (slightly unacceptable to acceptable) by day 60 of storage.

Interaction of storage day and sensory quality showed a non-significant ($p>0.05$) effect on microbial growth and odour (Figures 4.28 and 4.29) while overall quality (Figure 4.30) was significantly ($p<0.05$) affected. A slight correlation was also observed for sensory microbial growth and odour of fillets. Regression coefficients of 0.12 and 0.17 were obtained for microbial growth and odour, respectively. Regression coefficients for overall quality ranged from 0.10 (treatment 5) to 0.51 (treatment 7).

Table 4.12: Sensory properties of smoked chicken fillets cured with varying salt combinations

Parameters	Storage day	Treatments 1	2	3	4	5	6	7	8
Microbial growth	**0**	3.00^{a}	3.00	3.00^{a}	3.00^{a}	3.00	3.00	3.00^{a}	3.00
	15	2.80^{ab}	3.00	3.00^{a}	2.90^{a}	3.00	3.00	2.90^{a}	3.00
	30	2.90^{a}	2.90	2.80^{ab}	2.90^{a}	2.90	2.80	2.80^{ab}	2.80
	45	2.50^{a}	2.90	2.50^{b}	2.40^{b}	2.80	2.90	2.60^{ab}	2.90
	60	2.90^{a}	3.00	2.90^{a}	2.80^{a}	2.80	2.90	2.40^{b}	3.00
	SEM	0.05	0.03	0.05	0.06	0.04	0.04	0.07	0.03
Odour	**0**	3.00^{a}	3.00^{a}	3.00^{a}	3.00^{a}	3.00	3.00^{a}	3.00^{a}	3.00^{a}
	15	2.50^{ab}	2.70^{abc}	2.60^{ab}	2.50^{ab}	2.80	2.60^{a}	2.40^{bc}	2.80^{ab}
	30	2.10^{b}	2.20^{c}	2.50^{ab}	2.30^{bc}	2.60	1.90^{c}	1.90^{c}	2.30^{b}
	45	2.60^{ab}	2.80^{ab}	2.30^{b}	2.40^{b}	2.70	2.40^{ab}	2.30^{bc}	2.60^{ab}
	60	2.60^{ab}	2.30^{bc}	2.60^{ab}	1.80^{c}	2.80	1.90^{b}	2.50^{ab}	2.30^{b}
	SEM	0.09	0.09	0.08	0.09	0.07	0.11	0.10	0.08
Overall quality	**0**	3.00^{a}	3.00^{a}	3.00^{a}	3.00^{a}	3.00^{a}	3.00^{a}	3.00^{a}	3.00^{a}
	15	2.20^{b}	2.40^{b}	2.20^{b}	2.20^{b}	2.00^{c}	1.80^{bc}	1.80^{b}	2.50^{b}
	30	1.60^{c}	2.20^{bc}	1.90^{b}	2.10^{b}	2.60^{ab}	1.50^{bc}	1.70^{b}	2.20^{c}
	45	2.00^{b}	2.50^{b}	1.80^{b}	2.10^{b}	2.50^{b}	1.90^{b}	1.50^{b}	2.30^{b}
	60	1.90^{ab}	1.90^{c}	1.90^{b}	1.40^{c}	2.50^{b}	1.40^{c}	1.30^{b}	1.80^{c}
	SEM	0.09	0.08	0.10	0.10	0.08	0.10	0.11	0.08

^{a,b,c}: Columns having varying superscripts indicate significant ($p<0.05$) variations in means

Salt inclusion:

1 - 100%NaCl;

2 - 50%NaCl + 50%$CaCl_2$;

3 - 50%NaCl + 50%KCl;

4 - 50%NaCl + 50%$MgCl_2$;

5 - 50%NaCl + 25%$CaCl_2$ + 25%KCl;

6 - 50%NaCl + 25%$CaCl_2$ + 25%$MgCl_2$;

7 - 50%NaCl + 25%KCl + 25%$MgCl_2$;

8 - 25%NaCl + 25%$CaCl_2$ + 25%KCl + 25%$MgCl_2$

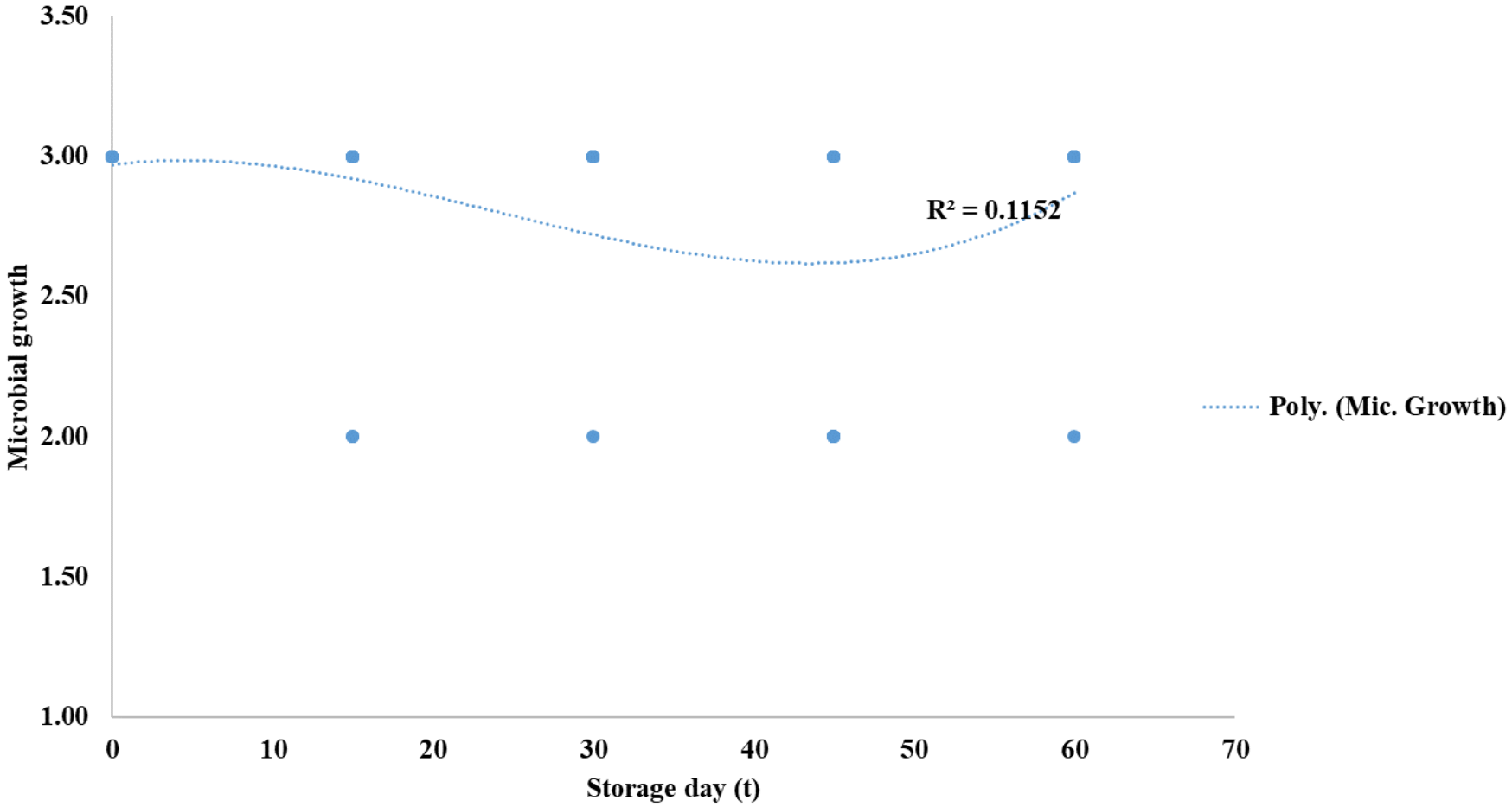

Figure 4.28: Relationship between storage duration and microbial growth of smoked chicken fillets cured with varying salt combinations

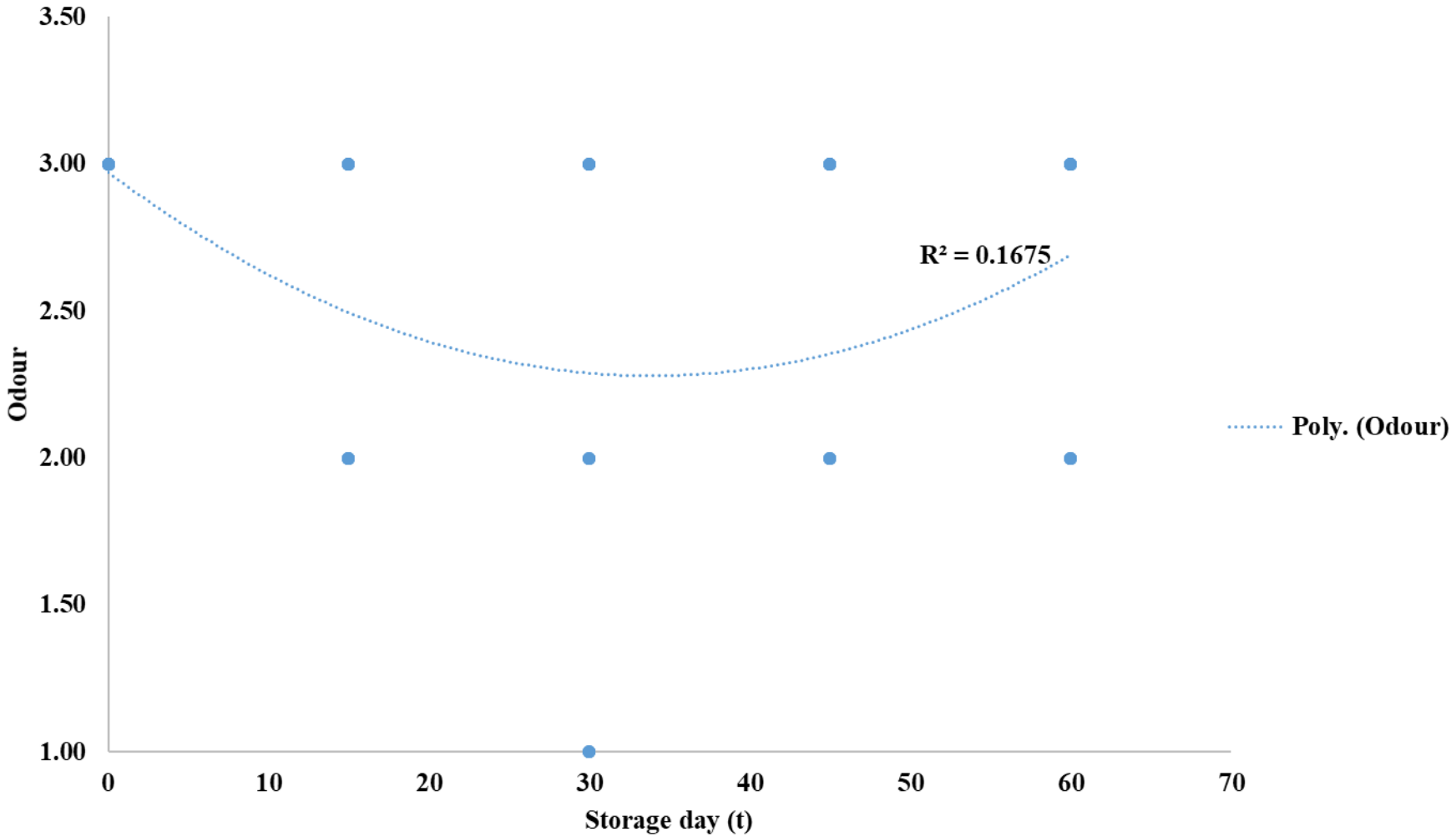

Figure 4.29: Relationship between storage duration and odour of smoked chicken fillets cured with varying salt combinations

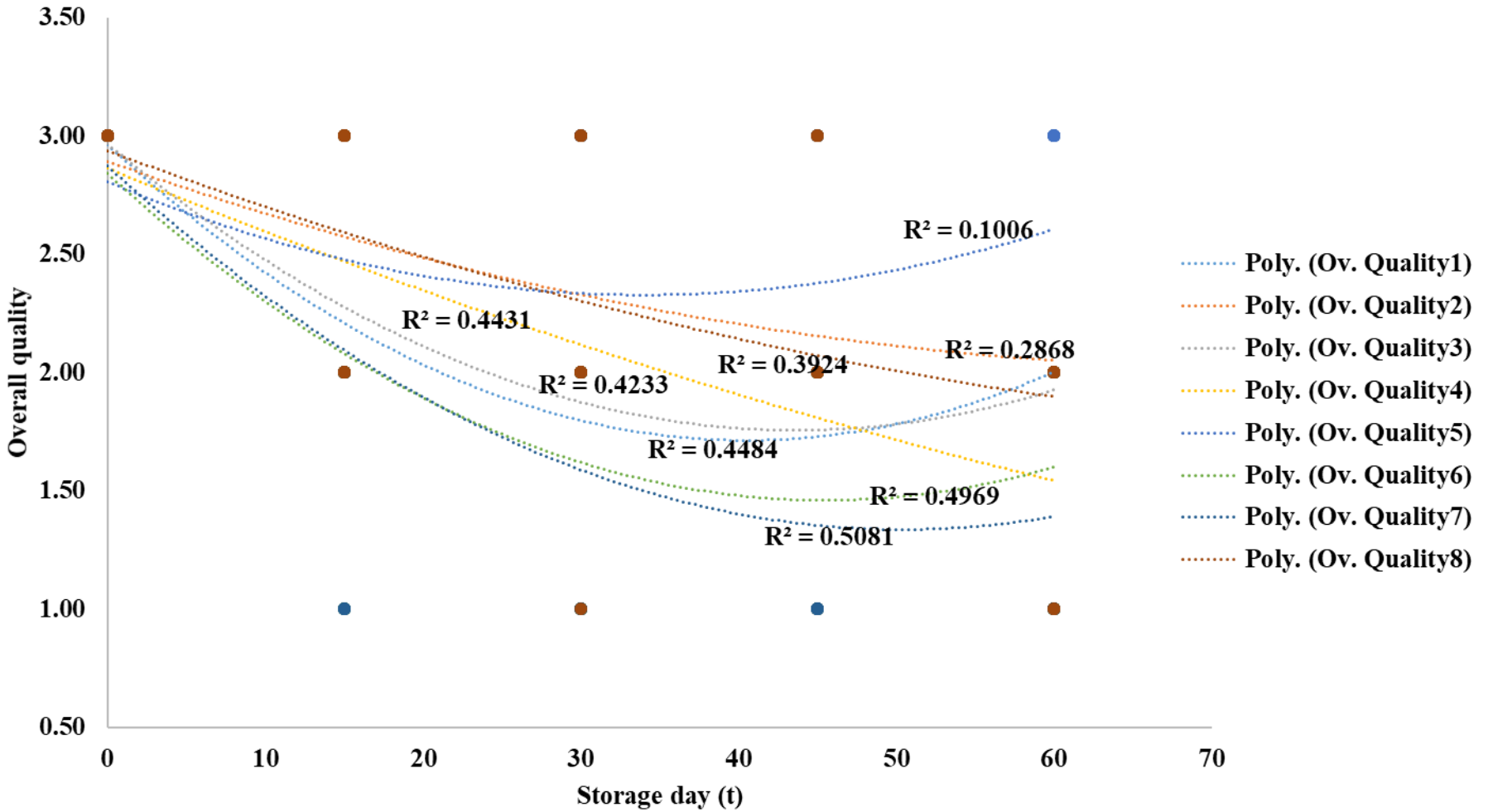

Figure 4.30: Relationship between storage duration and overall quality of smoked chicken fillets cured with varying salt combinations

4.3.7 Fatty acid profile of smoked fillets cured with varying salt combinations

Fatty acid composition of cured-smoked chicken fillets on day 0 of storage (Table 4.13) showed significant ($p<0.05$) effect of treatment on all parameters measured. Lauric acid was highest ($p<0.05$) in T4 (5.42%) and T8 (5.17%) while least value was observed in T1 (4.17%). Stearic acid was highest ($p<0.05$) in T4 (7.64%) and closely followed T5 (7.26%) and T8 (7.27%) while least value was observed in T1 (5.70%). Palmitic acid was highest ($p<0.05$) in T4 (6.85%) and least in T8 (5.38%) and T1 (5.31%) while arachidonic acid was highest ($p<0.05$) in T4 (8.18%) closely followed by T8 (7.81%) and T5 (7.73%). Least value was obtained in T1 (6.04%). Oleic (7.68%, 5.43%) and Margaric (7.24%, 5.54%) acids were highest in T4 and least in T1. Similar trends were observed for all other parameters measured.

A general decline in fatty acids composition of fillets was observed after storage for 60 days (Table 4.14). All parameters were also significantly affected by salt treatments except for butyric and propionic acids and significantly highest fatty acid values were observed in T1 for all other parameters measured except for valeric acid and caprylic acid. Lauric and stearic acids were least in T2 (1.82%) and T7 (2.10%) while palmitic (2.13%) and arachidonic (2.44%) acids were least in T2. No significant difference was observed within other treatments for oleic acid while margaric acids were significantly least in T2 (2.33%). No significant difference was also observed within other treatments for linoleic acid while ligoleric acid was least in T2 (2.94%), T6 (2.77%) and T7 (2.77%). Myristic acid was observed to be least in T2 (1.92%) while behemic acid was least in T7 (2.62%). Valeric acid was highest in T7 (1.79%) and least in T2 (1.18%) while palmtoleic acid was least in T4 (2.02%). Caprylic acid was significantly highest in T8 (2.35%) and least in T3 (1.11%) while acetic acid was least in T4 (1.77%).

Table 4.13: Fatty acid profile of smoked chicken fillets cured with varied salt combinations on day 0 of storage

	Treatments								
Parameters(%)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**SEM**
Lauric acid	4.17^{e}	4.71^{cd}	4.83^{bc}	5.42^{a}	5.14^{ab}	4.37^{de}	4.41^{de}	5.17^{a}	0.09
Stearic acid	5.70^{f}	6.64^{c}	6.68^{c}	7.64^{a}	7.26^{b}	6.26^{d}	5.98^{e}	7.27^{b}	0.13
Palmitic acid	5.31^{e}	5.81^{d}	6.14^{c}	6.85^{a}	6.50^{b}	5.62^{de}	5.38^{e}	6.57^{b}	0.12
Arachidonic acid	6.04^{f}	6.91^{d}	7.26^{c}	8.18^{a}	7.73^{b}	6.76^{d}	6.39^{e}	7.81^{b}	0.15
Oleic acid	5.43^{e}	6.54^{c}	6.63^{c}	7.68^{a}	7.14^{b}	6.32^{d}	5.92^{d}	7.35^{b}	0.15
Margaric acid	5.54^{f}	6.41^{cd}	6.39^{d}	7.24^{a}	6.73^{bc}	5.97^{e}	5.70^{ef}	6.92^{ab}	0.12
Linoleic acid	5.48^{f}	6.63^{c}	6.72^{c}	7.44^{a}	7.14^{b}	6.19^{d}	5.82^{e}	7.22^{b}	0.14
Ligoleric acid	7.31^{f}	8.65^{c}	8.85^{c}	9.82^{a}	9.34^{b}	8.17^{d}	7.77^{e}	9.63^{a}	0.18
Myristic acid	4.58^{f}	5.24^{d}	5.57^{d}	6.15^{a}	5.73^{bc}	5.09^{d}	4.75^{e}	5.89^{b}	0.11
Behemic acid	6.54^{e}	7.87^{c}	8.20^{c}	9.20^{a}	8.63^{b}	7.50^{d}	7.24^{d}	8.60^{b}	0.17
Butyric acid	1.73^{d}	2.08^{c}	2.19^{bc}	2.48^{a}	2.18^{bc}	1.88^{d}	1.88^{d}	2.30^{ab}	0.05
Valeric acid	2.10^{e}	2.41^{bc}	2.49^{b}	2.72^{a}	2.49^{b}	2.28^{cd}	2.19^{de}	2.70^{a}	0.05
Palmtoleic acid	5.10^{h}	5.90^{e}	6.16^{d}	6.76^{a}	6.41^{c}	5.65^{f}	5.37^{g}	6.56^{b}	0.12
Caprylic acid	2.76^{f}	3.41^{c}	3.44^{c}	3.88^{a}	3.65^{b}	3.24^{d}	3.08^{e}	3.75^{ab}	0.07
Propionic acid	2.37^{e}	2.74^{c}	2.85^{bc}	3.09^{a}	2.86^{bc}	2.52^{d}	2.50^{de}	2.97^{ab}	0.05
Acetic acid	4.37^{f}	5.04^{d}	5.23^{c}	5.78^{a}	5.48^{b}	4.84^{e}	4.49^{f}	5.55^{b}	0.10

[a,b,c,…]- Rows with different superscripts indicate significant ($p<0.05$) variations in means

SEM: Standard Error of Mean

T1- 100%NaCl;

T2- 50%NaCl + 50%$CaCl_2$;

T3- 50%NaCl + 50%KCl;

T4- 50%NaCl + 50%$MgCl_2$;

T5- 50%NaCl + 25%$CaCl_2$ + 25%KCl;

T6- 50%NaCl + 25%$CaCl_2$ + 25%$MgCl_2$;

T7- 50%NaCl + 25%KCl + 25%$MgCl_2$;

T8- 25%NaCl + 25%$CaCl_2$ + 25%KCl + 25%$MgCl_2$

Table 4.14: Fatty acid profile of smoked chicken fillets cured with varied salt combinations on day 60 of storage

	Treatments								
Parameters (%)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**SEM**
Lauric acid	2.91^{a}	1.82^{c}	2.42^{ab}	2.30^{bc}	2.32^{bc}	2.30^{bc}	2.57^{ab}	2.49^{ab}	0.08
Stearic acid	3.71^{a}	2.55^{bc}	2.72^{b}	2.75^{b}	2.63^{b}	2.73^{b}	2.10^{c}	2.83^{b}	0.10
Palmitic acid	3.52^{a}	2.13^{c}	2.65^{b}	2.66^{b}	2.53^{bc}	2.64^{b}	2.80^{b}	2.79^{b}	0.09
Arachidonic acid	4.20^{a}	2.44^{c}	2.45^{c}	2.71^{bc}	2.59^{bc}	2.78^{ab}	2.59^{bc}	3.30^{b}	0.13
Oleic acid	3.81^{a}	2.47^{b}	2.45^{b}	2.65^{b}	2.64^{b}	2.70^{b}	2.93^{b}	2.79^{b}	0.10
Margaric acid	3.66^{a}	2.33^{c}	2.77^{bc}	2.52^{bc}	2.62^{bc}	2.58^{bc}	2.86^{b}	2.81^{bc}	0.09
Linoleic acid	3.68^{a}	2.78^{b}	2.82^{b}	2.66^{b}	2.68^{b}	2.71^{b}	2.89^{b}	2.69^{b}	0.08
Ligoleric acid	4.69^{a}	2.94^{c}	3.30^{bc}	3.21^{bc}	3.13^{bc}	2.77^{c}	2.77^{c}	3.62^{b}	0.13
Myristic acid	2.96^{a}	1.92^{c}	2.53^{ab}	2.28^{bc}	2.53^{ab}	2.54^{ab}	2.79^{ab}	2.65^{ab}	0.08
Behemic acid	4.46^{a}	3.08^{bcd}	3.45^{b}	3.09^{bcd}	2.75^{cd}	3.38^{bc}	2.62^{d}	3.36^{bc}	0.13
Butyric acid	1.63	1.55	1.44	1.44	1.54	1.52	1.70	1.56	0.04
Valeric acid	1.51^{ab}	1.18^{b}	1.59^{ab}	1.66^{ab}	1.62^{ab}	1.60^{ab}	1.79^{a}	1.46^{ab}	0.06
Palmtoleic acid	3.39^{a}	2.27^{bc}	2.55^{bc}	2.02^{c}	2.56^{bc}	2.49^{bc}	2.73^{b}	2.61^{bc}	0.09
Caprylic acid	2.26^{a}	1.51^{bc}	1.11^{c}	1.78^{ab}	1.89^{ab}	1.80^{ab}	2.21^{a}	2.35^{a}	0.10
Propionic acid	1.95	1.41	1.71	1.70	1.71	1.71	1.66	1.65	0.06
Acetic acid	3.18^{a}	2.19^{bc}	2.43^{b}	1.77^{c}	2.29^{bc}	2.26^{bc}	2.66^{bc}	2.39^{ab}	0.10

a,b,c,...- Rows with different superscripts indicate significant ($p<0.05$) variations in means

SEM: Standard Error of Mean

T1- 100%NaCl;

T2- 50%NaCl + 50%$CaCl_2$;

T3- 50%NaCl + 50%KCl;

T4- 50%NaCl + 50%$MgCl_2$;

T5- 50%NaCl + 25%$CaCl_2$ + 25%KCl;

T6- 50%NaCl + 25%$CaCl_2$ + 25%$MgCl_2$;

T7- 50%NaCl + 25%KCl + 25%$MgCl_2$;

T8- 25%NaCl + 25%$CaCl_2$ + 25%KCl + 25%$MgCl_2$

4.3.8 Mineral composition of smoked fillets cured with varying combinations of chloride salts

Mineral composition of fillets on day 0 and after storage till day 60 is presented in Figures 4.31 and 4.32. Significant ($p<0.05$) effect of salt treatments were observed for the minerals assayed in the fillets. Sodium content was observed to increase with storage for most treatments. In treatment 1, sodium content increased from 23.7g/kg on day 0 to 28.2g/kg on day 60, while in treatment 2 calcium drastically reduced from 53.1g/kg on day 0 to 3.7g/kg on day 60 of storage while sodium increased slightly from 17.8g/kg on day 0 to 19.6g/kg on day 60. In treatment 3, potassium level reduced over storage from 35.7g/kg to 25.5g/kg while sodium slightly increased from 17.8g/kg to 19.6g/kg. In treatment 4, magnesium decreased from 7.1g/kg on day 0 to 1.4g/kg on day 60 while sodium increased from 17.2g/kg to 21.1g/kg. Treatment 5 recorded a drastic decrease in calcium and potassium values and a slight decrease in sodium value. Calcium reduced from 35.7g/kg to 2.9g/kg, potassium reduced from 23.3g/kg to 15.1g/kg and sodium reduced from 21.3g/kg to 21.0g/kg. In treatment 6, calcium drastically reduced from 30.3g/kg to 3.1g/kg, magnesium slightly decreased from 4.8g/kg to 4.2g/kg and sodium increased from 18.3g/kg to 22.5g/kg over storage. Sodium, magnesium and potassium in treatment 7 slightly decreased over storage from 22.5g/kg to 15.1g/kg, 5.1g/kg to 4.2g/kg and 20.1g/kg to 16.4g/kg, respectively. In treatment 8, no decrease was observed for magnesium level (4.7g/kg) during storage, potassium and sodium reduced slightly from 26.0g/kg to 21.8g/kg and 17.5g/kg to 16.4g/kg, respectively. Calcium however reduced considerably from 33.6g/kg on day 0 to 2.8g/kg on day 60.

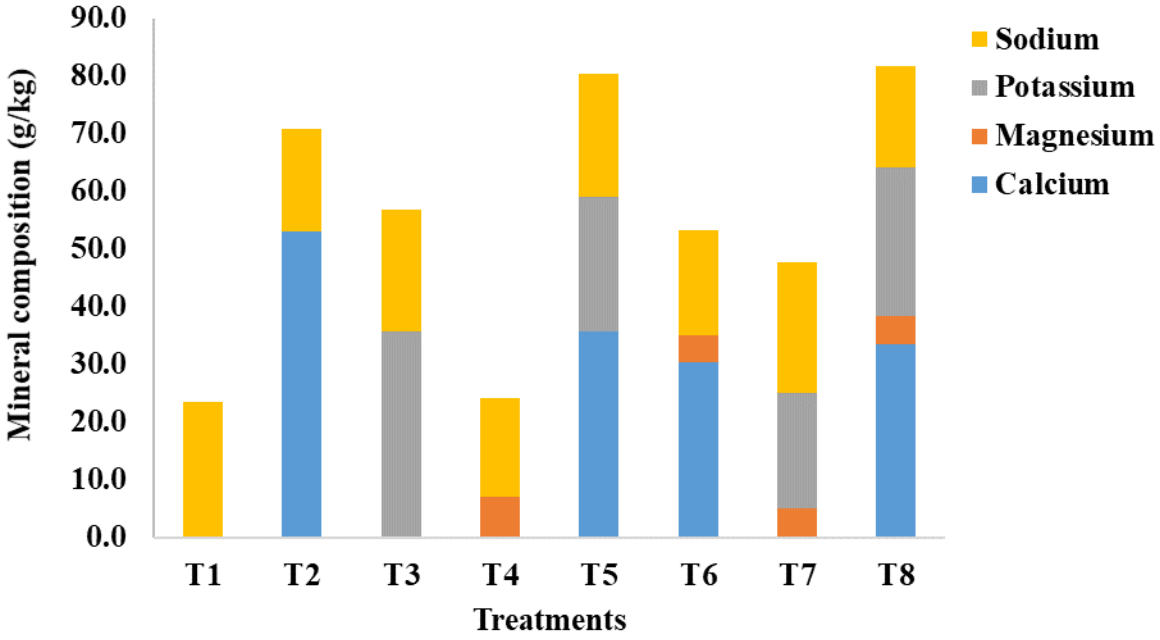

Figure 4.31: Mineral composition of smoked chicken fillets cured with varying salt combinations on day 0 of storage

T1- 100%NaCl;

T2- 50%NaCl + 50%$CaCl_2$;

T3- 50%NaCl + 50%KCl;

T4- 50%NaCl + 50%$MgCl_2$;

T5- 50%NaCl + 25%$CaCl_2$ + 25%KCl;

T6- 50%NaCl + 25%$CaCl_2$ + 25%$MgCl_2$;

T7- 50%NaCl + 25%KCl + 25%$MgCl_2$;

T8- 25%NaCl + 25%$CaCl_2$ + 25%KCl + 25%$MgCl_2$

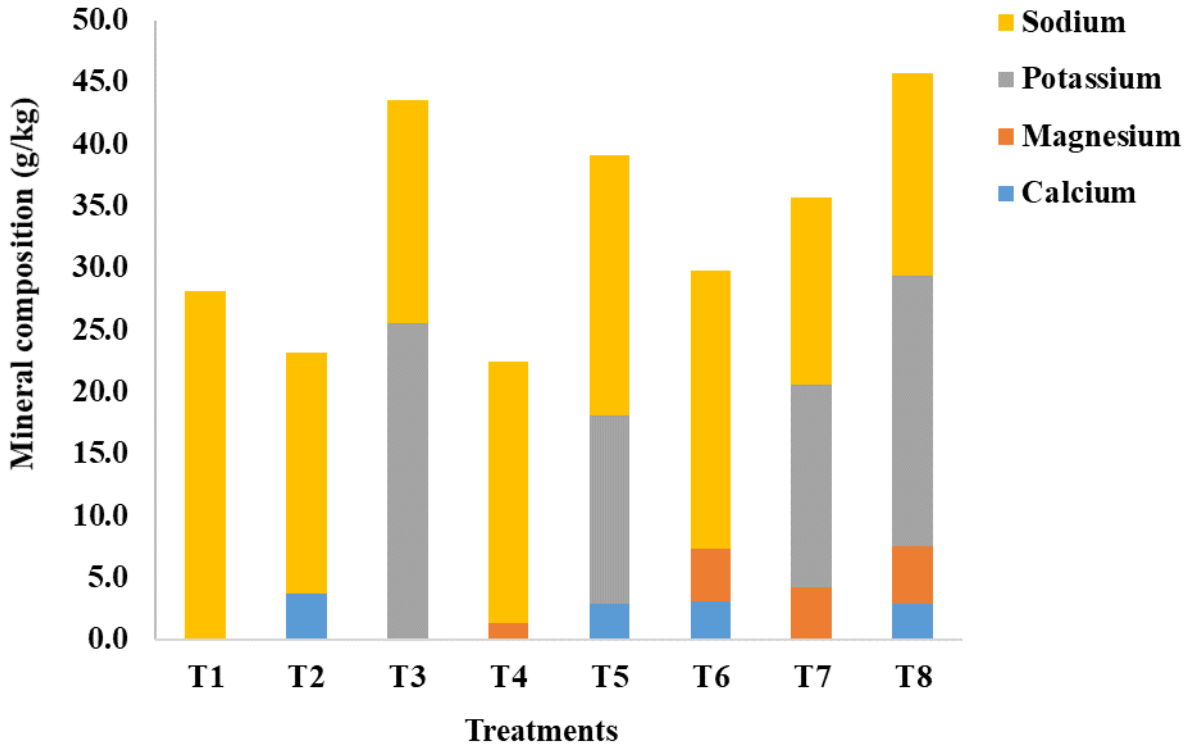

Figure 4.32: Mineral composition of smoked chicken fillets cured with varying salt combinations on day 60 of storage

T1- 100%NaCl;

T2- 50%NaCl + 50%$CaCl_2$;

T3- 50%NaCl + 50%KCl;

T4- 50%NaCl + 50%$MgCl_2$;

T5- 50%NaCl + 25%$CaCl_2$ + 25%KCl;

T6- 50%NaCl + 25%$CaCl_2$ + 25%$MgCl_2$;

T7- 50%NaCl + 25%KCl + 25%$MgCl_2$;

T8- 25%NaCl + 25%$CaCl_2$ + 25%KCl + 25%$MgCl_2$

4.4 Experiment Four: Consumer Acceptability and Keeping Quality of Cured Smoked Chicken Fillets Developed with Bell Pepper Extract and Reduced Sodium Level

4.4.1 Physicochemical properties of cured smoked chicken fillets stored at refrigerated and room temperature

Physicochemical properties of cured-smoked chicken fillets (Table 4.15) were significantly ($p<0.05$) affected by the condition of storage, either under refrigeration or at room temperature. The pH and shear force of fillets did not follow any particular pattern over storage while water holding capacity was observed to increase till day 60 of storage, after which a decline was observed till day 90. Highest ($p<0.05$) pH was observed in fillets stored at room temperature (5.92) on day 0 and least in fillets stored under refrigeration (5.86) on day 60. Water holding capacity was least (34.67) in fillets stored at refrigerated temperature on day 0 and highest (43.33) on day 90 in fillets stored at room temperature. Shear force was least (1.53) on day 0 and highest (1.92) on day 90 in fillets stored at room temperature.

Significant ($p<0.05$) interaction effect of storage duration and storage condition was observed for pH (Figure 4.33) of fillets while no significant effect was observed for water holding capacity (Figure 4.34) and shear force (Figure 4.35) of fillets. All physicochemical properties showed slight correlation with the storage duration. Regression coefficient was 0.35 in fillets stored at refrigerated temperature and 0.30 in fillets stored at room temperature. Regression coefficients for both water holding capacity and shear force were 0.16 and 0.27, respectively.

Table 4.15: Physicochemical properties of cured smoked chicken fillets stored at refrigerated and room temperature

		Treatments	
Parameters	**Storage day**	**1**	**2**
pH	**0**	5.69^{a}	5.92^{ab}
	15	5.81^{abc}	5.73^{c}
	30	5.65^{c}	5.88^{ab}
	45	5.81^{abc}	5.69^{c}
	60	5.70^{bc}	5.80^{abc}
	75	5.80^{abc}	5.77^{bc}
	90	5.86^{ab}	5.93^{a}
	SEM	0.03	0.02
WHC (%)	**0**	34.67^{c}	35.00^{ab}
	15	35.67^{c}	35.67^{ab}
	30	31.33^{c}	30.33^{b}
	45	54.00^{a}	48.33^{a}
	60	47.67^{b}	49.33^{a}
	75	28.67^{c}	34.33^{ab}
	90	37.33^{bc}	43.33^{ab}
	SEM	2.20	2.26
Shear force	**0**	1.58^{c}	1.53^{d}
(kg/m^3)	**15**	1.82^{bc}	1.58^{cd}
	30	1.70^{c}	1.78^{bc}
	45	1.70^{c}	1.70^{bcd}
	60	2.22^{ab}	2.70^{a}
	75	2.58^{a}	2.83^{a}
	90	1.78^{bc}	1.92^{b}
	SEM	0.09	0.11

$^{a,b,c\ldots}$- Columns having varying superscripts indicate significant ($p<0.05$) variations in means

SEM: Standard Error of Mean; WHC- Water holding capacity

T1: Refrigerated temperature

T2: Room temperature

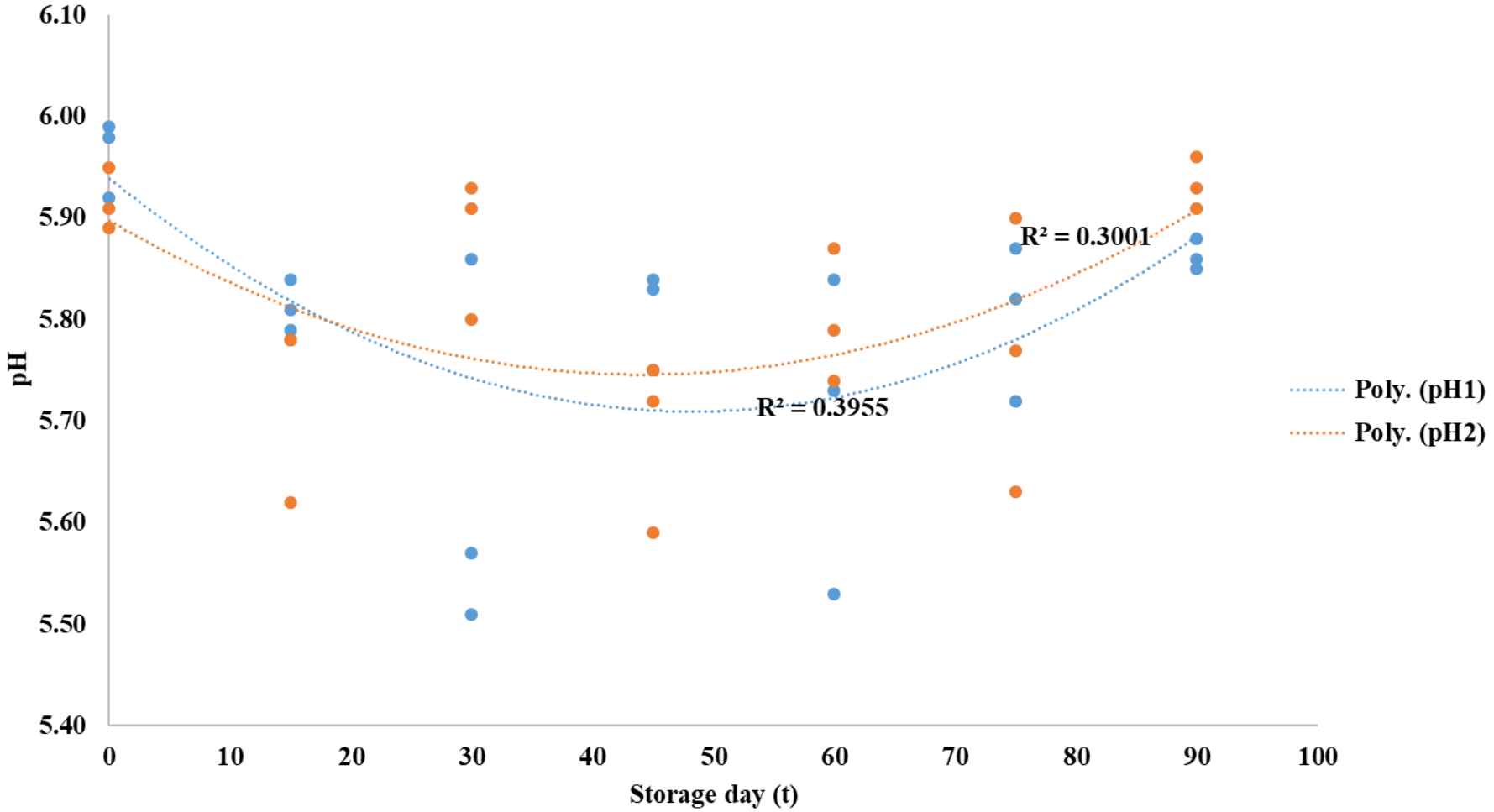

Figure 4.33: Relationship between storage duration and pH of cured-smoked chicken fillets stored at refrigerated and room temperature

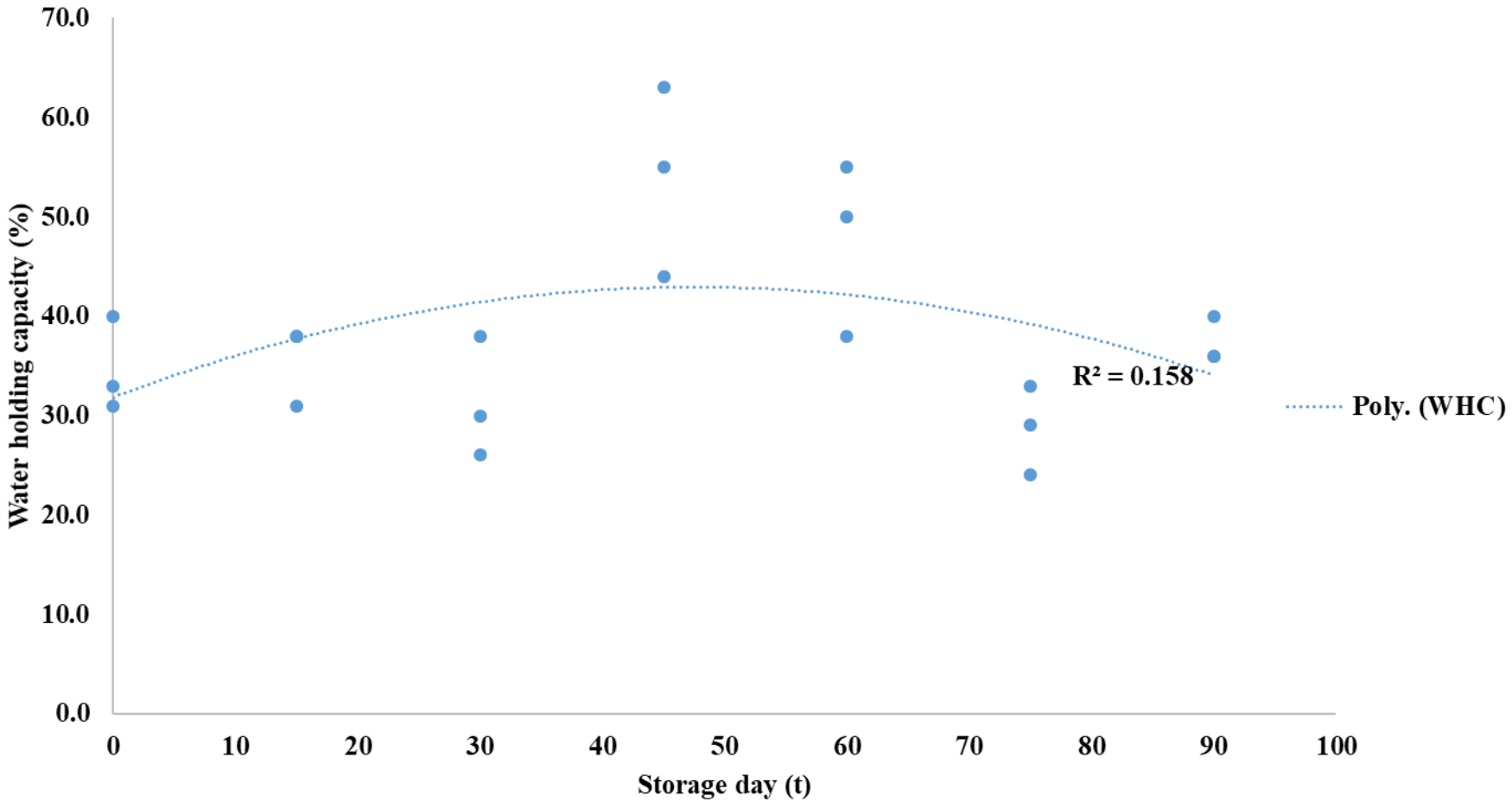

Figure 4.34: Relationship between storage duration and water holding capacity of cured-smoked chicken fillets stored at refrigerated and room temperature

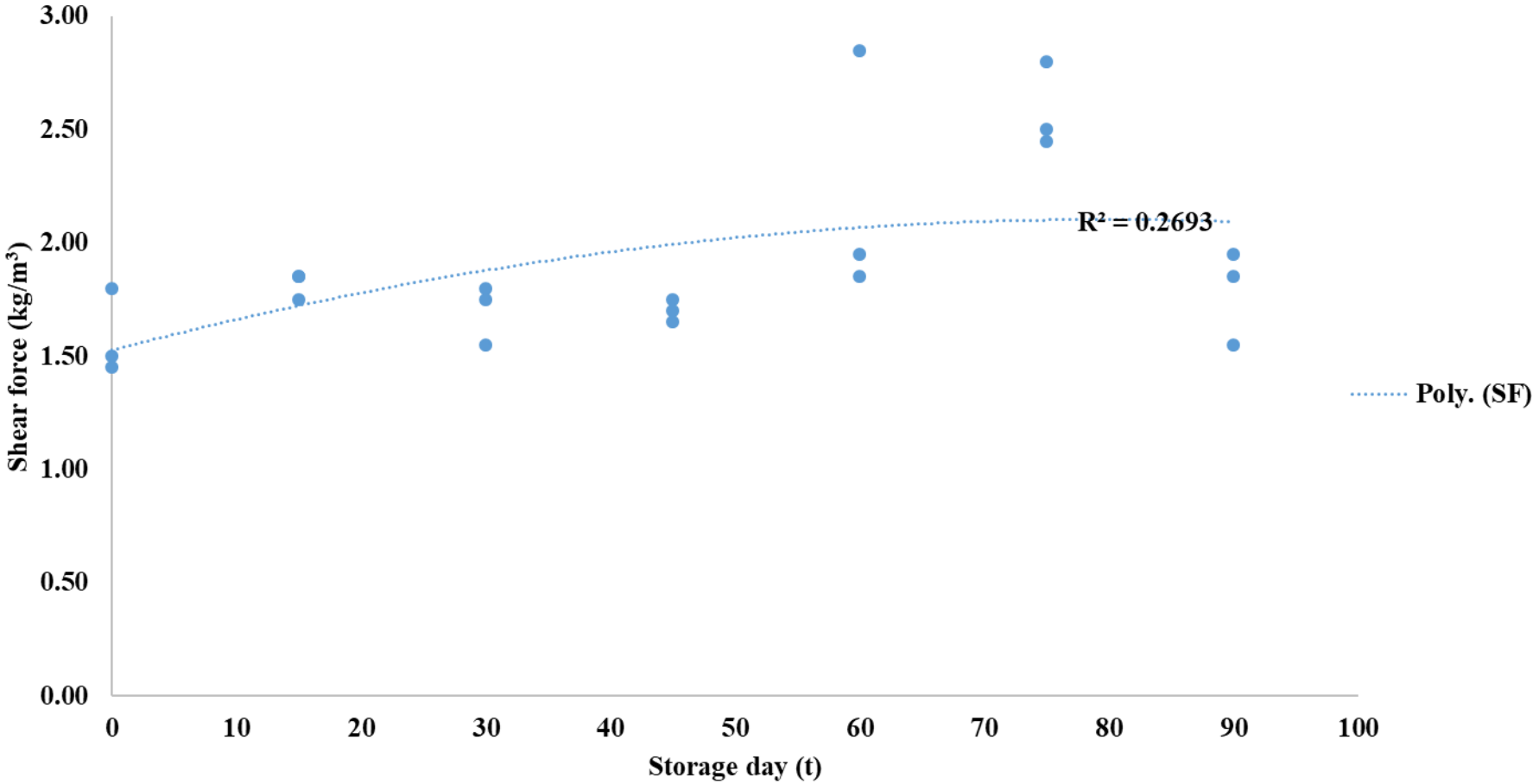

Figure 4.35: Relationship between storage duration and shear force of cured-smoked chicken fillets stored at refrigerated and room temperature

4.4.2 Lipid oxidation and protein deterioration of cured smoked chicken fillets stored at refrigerated and room temperature

Significant ($p<0.05$) effect of storage day was observed for all treatments for lipid oxidation while only fillets stored under refrigerated temperature was significantly ($p<0.05$) affected by storage condition for volatile basic nitrogen (Table 4.16). Lipid oxidation increased for all treatments till day 45 of storage and gradually stabilised till day 90 of storage. Highest value was observed on day 45 (1.25) in fillets stored at room temperature while least value was observed on day 90 of storage (0.59). Volatile basic nitrogen increased ($p<0.05$) gradually over storage for fillets stored under refrigeration from 39.67 on day 0 to 56.00 by day 75 of storage.

Interaction of storage duration and storage condition was significant ($p<0.05$) for lipid oxidation (Figure 4.36) of fillets but non-significant ($p>0.05$) for volatile basic nitrogen (Figure 4.37) of the fillets. Slight correlation between the storage duration and both parameters was observed. Regression coefficient was 0.31 and 0.18 for fillets stored at refrigerated and room temperature, respectively, while volatile basic nitrogen was 0.11.

Table 4.16: Lipid oxidation and volatile basic nitrogen of cured smoked chicken fillets stored at refrigerated and room temperature

		Treatments	
Parameters	**Storage day**	**1**	**2**
Lipid	**0**	0.90^{bc}	0.93^{b}
oxidation	**15**	1.02^{b}	0.85^{bc}
(mgMDA/100g)	**30**	0.88^{bc}	0.83^{bc}
	45	1.22^{a}	1.25^{a}
	60	0.87^{bc}	0.74^{c}
	75	0.80^{c}	0.93^{b}
	90	0.86^{bc}	0.59^{d}
	SEM	0.04	0.04
Volatile basic	**0**	39.67^{bc}	42.23
nitrogen	**15**	50.40^{ab}	40.60
(mg/100g)	**30**	40.60^{abc}	47.60
	45	45.73^{abc}	52.73
	60	42.00^{abc}	47.60
	75	56.00^{a}	43.40
	90	34.53^{c}	37.33
	SEM	2.10	1.80

$^{a,b,c,\cdots}$- Columns having varying superscripts indicate significant ($p<0.05$) variations in means

SEM: Standard Error of Mean

T1: Refrigerated temperature

T2: Room temperature

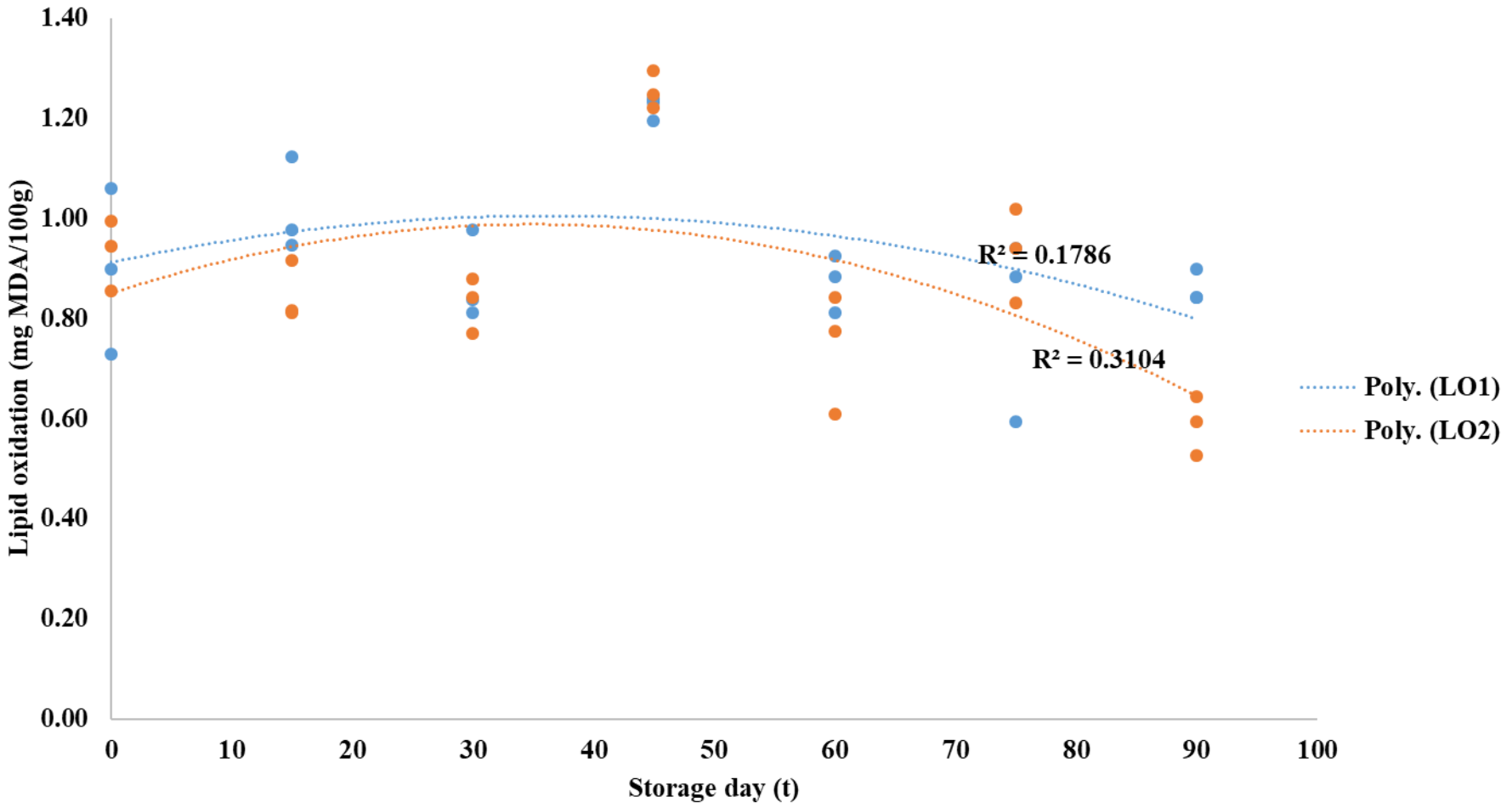

Figure 4.36: Relationship between storage duration and lipid oxidation of cured-smoked chicken fillets stored at refrigerated and room temperature

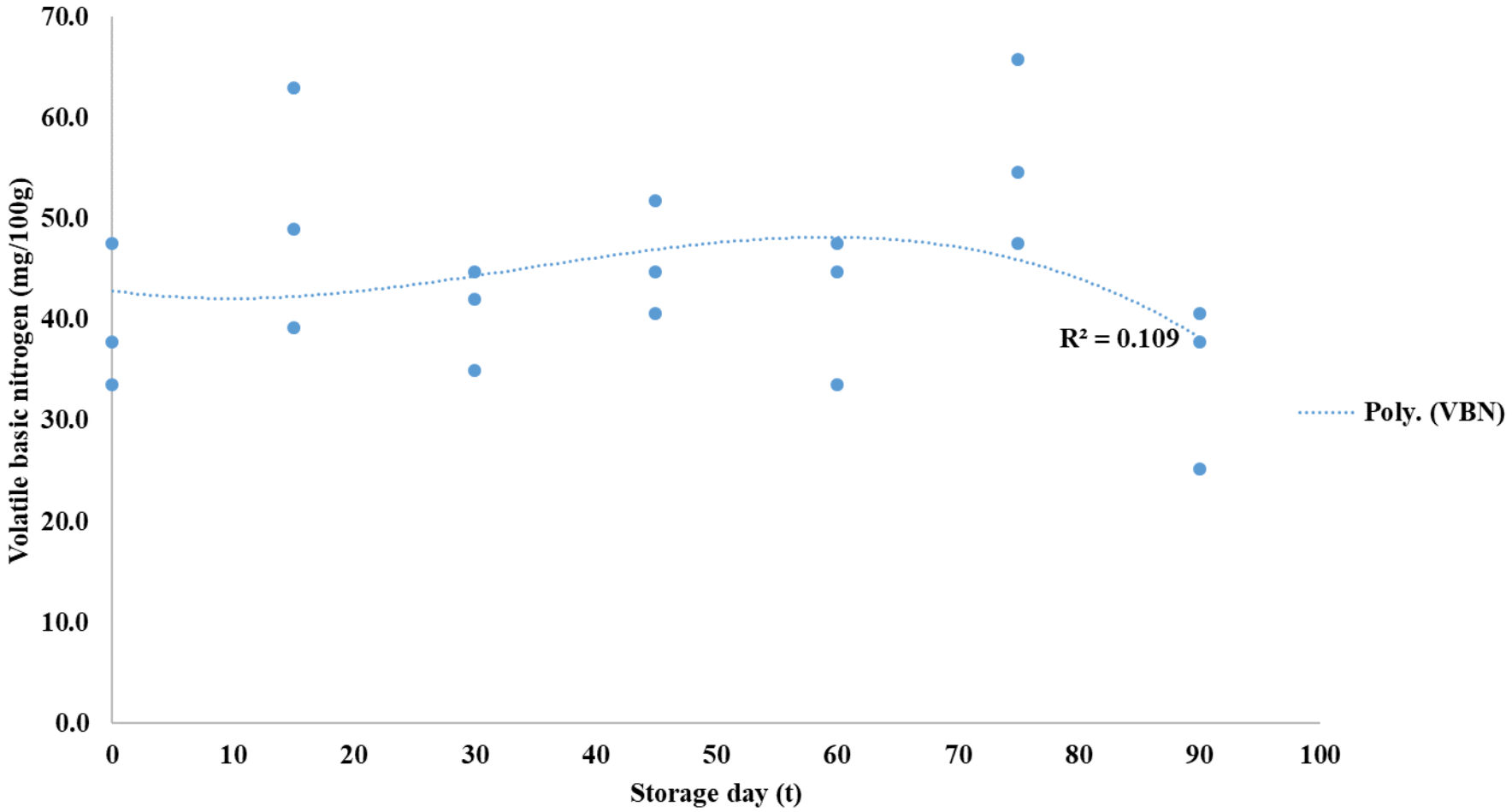

Figure 4.37: Relationship between storage duration and volatile basic nitrogen of cured-smoked chicken fillets stored at refrigerated and room temperature

4.4.3 Colour properties of cured smoked chicken fillets stored at refrigerated and room temperature

Lightness and redness of fillets were observed to significantly ($p<0.05$) decrease during storage while yellowness (Table 4.17) increased ($p<0.05$). Lightness reduced from 57.29 in fillets stored at room temperature to 40.19 for treatments stored under refrigeration by day 90 of storage while redness reduced from 20.96 to 3.51 in fillets stored at room temperature. Yellowness increased from 13.47 to 28.41 by day 60 of storage for both treatments, then a sharp decline was observed till day 90.

The interaction effect of storage duration and storage condition was not significant for all colour properties of fillets. Low correlation was also observed for the colour properties. Regression coefficients was 0.16, 0.41 and 0.29 for lightness (Figure 4.38), redness (Figure 4.39) and yellowness (Figure 4.40), respectively.

Table 4.17: Colour properties of cured smoked chicken fillets stored at refrigerated and room temperature

		Treatments	
Parameters	**Storage day**	**1**	**2**
Lightness	**0**	56.35^{b}	57.29^{ab}
	15	51.52^{bc}	51.49^{b}
	30	46.78^{bcd}	48.32^{bcd}
	45	48.28^{bcd}	50.21^{bc}
	60	68.53^{a}	63.76^{a}
	75	42.44^{cd}	41.06^{cd}
	90	40.19^{d}	40.36^{d}
	SEM	2.20	1.96
Redness	**0**	17.35^{b}	20.96^{a}
	15	16.50^{b}	19.46^{ab}
	30	14.31^{bc}	14.08^{c}
	45	11.57^{c}	15.80^{bc}
	60	23.35^{a}	20.30^{a}
	75	5.53^{d}	6.58^{d}
	90	5.00^{d}	3.51^{d}
	SEM	1.42	1.49
Yellowness	**0**	13.47^{bcd}	17.49^{bc}
	15	18.87^{b}	24.75^{ab}
	30	16.44^{bc}	17.19^{bc}
	45	13.09^{bcd}	20.04^{ab}
	60	28.41^{a}	26.38^{a}
	75	8.90^{cd}	9.22^{cd}
	90	7.76^{d}	5.59^{d}
	SEM	1.65	1.79

$^{a,b,c\ldots}$- Columns having varying superscripts indicate significant ($p<0.05$) variations in means

SEM: Standard Error of Mean

T1: Refrigerated temperature

T2: Room temperature

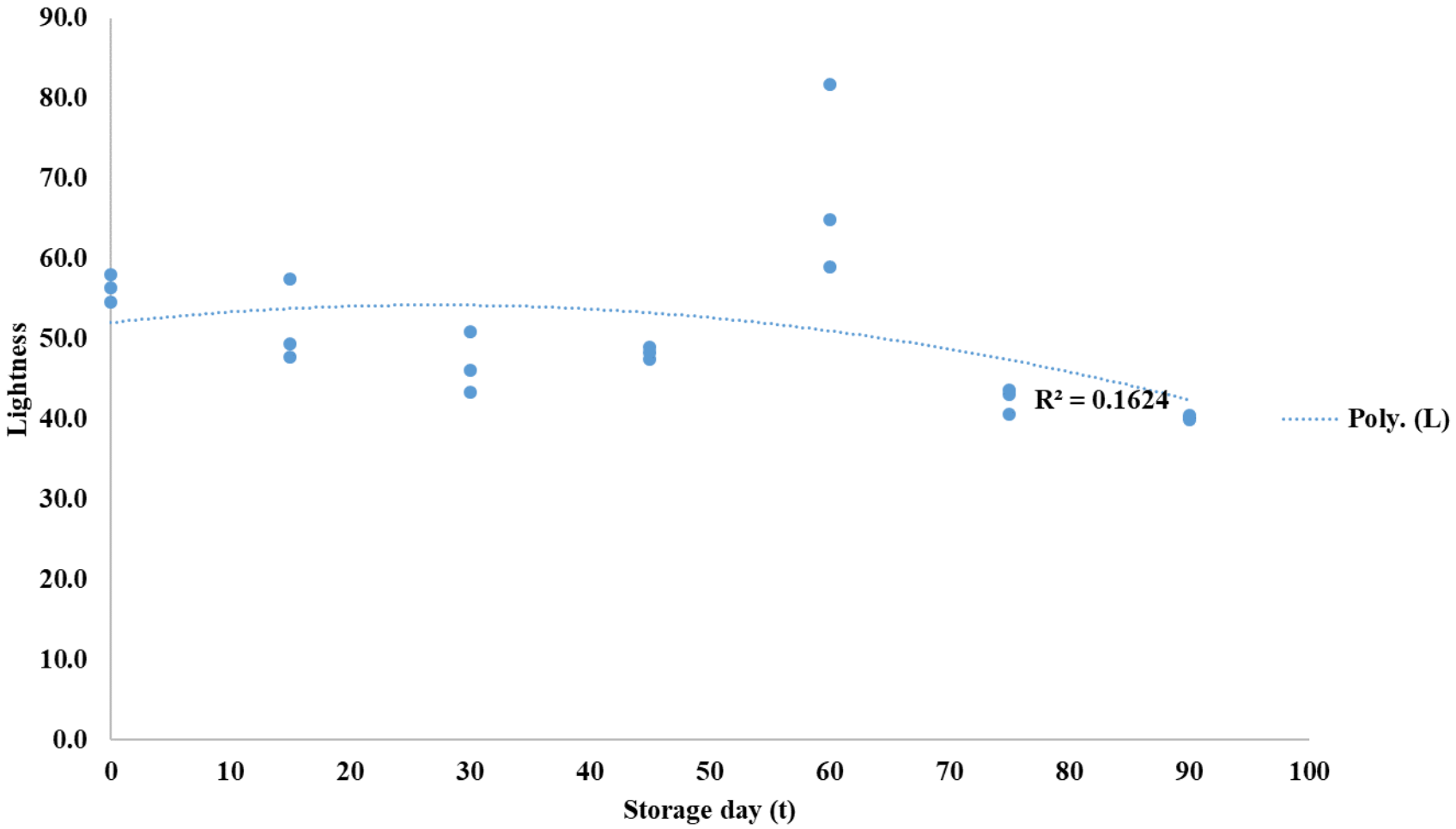

Figure 4.38: Relationship between storage duration and lightness of cured-smoked chicken fillets stored at refrigerated and room temperature

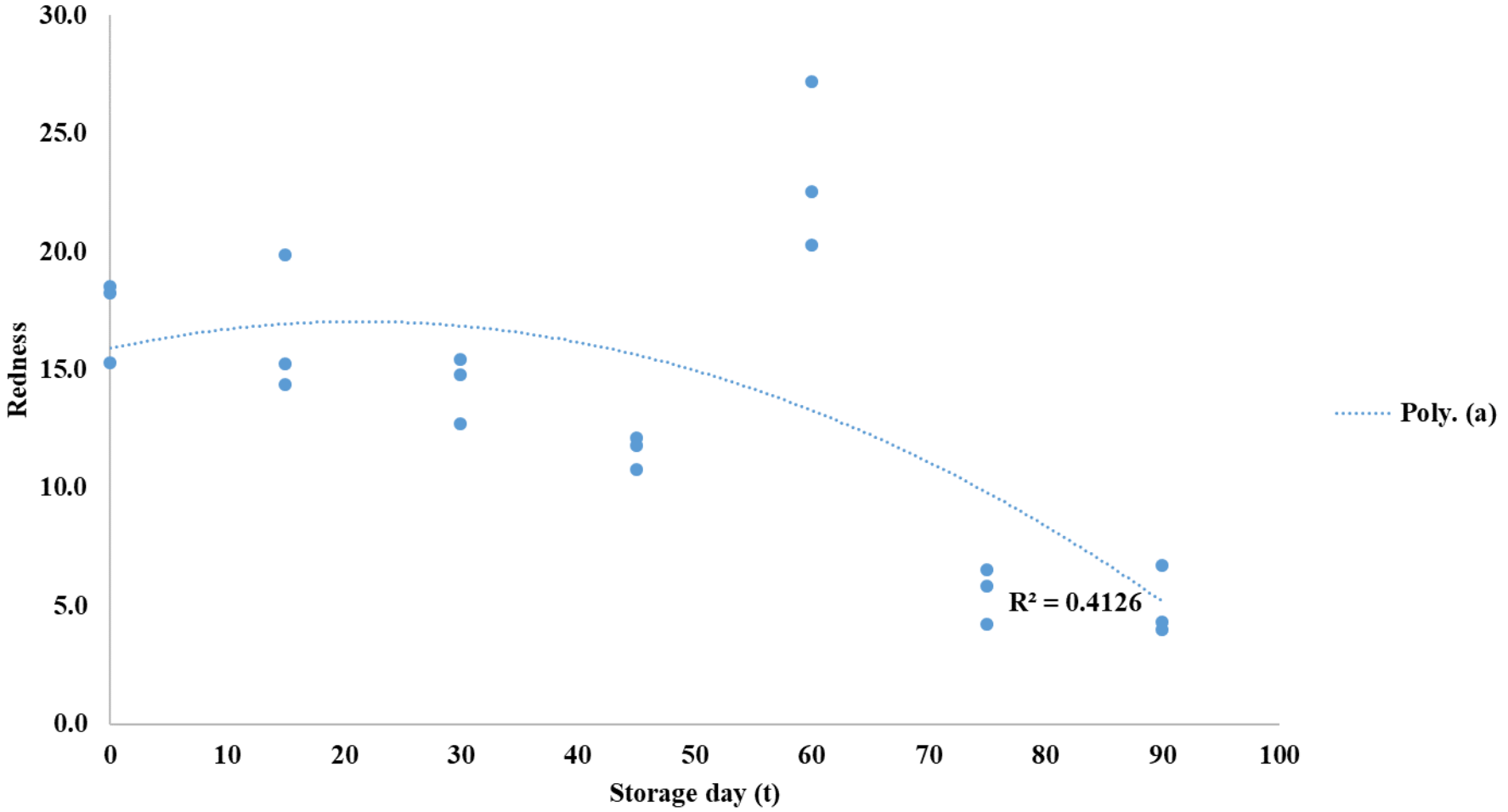

Figure 4.39: Relationship between storage duration and redness of cured-smoked chicken fillets stored at refrigerated and room temperature

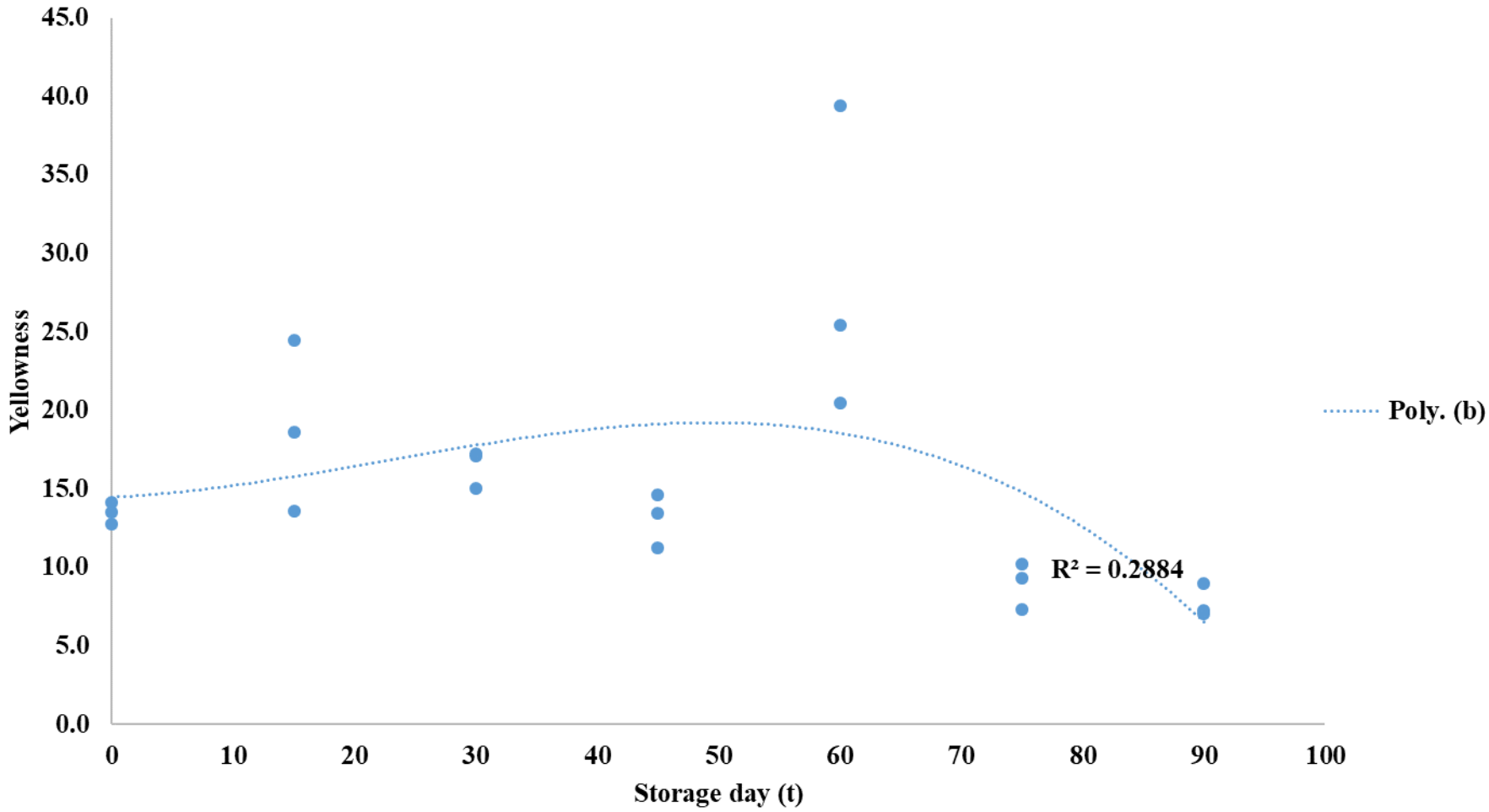

Figure 4.40: Relationship between storage duration and yellowness of cured-smoked chicken fillets stored at refrigerated and room temperature

4.4.4 Microbial load of cured smoked chicken fillets stored at refrigerated and room temperature

Microbial load of fillets was observed to be significantly ($p<0.05$) affected by storage day for both treatments (Table 4.18). An increase was observed over storage for both total anaerobic bacteria and lactic acid bacteria counts. Total anaerobic bacteria count increased ($p<0.05$) from 2.42 on day 0 to 3.76 by day 90 of storage in fillets stored at room temperature while lactic acid bacteria count increased ($p<0.05$) from 0.67 on day 0 in fillets stored under refrigeration to 3.33 on day 90 in fillets stored at room temperature.

Significant ($p<0.05$) interaction effect between storage duration and storage condition was observed for only total anaerobic bacteria count (Figure 4.41) of fillets while lactic acid bacteria count (Figure 4.42) was not significantly affected ($p>0.05$). A high correlation was observed between storage duration and the microbial counts with regression coefficients of 0.59 in fillets stored under refrigeration and 0.82 for fillets stored at room temperature for total anaerobic bacteria counts. Lactic acid bacteria count has a coefficient of 0.45

Table 4.18: Microbial counts (log_{10}cfu/g) of cured smoked chicken fillets stored at refrigerated and room temperature

		Treatments	
Parameters	**Storage day**	**1**	**2**
TAB counts	**0**	2.42^{e}	2.50^{d}
	15	3.91^{a}	4.03^{a}
	30	3.45^{cd}	3.75^{b}
	45	3.27^{d}	4.09^{a}
	60	3.63^{bc}	3.83^{b}
	75	3.90^{a}	3.69^{b}
	90	3.76^{ab}	3.52^{c}
	SEM	0.11	0.11
LAB counts	**0**	0.67^{c}	1.33^{b}
	15	3.76^{a}	3.70^{a}
	30	2.60^{b}	4.05^{a}
	45	2.52^{b}	3.26^{a}
	60	3.46^{ab}	3.53^{a}
	75	3.11^{ab}	3.96^{a}
	90	2.68^{b}	3.33^{a}
	SEM	0.23	0.21

$^{a,b,c\cdots}$- Columns having varying superscripts indicate significant ($p<0.05$) variations in means

TAB counts: Total Anaerobic Bacteria counts; LAB counts: Lactic Acid Bacteria counts

SEM: Standard Error of Mean

T1: Refrigerated temperature

T2: Room temperature

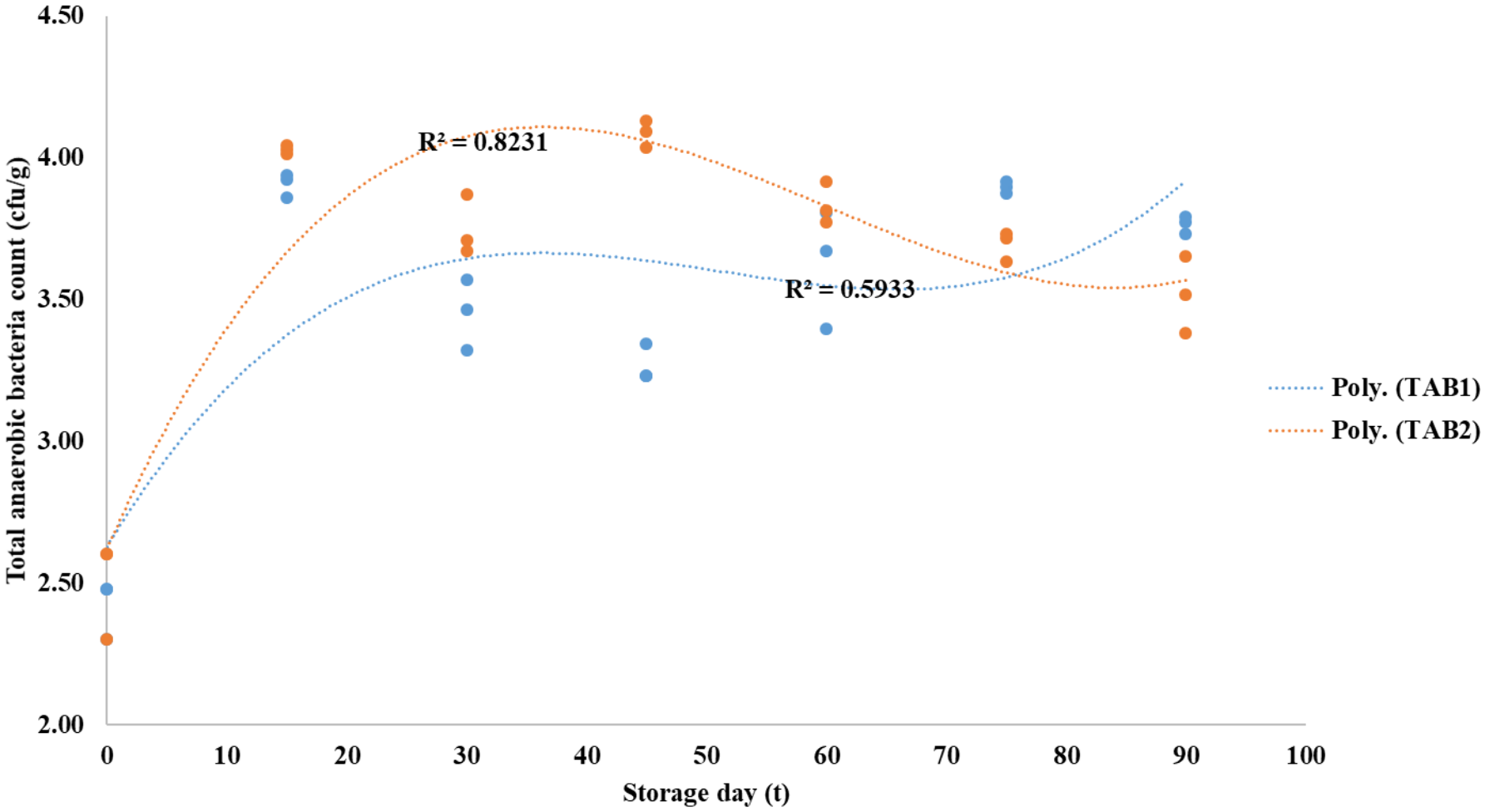

Figure 4.41: Relationship between storage duration and total anaerobic bacteria count of cured-smoked chicken fillets stored at refrigerated and room temperature

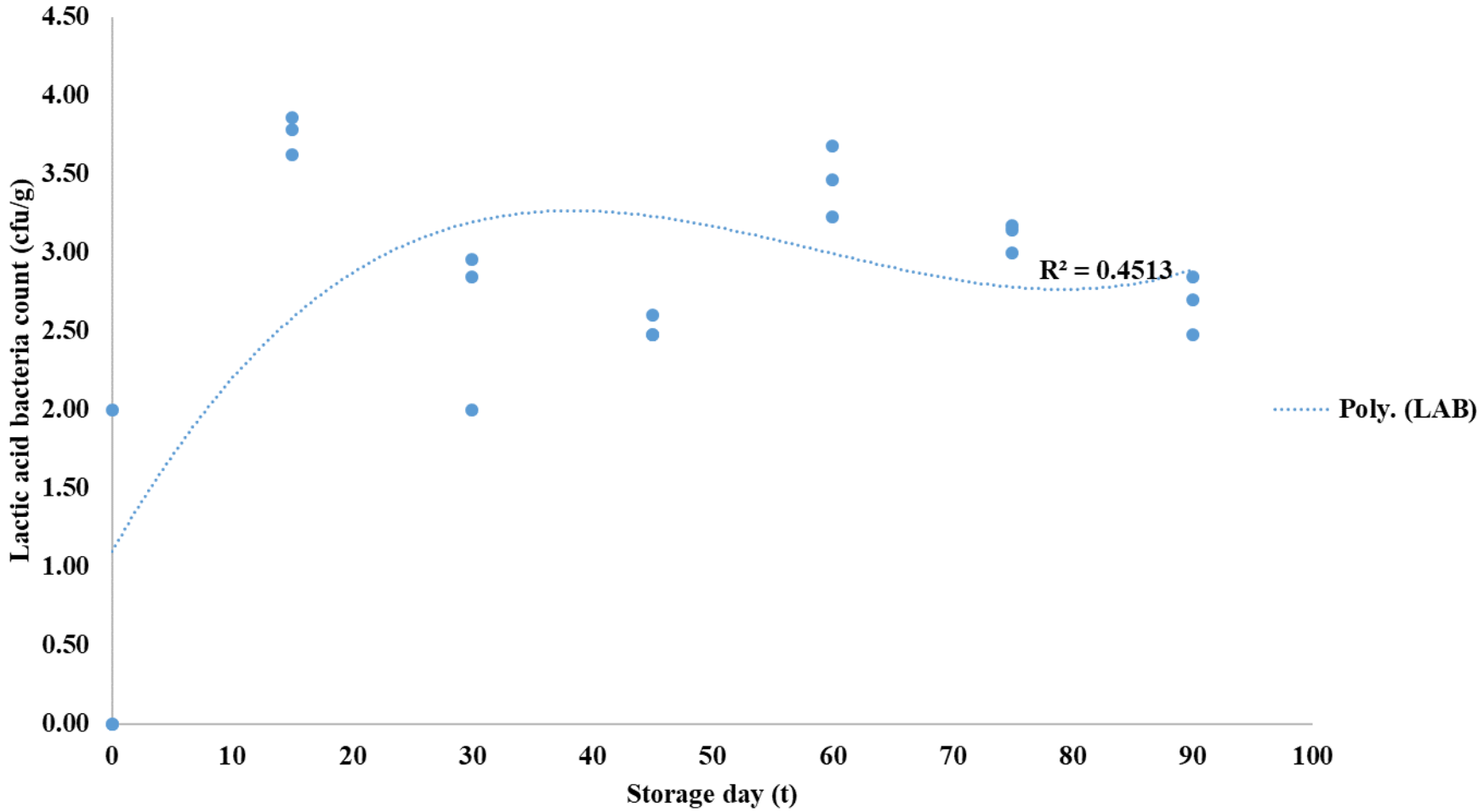

Figure 4.42: Relationship between storage duration and lactic acid bacteria count of cured-smoked chicken fillets stored at refrigerated and room temperature

4.4.5 Sensory quality of cured smoked chicken fillets stored at refrigerated and room temperature

Sensory quality of fillets was also observed to be significantly ($p<0.05$) affected by storage day, with a gradual decrease ($p<0.05$) in quality over the storage period for all treatments (Table 4.19). Visible microbial growth on fillets during storage increased gradually with highest quality observed on day 0 (no visible microbial growth on all surfaces) to a range of 1.70 to 1.80 (microbial growth on some surfaces) by day 90. Fillets under both storage conditions had characteristic cured smoked odour (3.00) on day 0 of storage which reduced to a range of 1.70 to 1.80 (off or rancid odour) by day 90 of storage. Overall quality of fillets under both storage conditions also reduced from 3.00 (excellent) to 1.60 to 1.70 (slightly unacceptable) on day 90 of storage.

Interaction effect between storage duration and storage condition was not significant ($p>0.05$) for sensory properties of both treatments. A high positive correlation was however observed for the sensory properties of the fillets with regression coefficients of 0.53, 0.57 and 0.62 for microbial growth (Figure 4.43), odour (Figure 4.44) and overall quality (Figure 4.45) of fillets.

Table 4.19: Sensory properties of cured smoked chicken fillets stored at refrigerated and room temperature

		Treatments	
Parameters	**Storage day**	**1**	**2**
Microbial growth	**0**	3.00[a]	3.00[a]
	15	2.90[a]	2.80[a]
	30	2.90[a]	2.90[a]
	45	2.60[ab]	2.60[a]
	60	2.20[bc]	2.00[b]
	75	1.80[c]	1.70[c]
	90	1.80[c]	1.70[c]
	SEM	0.08	0.08
Odour	**0**	3.00[a]	3.00[a]
	15	3.00[a]	3.00[a]
	30	2.70[a]	2.70[a]
	45	2.80[a]	2.70[a]
	60	2.30[b]	2.10[b]
	75	2.00[bc]	1.90[c]
	90	1.80[c]	1.70[c]
	SEM	0.07	0.08
Overall quality	**0**	3.00[a]	3.00[a]
	15	3.00[a]	3.00[a]
	30	2.80[a]	2.60[ab]
	45	2.70[ab]	2.50[b]
	60	2.40[b]	2.40[b]
	75	1.70[c]	1.70[c]
	90	1.70[c]	1.60[c]
	SEM	0.08	0.08

[a,b,c…]- Columns having varying superscripts indicate significant ($p<0.05$) variations in means

SEM: Standard Error of Mean

T1: Refrigerated temperature

T2: Room temperature

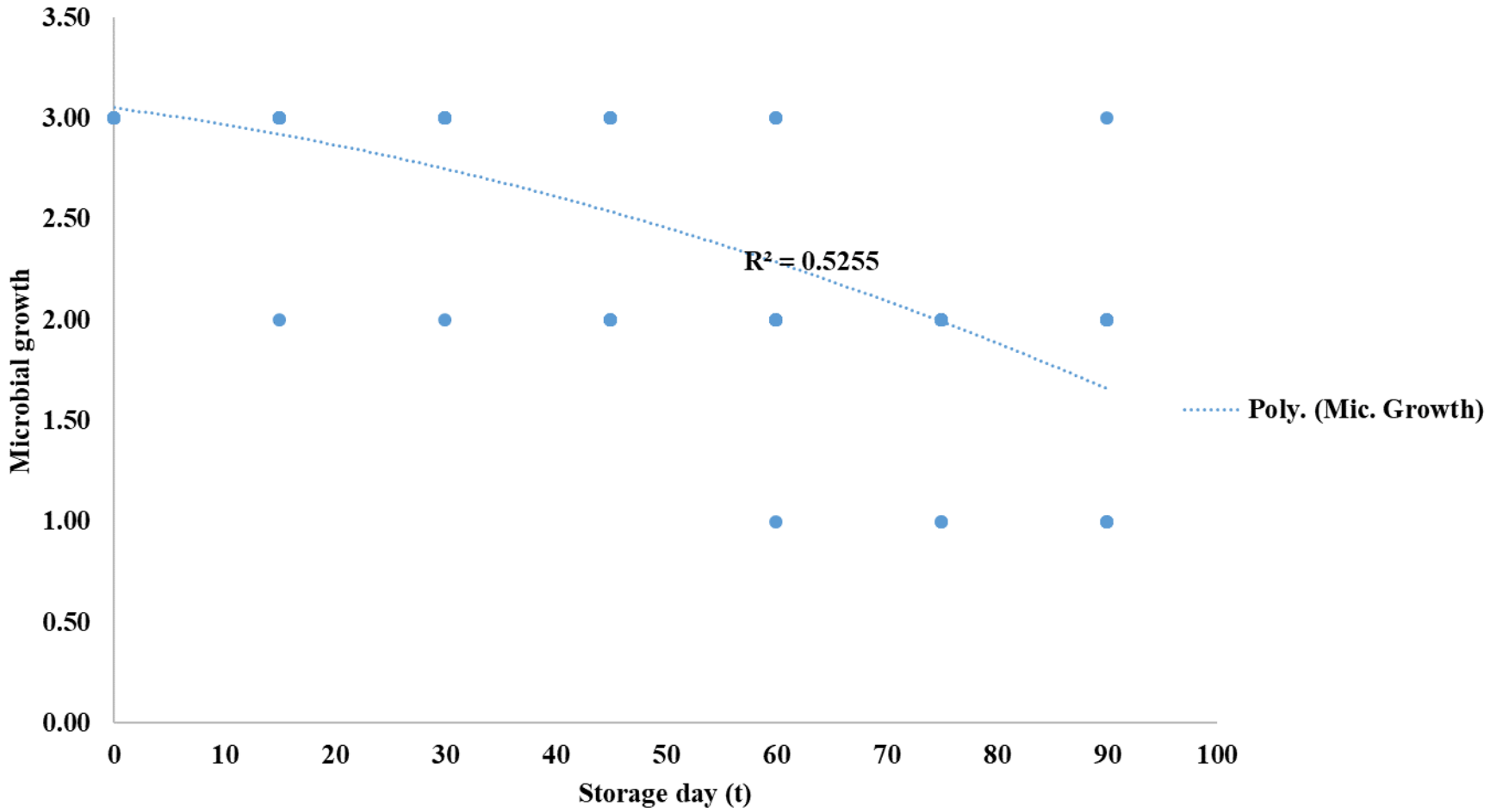

Figure 4.43: Relationship between storage duration and microbial growth of cured-smoked chicken fillets stored at refrigerated and room temperature

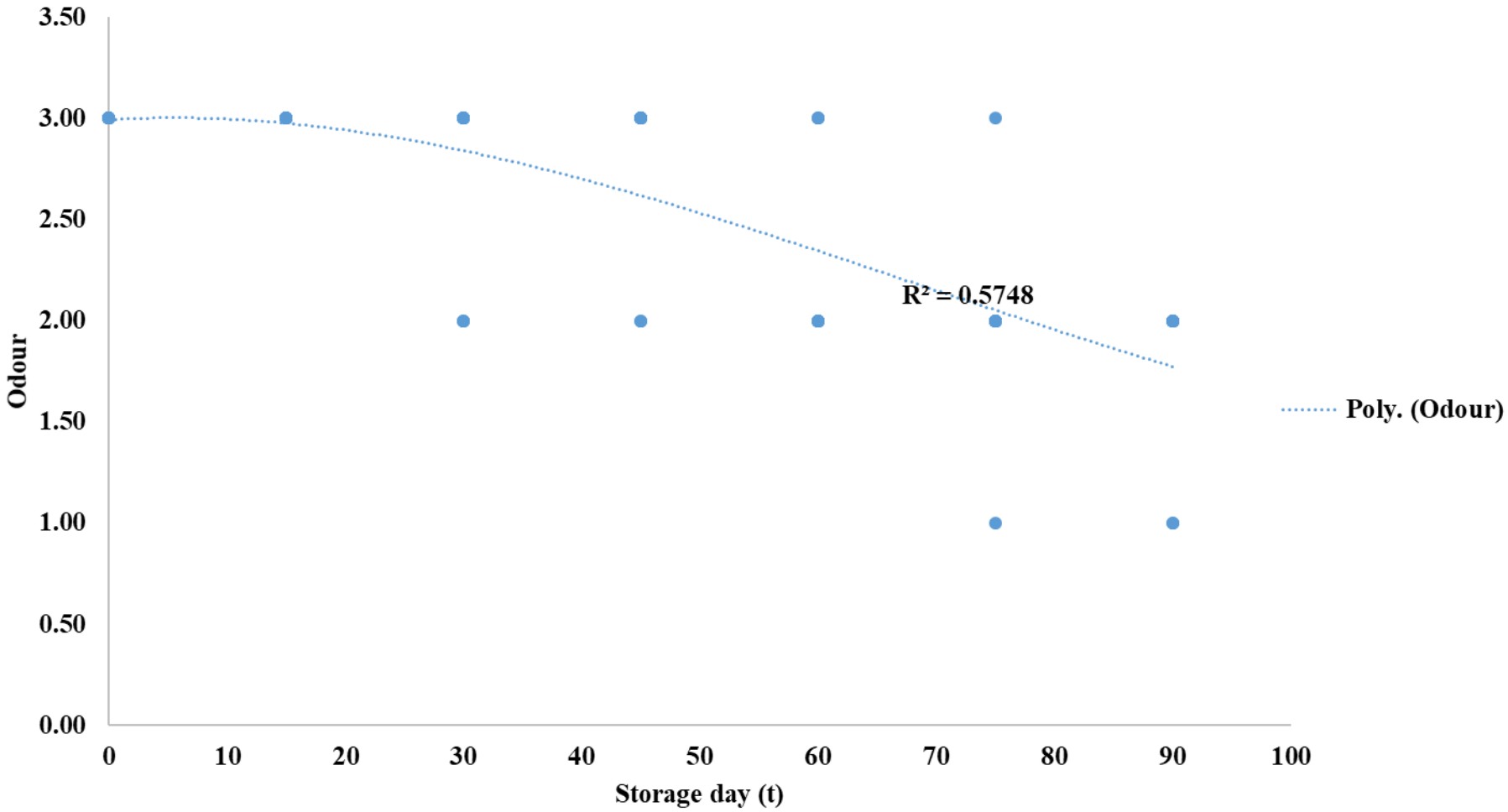

Figure 4.44: Relationship between storage duration and odour of cured-smoked chicken fillets stored at refrigerated and room temperature

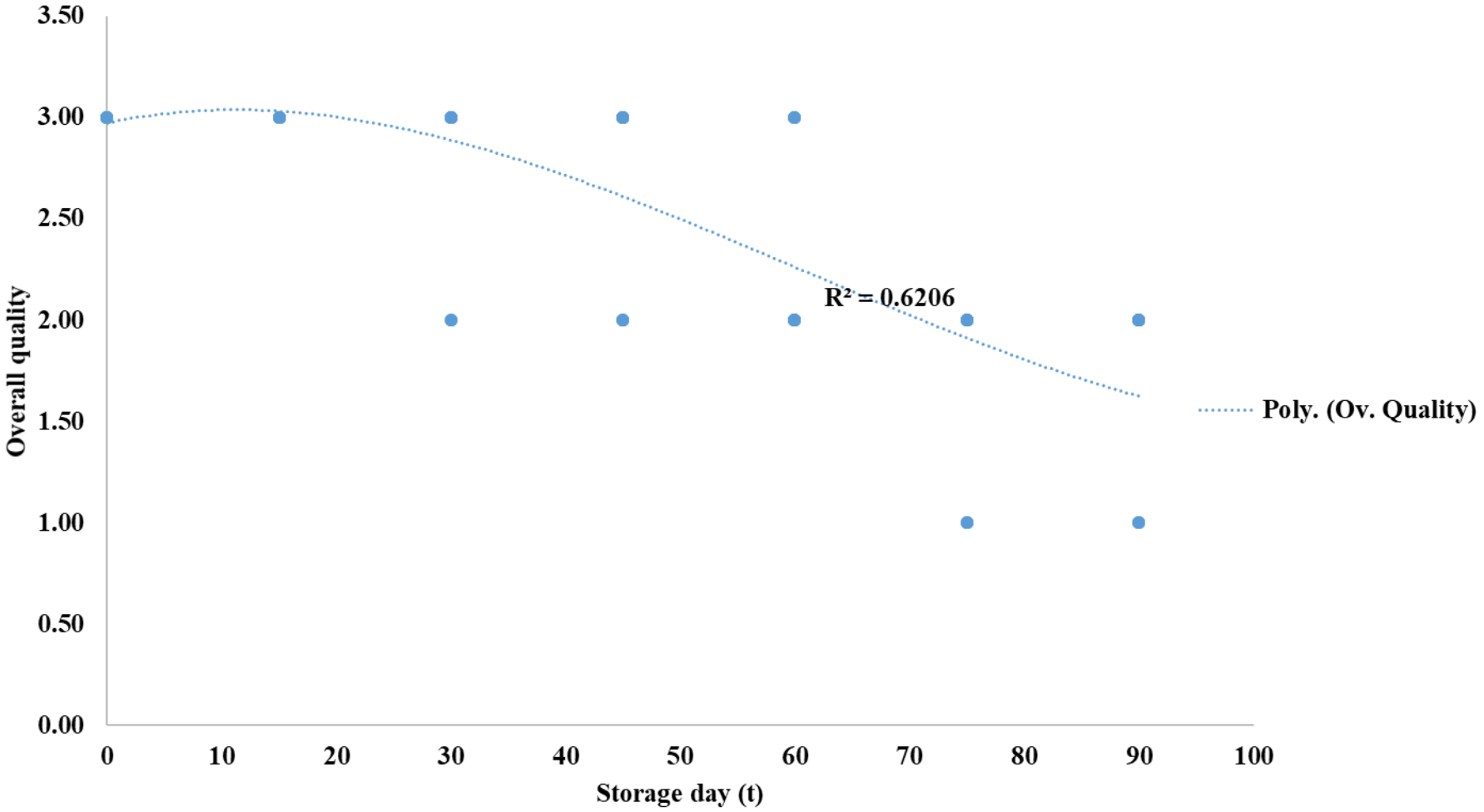

Figure 4.45: Relationship between storage duration and overall quality of cured-smoked chicken fillets stored at refrigerated and room temperature

4.4.6 Fatty acid profile of cured-smoked chicken fillets stored at refrigerated and room temperature

Fatty acid profile of fillets stored at refrigerated and room temperature on day 0 (Table 4.20) showed significant ($p < 0.05$) effect of storage condition on only acetic acid with fillets stored at room temperature having 3.37% while those stored at refrigerated temperature was 3.25%. Non-significant effect of storage condition was however observed for other parameters measured with treatment 2 having highest values for most of the parameters - Lauric acid - 3.09, stearic acid – 4.38, palmitic acid – 3.81, arachiconic acid – 4.70, oleic acid – 4.55, margaric acid – 4.22, linoleic acid – 4.35, ligoleric aicd – 5.69, behemic acid – 5.25, butyric acid – 1.38, palmtoleic acid – 3.88 and caprylic acid – 2.23. Treatment 1 has highest values in myristic acid (3.55), valeric acid (1.57) and propionic acid (1.80).

On day 90, significant effect of storage condition (Table 4.21) was observed only in behemic acid with 4.84 in treatment 1 and 3.10 in treatment 2. Most parameters had highest values in treatment 1: lauric acid – 2.22, stearic acid – 3.14, palmitic acid – 2.87, arachidonic acid – 3.42, oleic acid – 3.14, margaric acid – 3.01, linoleic acid – 3.17, ligoleric acid – 4.10, myristic acid – 2.51, butyric acid – 0.95, valeric acid – 1.17, palmtoleic acid – 2.79, caprylic acid – 1.62, propionic acid – 1.32 and acetic acid – 2.49.

Table 4.20: Fatty acid profile of cured-smoked chicken fillets stored at refrigerated and room temperature (day 0)

	Treatments		
Parameters (%)	**1**	**2**	**SEM**
Lauric acid	3.02	3.09	0.02
Stearic acid	4.28	4.38	0.03
Palmitic acid	3.68	3.81	0.12
Arachidonic acid	4.48	4.70	0.12
Oleic acid	4.30	4.55	0.13
Margaric acid	4.14	4.22	0.09
Linoleic acid	4.26	4.35	0.05
Ligoleric acid	5.54	5.69	0.06
Myristic acid	3.55	3.47	0.08
Behenic acid	5.18	5.25	0.05
Butyric acid	1.30	1.38	0.02
Valeric acid	1.57	1.55	0.03
Palmtoleic acid	3.75	3.88	0.07
Caprylic acid	2.16	2.23	0.04
Propionic acid	1.80	1.75	0.04
Acetic acid	3.25^{b}	3.37^{a}	0.10

$^{a,b,c\cdots}$- Rows with different superscripts indicate significant ($p<0.05$) variation in means

SEM: Standard Error of Mean

T1: Refrigerated temperature
T2: Room temperature

Table 4.21: Fatty acid profile of cured-smoked chicken fillets stored at refrigerated and room temperature (day 90)

	Treatments		
Parameters (%)	**1**	**2**	**SEM**
Lauric acid	2.22	1.84	0.04
Stearic acid	3.14	2.60	0.05
Palmitic acid	2.87	2.34	0.04
Arachidonic acid	3.42	2.78	0.05
Oleic acid	3.14	2.57	0.03
Margaric acid	3.01	2.47	0.04
Linoleic acid	3.17	2.46	0.09
Ligoleric acid	4.10	3.36	0.04
Myristic acid	2.51	2.09	0.04
Behenic acid	4.84^{a}	3.10^{b}	1.05
Butyric acid	0.95	0.80	0.03
Valeric acid	1.17	0.93	0.04
Palmtoleic acid	2.79	2.33	0.06
Caprylic acid	1.62	1.38	0.03
Propionic acid	1.32	1.07	0.03
Acetic acid	2.49	1.91	0.10

$^{a,b,c,\cdots}$- Rows with different superscripts indicate significant ($p<0.05$) variation in means

SEM: Standard Error of Mean

T1: Refrigerated temperature
T2: Room temperature

4.4.7 Mineral composition of cured smoked chicken fillets stored at refrigerated and room temperature

Mineral composition of cured-smoked chicken fillets on days 0 and 90 of storage (Figures 4.46 and 4.47) showed a non-significant effect of treatments on the minerals analysed. A sharp decrease was observed for most minerals, however sodium increased slightly during storage. Fillets stored at refrigerated temperature had highest value of 18.33mg/kg while fillets stored at room temperature had least sodium content of 17.67 on day 0. Also, on day 0, highest calcium (19.27mg/kg), magnesium (3.92mg/kg) and potassium (16.17mg/kg) values were observed for fillets stored at refrigerated temperature while fillets stored at room temperature has values of 15.98mg/kg, 3.65mg/kg and 15.5mg/kg for calcium, magnesium and potassium, respectively. On day 90 of storage, sodium content (20mg/kg) was observed to be the same in both fillets. Calcium decreased to 12.85mg/kg and 16.65mg/kg for fillets at refrigerated and room temperatures. Magnesium also decreased to 3.14mg/kg and 2.36mg/kg for both treatments. An increase was however observed for potassium to 20mg/kg and 20.83mg/kg for fillets subjected to the different storage conditions.

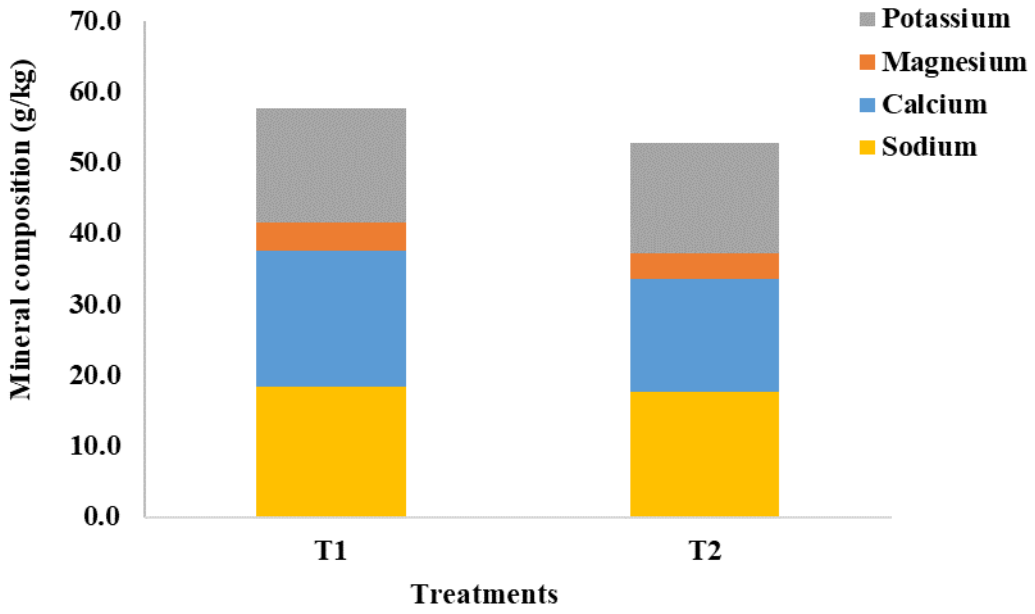

Figure 4.46: Mineral composition of cured-smoked chicken fillets stored at refrigerated and room temperature (day 0)

T1: Refrigerated temperature

T2: Room temperature

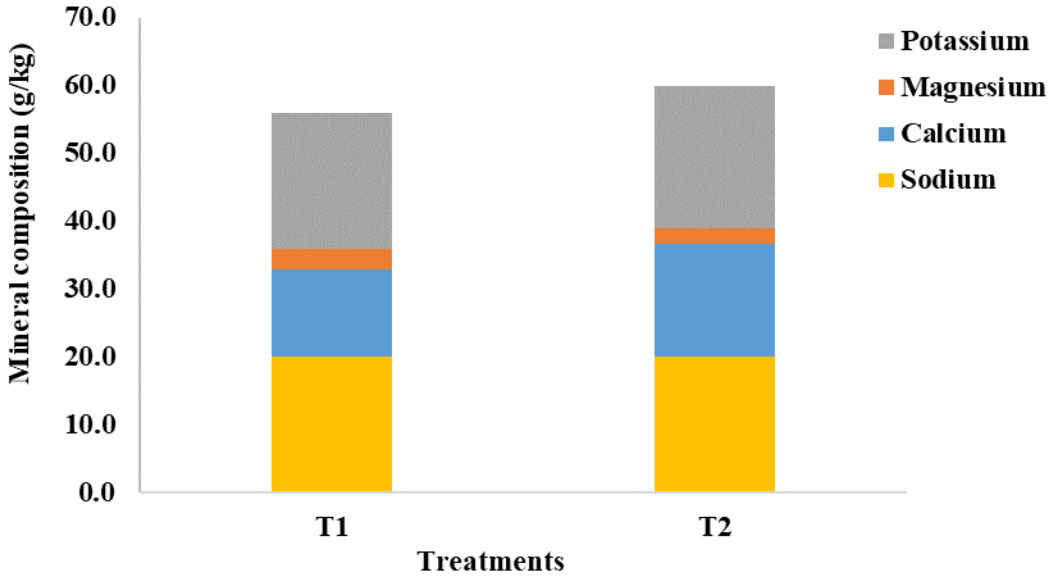

Figure 4.47: Mineral composition of cured-smoked chicken fillets stored at refrigerated and room temperature (day 90)

T1: Refrigerated temperature

T2: Room temperature

4.4.9 Consumer acceptability of cured-smoked chicken fillets prepared with *Capsicum* extract and reduced sodium level

Consumer acceptability of cured-smoked chicken fillets prepared with *Capsicum* extract and reduced sodium level (50% sodium chloride + 25% potassium chloride + 25% magnesium chloride) is shown in Table 4.23. A total of eighty-four (84) respondents took part in the assessment of prepared chicken fillets for consumer acceptability. For consumers' demography, most respondents were males (46) having a percentage of 54.80 while females (38) had a percentage of 45.20. Number of respondents within the age range of 11-20 was 3 (3.60%), 21-30 was 64 (76.20%), 31-40 was 6 (7.10%), 41-50 was 5 (6.00%) and 51-60 was 6 (7.10%). 77.40% of respondents were single (65) while 22.60% were married (19). Educational status of respondents was mostly tertiary (undergraduate-59) – 70.20% while 29.80% (25) were tertiary-postgraduate. For income level, number of respondents earning between ₦0.00 - ₦5,000.00 monthly was 12 (14.30%), ₦5,001.00 - ₦10,000.00 was 30 (35.70%), ₦10,000.00 – ₦50,000.00 was 28 (33.30%), ₦50,001.00 - ₦100,000.00 was 4 (4.80%) and Above ₦100,000.00 was 10 (11.90%). As regards consumption pattern of respondents, all respondents (84) do consume meat products (100%) and the types of meat product consumed were *suya* (76 – 90.50%), *kilishi* (63 – 75.00%), *asun* (63 – 75.00%), *kundi* (34 – 40.50%), corned beef (45 – 53.60%), hotdog sausage (38 – 45.20%) and minced meat (34 – 40.50%). Frequency of consumption was mostly occasional (73.80%), and 26.20% of respondents consume it frequently. A large percentage (90.50%) of the respondents were willing to pay/buy the product if sighted on shelf. Cost of product ranged from ₦1,200.00 to ₦2,500.00. Most of the respondents (67.90%) would buy at a cost range of ₦1,200.00 to ₦1,500.00, 47.60% will buy at between ₦1,500.00 to ₦1,800.00, 16.70% will buy at a cost between ₦1,800.00 to ₦2,100.00 while only 1.20% will buy at ₦2,100.00 - ₦2,500.00.

Table 4.22: Consumer acceptability of cured-smoked chicken fillets prepared with Capsicum extract and reduced sodium level

Description		Frequency	%
Consumers' demography			
Sex	Male	46	54.80
	Female	38	45.20
Age	11-20	3	3.60
	21-30	64	76.20
	31-40	6	7.10
	41-50	5	6.00
	51-60	6	7.10
Marital status	Single	65	77.40
	Married	19	22.60
Educational status	Tertiary-Undergraduate	59	70.20
	Tertiary-Postgraduate	25	29.80
Income	₦0.00 - ₦5,000.00	12	14.30
	₦5,001.00 - ₦10,000.00	30	35.70
	₦10,000.00 - ₦50,000.00	28	33.30
	₦50,001.00 - ₦100,000.00	4	4.80
	Above ₦100,000.00	10	11.90
Consumption pattern			
Do you consume meat products?	Yes	84	100
	No	0	0
Types consumed	*Suya*	76	90.5
	Kilishi	63	75
	Asun	63	75
	Kundi	34	40.5
	Corned beef	45	53.6
	Hotdog sausage	38	45.2
	Minced meat	34	40.5
Frequency of consumption	Frequently	22	26.2
	Occasionally	62	73.8
	Never	0	0

Willingness to pay/buy product	Yes	76	90.5
	No	8	9.5
Cost of product	₦1,200.00 - ₦1,500.00	57	67.9
	₦1,500.00 - ₦1,800.00	40	47.6
	₦1,800.00 - ₦2,100.00	14	16.7
	₦2,100.00 - ₦2,500.00	1	1.2

Chapter 5 CHAPTER FIVE

5.0 DISCUSSION

Experiment One

Chemical Properties of Selected Red Pepper Extracts

Red peppers (*Capsicum spp.*) are spices of the genus of plants from the *Solanaceae* family containing a mixture of phytochemicals like polyphenols, flavonoids and ascorbic acid (Nadeem *et al*., 2011) with biochemical and pharmacological properties such as antioxidant, anti-inflammatory, antimicrobial, anti-allergic etc. (Lee *et al*., 2005). Red peppers are also rich sources of carotenoids which play antioxidant and colour-impacting roles, thereby reducing the risk of diseases in humans as well as preserving and increasing acceptability of food products (Rao and Rao, 2007).

Most plant extracts contain antimicrobial and antioxidant compounds such as phenolic compounds, organic acids, and flavonoids. These compounds can cause cell membrane destruction, which can result in microorganisms being inactivated or killed due to cellular part leakage. These compounds' antioxidant properties can be due to their ability to serve as donors in the free-radical chain reaction of lipid oxidation (O'Grady *et al*., 2006). This study therefore assessed the chemical profile of methanolic extracts of Bell pepper (*tatase*), Bird pepper (*ata wewe*), Cayenne pepper (*bawa*) and Scotch bonnet (*ata rodo*) as antioxidant, antimicrobial and colour-fixing ingredients in the production of cured-smoked chicken fillets.

High total phenol content observed for Bird pepper with similar values obtained for Cayenne pepper confirmed that these peppers have significant levels of antioxidant properties. The values obtained are comparable to those stated by Alvarez-Parrilla *et al*. (2011) who observed a range of 0.33 to 2.5 mgGAC/g of phenol contents in different hot and sweet peppers. Bell pepper showed higher DPPH (1, 1-diphenyl 2-picrylhyrazyl) radical scavenging activity compared to others. The rate at which a plant extract scavenges the DPPH radical has been reported to show the level of antioxidant capability it possesses. The scavenging activity

levels reported in this study are related to those observed by Shaha *et al*. (2013) when bioactive compounds in chilli peppers were assessed.

Components of plant extracts such as saponins, glucosides, tannins, alkaloids etc. which were used as defence mechanisms by plants are responsible for the antimicrobial activities they possess (Ceylan and Fung, 2004). Extracts' tendency to suppress the development of microorganisms is therefore dependent on the levels of these compounds present in them. Red peppers (*Capsicum spp*) have been reported to render inactive or slow down the growth of spoilage and disease-causing microorganisms (Cichewicz and Thorpe, 1996). The trend observed in this study agrees with reports of Careaga *et al*. (2003) who tested the effectiveness of Capsicum extracts on *P. aeruginosa* and Kalia *et al*. (2012) who worked on the possibility of inhibiting the growth of *S. aureus, B. subtilis* and *E. coli* using capsaicin isolated from *Capsicum* spp. Plant extracts and oils have been reported to exert their antimicrobial mechanism on target spoilage and pathogenic bacteria by decreasing their cytoplasmic pH, as well as disrupting the cell wall (Gonelimali *et al*., 2018).

Red peppers are also high in carotenoids, composition of which could vary due to variety (genetics), maturation, climate and geographical area of cultivation, soil type, conditions of growth and harvest, processing and storage (Conforti, 2007). All extracts of selected peppers showed considerable amounts of carotenoids. Higher values obtained for Cayenne pepper, closely followed by bell pepper for yellow pigment, red pigment and total carotenoid contents could be as a result of the visible deep red colour of the two varieties compared to the other varieties assessed. The red colour of matured peppers has been reported to be due to the presence of carotenoid pigments, with Capsanthin and Capsorubin making up most of the pigments in the fruits, 30-60% and 6-18%, respectively. The intensity of the red colouration is primarily due to these two pigments (Govindarajan, 1985). The results of this analysis were higher than those published by Topus and Ozdemir (2007) when carotenoid contents of five cultivars of *Capsicum annuum* were assessed. Studies by Blanco-Ríos *et al*. (2013) on red and orange peppers, on the other hand, had elevated levels of total carotenoids equal to those found in this research.

Experiment Two

Effects of *Capsicum* spp. Extract on Quality of Ready-To-Eat Cured-Smoked Chicken Fillets

The cooking loss and product yield of chicken fillets showed no specific trend due to the effect of bell pepper extract treatment. The cooking loss values were also observed to be high. This could be as a result of the effect of the cooking method used (smoking) leading to moisture loss than that of the extract treatment itself. Also, muscle and connective tissues shrink and expel exudate upon application of heat to meat (Tornberg, 2005), irrespective of the extract or nitrite treatment.

Meat pH has significant effect on many of its qualities. It influences meat colour, water holding capacity and tenderness (Aberle *et al*., 2001). The lower pH values obtained over storage for cured smoked fillets demonstrated the effectiveness of nitrite and extract to reduce the pH of meat products. This shows a level of preservative effect on the fillets, as the shelf life of vacuum-packed products is prolonged by decreased pH values (Korkeala and Björkroth, 1997). The higher pH observed for fillets cured with no nitrite/extract on the regression chart when compared with other treatments could be due to protein degradation brought about by the accumulation of metabolites from the activities of anaerobic bacteria present in the fillets, as well as deamination of proteins. The action of bacteria would have increased due to the absence of nitrite or the pepper extract which are expected to act as antibacterial agents during the curing process (Jay, 1996). It may also be the result of basic nitrogen compounds developing in the fillets during storage. The increased acidity level (low pH) of fillets over storage for all treatments may be due to an increase in the number of lactic acid bacteria, continued anaerobic storage status, and the carbonic acid development in vacuum packaging (Jay *et al*., 2005). As reported by Korkeala and Björkroth (1997), this pH decrease is typical of vacuum-packed products. However, this trend negates the report by Karabagias *et al*. (2011) during the preservation of lamb meat treated with essential oils stored under modified atmosphere packaging. Deuri *et al*. (2016) reported a similar reduction of pH values for vacuum-packed RTE smoked pork product stored under refrigerated condition for up to 45 days. Osterlie *et al*. (2005) also published a decreased pH values of minced meat over storage, thus leading to slower growth of meat product microorganisms.

Shear force measurement gives an impression of the hardness or toughness of meat or meat product. Shear force values, measured by the total cutting force, shows the variability in texture of meat products. It can be correlated with overall tenderness of muscle even though it could be highly variable due to many inherent and acquired factors of meat, as well as their interaction. The reduced values observed over storage for all fillets irrespective of the extract treatments showed that the fillets' toughness decreased with storage. This may be due to the decreased antioxidant effect of nitrite and bell pepper extract during storage, resulting in increased spoilage of fillets and decreased tenderness (Huff-Lonergan and Lonergan, 2005). Antioxidants have been seen to provide some protection against endogenous protease oxidation during meat and meat products storage. When physicochemical and sensory properties of beef jerky prepared with soy sauce, red pepper paste, and soybean paste were tested, Lim *et al*. (2014) discovered similar results. Shear force values of chicken meat have also been observed to decrease over cold storage, resulting in greater tenderness.

Water holding capacity refers to the ability of post-mortem meat to retain water after being subjected to external forces such as chopping, grinding, slicing, and heating. This has an effect on meat products' quality during production (Oeckel *et al*., 1996). Well after the isoelectric protein stage is achieved, the water holding ability of meat products is expected to decline as the pH decreases. This will also reduce the product's moisture content. This was however not observed in this study as the water holding capacity of the fillets in vacuum storage increased initially till day 45 during storage, after which a reduction was observed for all treatments. This trend of result could probably be because of the vacuum packaging medium used, which might not have allowed for the loss of moisture during storage.

Off-flavour, rancidity and degradation are caused by lipid oxidation of food products, making them unfit for human consumption. It is one of the causes of quality degradation during storage. The presence of free radicals during the lipid oxidation process contributes to the formation of aldehydes, which are responsible for the formation of rancid flavours and undesirable colour changes in meat products (Guillén-Sans and Guzmán-Chozas, 1998). This lipid oxidation process is multifaceted, it is dependent on a variety of variables, such as the meat's chemical composition, light and oxygen access, storage temperature etc. Significant effects of treatment and day of storage for lipid oxidation of all treatments were observed. Fillets cured without the inclusion of nitrite or extract had the highest lipid oxidation values while the lowest values were obtained as bell pepper extract inclusion levels increased. This

seems to be expected as the use of nitrite or pepper extract has been stated to inhibit or decrease lipid oxidation rate in meat products (Ozgur and Ozcan, 2011) and there is a high propensity for rancidity in the fillets cured with none of these substances. It was observed that lipid oxidation of fillets increased over the storage period. However, those values were low for all over-storage treatments and this may be due to the impact of the vacuum packaging system used, which excludes air from the product during storage. Lipid oxidation is highly dependent on oxygen availability and the reaction of the substance to oxygen during storage was significantly reduced, resulting in the fillets' general low lipid oxidation rates. This coincides with reports by Østerlie and Lerfall (2005) that added lycopene, from tomato paste, to minced meat during the storage period, resulted in a reduced rate of lipid oxidation.

The rate of protein deterioration is measured by the formation of volatile basic nitrogen compounds in meat products during storage. Examples of which are ammonia and some amines such as dimethyl and tri-methyl amines. The formation of volatile basic nitrogen shows the rate of protein decomposition in meat products by micro-organisms during storage and it is related to the activity of the decarboxylase enzyme of micro-organisms (Lin and Lin, 2002). During storage, volatile basic nitrogen formation in stored fillets did not follow a specific pattern but a regression map of the relationship between the storage period and volatile basic nitrogen showed a peak by day 30 of storage for most treatments, after which a gradual drop was observed until day 60. Protein breakdown as a result of increased microbial and proteolytic enzyme activity could account for this increase as volatile basic nitrogen formation has been related to an increase in microbial spoilage during meat product storage (Wang, 2000). Reports by Arashisar *et al*. (2004) showed a similar steady increase in the volatile basic nitrogen formation in modified atmosphere and vacuum-packed rainbow trout fillets with increased storage time. Increasing trends in volatile basic nitrogen values were also observed by Wang (2000) in vacuum-packed Chinese-style sausage over storage. In vacuum-packed beef stored for up to 3 months, high total volatile basic nitrogen values were also observed by Stella *et al*. (2013). This was due to a rise in the amount of Lactobacilli during storage, as they have in vitro proteolytic capabilities through multiple peptidases and proteases that can target meat proteins, both myofibrillar and sarcoplasmic (Katikou *et al*., 2005). However, these observations contradicted those of Choi *et al*. (2003), who found a decline in lipid oxidation and reactive basic nitrogen values of pork sausages when green powder was used in partial nitrite substitution.

The colour of meat and meat products is used by consumers to determine its freshness and wholesomeness. Colour also influences the acceptability and purchasing decision of consumers at points of sale. The colour and its intensity is dependent on the quantity and characteristics of red pigments in the meat. The significant effect of storage duration was observed for colour properties of fillets. The gradual significant decrease observed for lightness and redness for all treatments could be as a result of gradual fading of the colour of the fillets or degradation of haem and the bell pepper extract colour (Rohlík *et al*., 2013), thereby resulting in their darker brown colour as expressed in the increasing yellowness observed for the fillets. Significant effect of extract treatment and storage day were observed for colour parameters measured on stored cured-smoked chicken fillets. Also, the regression chart showed higher lightness and redness values for fillets with increasing levels of bell pepper extract inclusion, notwithstanding the general decrease was observed over storage. This could be as a result of the antioxidant capability of the bell pepper extract to inhibit oxidative reactions which help maintain the pepper pigment in the fillets during storage (Rohlík *et al*., 2013). Fillets cured with no nitrite or extract inclusion had expectedly low lightness value showing that either nitrite or the pepper extract had colour imparting effect. It also shows that the bell pepper extract has the ability to impart colour on the cured meat product as nitrite and increasing concentration showed better effect.

For yellowness of fillets, lowest value was observed in fillets with no nitrite or extract while highest value was observed in fillets as inclusion of bell pepper extract increased. The trend found for colour parameters in this study can also be linked to the gradual aggregation of metmyoglobin with storage time (Mancini and Hunt, 2005). The presence of reduction systems and the rate of lipid oxidation of meat products could have resulted in this accumulation on the meat surface and subsequent decolouration (Faustman *et al*., 2010). The observed trend in this study also agrees with reports by Fernández-Ginés *et al*. (2003), who observed a reduction in redness values over a 28-day shelf storage period in bologna sausage. However, Lorenzo *et al*. (2014) found that under modified atmosphere packaging and refrigerated conditions, the use of grape extracts on pork patties aids in the preservation of the colour of freshly made patties, although a slight decrease in colour parameters was observed.

Duration of storage significantly affected the microbial counts of the chicken fillets during storage. The slight increase observed in total anaerobic plate count and lactic acid bacteria count over storage is characteristic of vacuum-packed stored meat product. The observed

bacteria counts were all below the maximum permissible limits (MPL) of 7 $\log_{10}$ cfu/g for micro-organisms in meat products (ICMSF, 2002). This therefore shows that the effect of either the nitrite or extract in reducing microbial load in the meat product was evident, with additional barrier presented by the packaging medium used, resulting in reduced count even in the fillets cured no extract/nitrite. Vacuum packaging can be considered an additional protective factor, as it can preserve packed meat products by reducing oxidative reactions and damage, and also allowing a selective growth of anaerobic bacteria on the meat surface (Fu *et al*., 1992). Lactic acid bacteria have been reported to exhibit low spoilage potential and its dominance will depend on the initial flora of lactics, the meat pH, initial level of contamination and absence/presence of facultative anaerobic microbiota (Grau, 1981). Comparable results were reported by Babji *et al*. (2000). This low occurrence of anaerobic bacteria during storage could be as a result of the pH which decrease over storage, as well as a possible growth dominance by facultative anaerobic microbiota. The total anaerobic bacteria counts in vacuum-packed luncheon sausages reported by Shaltout *et al*. (2016) were however slightly better than the findings of this study.

Sensory assessment of meat products assists a representative of the product's intended consumers in assessing the shelf life and acceptability of the products. The shelf life of meat and meat products is the amount of time that the consistency characteristics of the products are retained before spoilage starts. AccepTable and unaccepTable bacterial contamination, based on the quantity and form of microorganisms initially present and their subsequent growth, determines odours, tastes, discoloration, and the general undesirable appearance of the product throughout its shelf existence (Iulietto *et al*., 2015).

Sensory assessment of chicken fillets cured with bell pepper extract showed a significant and gradual decrease in sensory quality parameters over the storage period. Presence of microbial growth on surface of fillets, an external property, was only visible on some surfaces of the fillets during storage. Level of occurrence showed ‘no microbial growth on all surface’ on day 0 to ‘microbial growth present on some surfaces’ by day 60 of storage. This occurrence could be attributed to the prolonged storage of the product, as this microbial growth was only visible by day 45 of storage, and even not in all treatments. This could also be because bacterial patina or visibility of microbes on meat surface is only visible when the microbial population is between $10^{7.5}$-10^{8}cfu per cm^2 (Iulietto *et al*., 2015), a stage that was not reached in the chicken fillets during storage. Odour of fillets reduced from normal characteristic

odour on day 0 to off odour-slightly sulphurous by day 60 while overall quality was excellent at the beginning of storage to acceptable quality by mid-storage, that is day 30 and reduced to slightly unacceptable quality by the end of storage (day 60). Significant detectable changes in vacuum-packed meat products as a result of spoilage, regardless of antimicrobial or antioxidant treatment, include visible growth (colonies of slime and bacteria), textural changes, noxious odours and flavours (Borch *et al*., 1996; Nychas *et al*., 2008).

The odour in stored chicken fillets from day 45 of storage was found to be slightly off or sulphuric. This delay in the incidence of off odour in fillets may be due to the fact that the development of the off odour in vacuum- and modified atmosphere - packed meat and meat products is less severe and may have a sour, acidic aroma, which is a result of spoilage caused by the aggregation of anaerobic bacteria, especially lactic acid bacteria, during storage (Pin *et al*., 2002). The decrease in the overall quality observed by panellists could be due to the overall effect of increased microbial growth in storage, off-odour production, decreased textural properties and overall fillet appearance. It however did not pass the limit of unacceptability, which was score one, for all treatments. The trend of this result is related to reports of Deda *et al*. (2007) who observed a reduced quality in appearance, taste and development of off odour in frankfurter sausages from the 3rd week of storage till the storage period lapsed, which lasted for 30 days. Babji (2000) also observed the development of sour odour in vacuum packed minced goat meat after storage for 40 days, depicting a decline in the quality of the meat product.

Experiment Three

Effects of Sodium Chloride Replacement on Quality of Ready-To-Eat Cured Smoked Chicken Fillets

The global call and advocacy for reduction of sodium chloride in foods and most importantly processed meat products because of its role in the incidence of hypertension that can lead to the development of coronary heart diseases (McCarty, 2004) has greatly intensified. This therefore necessitated the need to assess the use of other edible chloride salts in the development of cured chicken fillets. The chloride salts of interest in this study were Calcium chloride, Magnesium chloride and Potassium chloride. The ions of these chloride salts (Ca^{2+}, Mg^{2+} and K^{-}) have additional health benefits, beyond their possible roles in reducing ingoing

sodium in low-sodium meat product. such health benefits are building and maintenance of strong bones, support of muscle and nerve function, fluid balance, reduction in blood pressure etc. (He and MacGregor, 2007; Cormick and Belizán, 2019).

Significant effect of salt treatment was observed for cooking loss and product yield of freshly prepared cured-smoked chicken fillets. Cooking loss increased with more combinations of salts, resulting also in a reduced product yield with more salt combinations. That is, as the concentration of sodium chloride decreased. This trend could be as a result of the general moisture draining effect of the salts which can be related to the ability of salts to interact with the meat matrix thereby increasing solubility and extraction of meat protein. The presence of divalent cations (from magnesium and calcium ions) that can lead to increased myofibrillar protein extraction may also have resulted in the increased cooking loss observed in the fillets with an increase in the salt combination (Barbut, 1995). In reduced-fat mortadella prepared with calcium, magnesium, and potassium chloride blends as a partial substitute for sodium chloride, Horita *et al*. (2011) also reported a decrease in product yield with increasing divalent salt combinations. According to Ruusunen ansd Puolanne (2005), sodium chloride improves the water-binding capacity of meat, so reducing sodium chloride with increasing combinations of other chloride salts can result in lower product yield or higher cooking loss. In moderately reduced sodium chloride sausage samples, Aaslyng *et al*. (2014) found no impact of salt reduction, although significantly reduced sodium chloride sausages showed increased cooking loss/reduced product yield relative to the control. In conjunction with the salt treatments, the heat treatment method used for cooking (smoking) may also have affected the rate of moisture loss in the fillets.

Significant reduction in pH values over storage was observed for all treatments. This decrease may be attributed to the packaging used rather than the salt treatment. The pH reduction has been documented as a natural phenomenon in vacuum-packaged meat products due to the growth of lactic acid bacteria. In the literature, conflicting results have been reported about the impact of various salt combinations on the pH of meat products as a replacement for sodium chloride. Flores *et al*. (2005) found that adding varying amounts of calcium chloride to replace sodium chloride has no significant effect on the pH of dry fermented sausages. When sodium chloride was replaced with a combination of potassium chloride, magnesium chloride, and calcium chloride, Gimeno *et al*. (2001) however observed a significant drop in pH values for prepared dry-fermented sausages relative to standard ones. When sodium

chloride was substituted for other salts in the manufacture of mortadella. Horita *et al*. (2011) also found a similar decrease in pH. The association between the length of storage and the pH of the fillets indicates that a high correlation was seen for all treatments during the storage period, indicating that the decrease in pH was primarily due to the storage period.

Shear force values of stored fillets was observed to be significantly affected by salt treatments except for those fillets with combinations of calcium chloride and other salts. This effect however did not follow any specific pattern. A significant increase was observed by Lorenzo *et al*. (2015) after the salting stage to the end of processing of dry-cured laćon, with those treated with 50% sodium chloride and 50% potassium chloride having the highest values. The shear force value could be influenced by moisture content of the product as those with higher moisture content tend to have higher shear force. Aliño *et al*. (2010) also observed significant effect of sodium chloride replacement with blends of calcium, potassium and magnesium chlorides on texture properties of dry-cured loin. There was significant interaction of storage duration and shear force values of stored fillets. The correlation of the model was however observed to be low, suggesting that the slight increase over storage could also be to other factors other than the storage duration.

Sodium chloride performs a number of important functions in meat products, including preservative action, enhancement of the water holding capacity of the meat's myofibrillar proteins, which is aided by ionic force, reduction of water loss during transportation, and increased meat emulsion stability (Terrell, 1983). Sodium chloride also contributes to the meat product's protein binding, colour, taste, texture, and traditional salty taste (Lorenzo, 2014). Significant reduction in water holding capacity of the fillets cured with varying salt combinations could be as a result of reduced quality of the fillets with increasing storage period. This reduced quality could be due to increased microbial growth and formation of volatile basic nitrogen thereby leading to decreasing meat texture. Also, the reduction in the salt content over storage, evident by the reduced mineral levels observed in the fillets by the end of storage could have resulted in decreasing water holding capacity values. This is because sodium chloride has the capability to maintain water binding properties of meat proteins through its ionic properties and its reduction might result in reduced binding of the meat proteins thereby resulting in fillets with loose texture (Desmond, 2006; Choi *et al*., 2014). Horita *et al*. (2011) also observed decreasing water holding capacity with storage in mortadella when sodium chloride was substituted with combinations of calcium chloride and

potassium chloride. The reduced water holding capacity of the fillets with storage duration could also have been as a result of the decreasing pH values observed for the fillets during storage since high pH enhances the meat products' water holding capacity (Song *et al*., 2020). The significant interaction of storage duration and salt treatment with the slight correlation between storage duration and water holding capacity observed for the fillets however suggests that not only the storage duration but other factors such as the salt treatment, as earlier stated, could have affected the reduced water holding capacity values observed for the fillets during the storage period.

As a consequence of increased malondialdehyde formation induced as a result of the breakdown of polyunsaturated fatty acids and additional reactions involving amino acids expressed from muscle protein during storage (Ercoşkun and Özkal, 2011), a gradual increase in lipid oxidation of stored fillets by day 60 was observed. However, the observed values were low, representing a low degree of lipid oxidation incidence. Since oxygen, which is essential for lipid oxidation, was omitted during preparation, the vacuum packaging medium used may have contributed to this low incidence. Wu *et al*. (2016) also observed that partial sodium chloride substitution with potassium chloride resulted in lower lipid oxidation values in dry-cured bacon. Increasing lipid oxidation in salamis prepared with 50 percent sodium chloride and combinations of calcium and potassium chlorides until day 60 of storage was also observed by Dos Santos *et al*. (2017) also observed increasing lipid oxidation in salamis prepared with 50% sodium chloride and combinations of calcium and potassium chlorides till day 60 of storage. It was also observed that higher lipid oxidation values were observed in treatments with more combinations of chloride salts. This may be due to the improved ionic activity of the salts in the curing media as the salt compositions in the treatments became more complex. Zanardi *et al*. (2010) also found a rise in the oxidation of lipids with an increase in ionic strength when sodium chloride was substituted in Italian-type salami by potassium, calcium, and magnesium chloride mixture. By decomposing vitamins and unsaturated fatty acids, increasing lipid oxidation can have a detrimental effect on the sensory quality of cured meat products, as well as a significant influence on the nutritional value of the products.

The formation of volatile basic nitrogen in meat products during storage is an index of spoilage because its occurrence is as a result of enzymatic degradation of protein by microorganisms in the meat or meat product (Liu *et al*., 2009). The formation of volatile

basic nitrogen in fillets did not follow a specific pattern during storage however significant effect of salt treatment was observed. A continuous increase was observed for cooked sausages during storage by Jin *et al*. (2020) when sodium chloride was partially reduced by addition of potassium chloride in the preparation of the sausages. The volatile basic nitrogen values obtained mirrored the increase in microbial counts of the fillets over storage. And the rise observed during storage may be due to an increase in the product's microbial load, as higher microbial counts could lead to higher volatile basic nitrogen values. Similar results were obtained by Wang (2000) for vacuum packaged Chinese-style sausage. Armenteros *et al*. (2009) also reported a reduction in proteolysis (breakdown of protein by action of microbes and endogenous proteolytic enzymes) in dry-cured beef loins when sodium chloride was replaced with other chloride salts. This proteolysis results in meat products with excessive softness and unpleasant odour and flavours. The relationship between the storage duration and the volatile basic nitrogen values of the fillets shows a weak correlation between both. This suggests that it was not only the storage duration that had influence on the volatile nitrogen values of the fillets, but other factors such as the microbial growth and protein breakdown could have influenced these values as well.

Consumers' purchasing decisions are heavily influenced by the colour of meat and meat products. It has been documented that the use of sodium chloride in meat products has a major effect on texture, flavour, and colour (Lorenzo, 2014). The colour of cured meat products is primarily determined by the chemical transformations of natural pigments in meat caused by their reaction with sodium chloride and curing salts. Significant reduction in lightness and redness of fillets over storage could be attributed to the fading of the luminosity of fillets that occurred during storage. It could also be attributed to the reduction in salt content, and the ionic strength of the salt mixtures during storage, as colour has been reported to be sensitive to ionic strength of salt (Møller and Skibsted, 2006). This reduction in lightness and redness of the fillets resulted in an increased yellowness of the fillets, depicted by their increased brownish colour during storage (Mancini and Hunt, 2005). This shows a development of metmyoglobin pigment in replacement of the nitrosylmyogobin pigment observed in the fillets at the beginning of storage. When sodium chloride was substituted with potassium lactate and calcium ascorbate, Choi *et al*. (2014) found a substantial decrease in the lightness and redness, as well as increased yellowness of emulsified sausages. This outcome does not align with those noted by Ferrini *et al*. (2012) who, when sodium chloride was reduced in dry-cured meat, did not find any colour variations. Alino *et al*. (2010) also did

not record any significant variation in treatments during dry-cured ham processing and storage. The decrease in fillet lightness and redness could also be attributed to the softer texture of the storage fillets, with lower pH because the pH of the meat has a large influence on the colour of the meat, and higher pH has been confirmed to reduce the brightness of meat products.

Microbial counts significantly increased gradually over storage, irrespective of the salt treatments. Salts have been linked to changes in the microbial flora of vacuum-packed meat products towards the anaerobic bacteria (Yamanaka *et al*., 2005), as evidenced with higher values obtained for total anaerobic bacteria and lactic acid bacteria counts of the fillets. This shift can help maximise products' shelf life. The observed values were however below $7\log_{10}$ cfu/g, the Maximum Permissible Limit of microbial growth in food products ((ICMSF, 2002). This can be linked to the antimicrobial effect of bell pepper extract used in the formulation. The trend observed in this study followed the reports by Bower (2016) when sodium was reduced in the production of roast beef and turkey stored for 18 weeks. Increase in total aerobic and lactic acid bacteria counts were also observed by Aaslyng *et al*. (2014) in moderately and greatly reduced sausage and bacon samples. However, Alino *et al*. (2010) discovered no major impact of sodium chloride on dry-cured loin counts of lactic acid bacteria during storage. Sodium chloride can induce salt-tolerant and lactic acid bacteria to selectively multiply, while suppressing coliform growth (Yamanaka *et al*., 2005), this was also observed in this study. For all treatments, a significant relationship between storage length and fillet counts of lactic acid bacteria was observed. There was also a clear positive association between the period of storage and the counts of lactic acid bacteria, suggesting that, apart from other factors, the duration of storage had a major influence on the growth of the bacteria during storage. However, studies have shown that it is difficult to predict the antimicrobial activity of salt mixtures, as their growth may be affected by other factors such as pH, storage temperature, dissolved solutes in the medium and the microorganism strain (Gimeno *et al*., 2001). The observed reduced microbial counts of the fillets below the maximum permissible level could also be as a result of the smoking method used for cooking, before packaging. Deuri *et al*. (2016) indicated that the reduction in microbial counts of prepared Vawska rep (a RTE smoked pork product) can be attributed to smoking, antimicrobial activity of the curing ingredients and vacuum packing.

Sensory properties of fillets cured with varying salt combinations showed a slight decrease in quality over storage. The presence of microbial growth on surface was almost non-existent in the fillets in all treatments during storage. The creation of colour variations, off odours, and off flavours in meat products indicates spoilage and changes in texture of the product, with microbial growth being the main cause (Schirmer *et al*., 2009). The non-existence observed microbial growth on the surface of fillets during storage could be due to reduced incidence of visible colonies of bacteria as a result of the vacuum-packaging. It could also be due to antimicrobial activity of the bell pepper extract used. Fillets cured with 50% sodium chloride and magnesium chloride, as well as those cured with 50% sodium chloride and 25% each of Calcium chloride and magnesium chloride were observed to have lowest scores for odour. This shows a higher development of off odour compared to other treatments. This may be explained by the inclusion of magnesium chloride in the salt mixture. Also, the development of rancid odour or off odour could be as a result of increased oxidative rancidity and volatile compound formation due to protein denaturation during storage (Deuri *et al*., 2016). Devatkal and Mendiratta (2001) reported off odour in stored restructured pork rolls due to deterioration of flavour which could be as a result of microbial growth, oxidative rancidity. It can also be due to liberation of fatty acids. Sahoo and Anjaneyulu (1997) also reported similar results as obtained in this study, as smoked buffalo meat showed loss of colour, texture and development of spoilage odour during storage. Overall quality scores of fillets which dropped from excellent on day 0 of storage to slightly unaccepTable by day 60 could be as a result of development of off odour, spoilage brought about by microbial deterioration and some level of lipid oxidation occurring in the product during storage, irrespective of the packaging medium, antioxidant or antimicrobial treatment (Borch *et al*., 1996). This finding contradicts the findings of Jin *et al*. (2020) who found no major impact of sodium chloride reduction on the overall consistency of cooked sausages during storage. Zanardi *et al*. (2010) observed no major difference in spoilage odour between low sodium chloride salamis and conventional salamis (Cacciatore salamis). The interaction impact of storage time and salt treatment was only important for overall fillet quality during storage (Figure 30), with quality steadily declining. Although, the unaccepTable mark of 1 was not hit for all treatment during storage. There was slight correlation between the storage day and the quality of the fillets depicting that other factors could have affected the overall quality of the fillets, beyond the storage duration. These factors could include conditions of storage, lipid oxidation, development of volatile compounds and increased microbial growth (Nychas *et al*., 2008).

Mineral composition of fillets was determined on day 0 and day 60 of storage. A significant reduction was observed for sodium content in other treatments with the partial replacement of the sodium chloride with the other chloride salts, this being the aim of the study. Also, the fillets with other combination of chloride salts also has levels of magnesium, calcium and potassium, which are required for proper functioning of body functions, example of which is adequate bone health. Wu *et al*. (2014, 2015) reported high levels of sodium and potassium in dry-cured fillets in accordance to the level of inclusion during the curing process. This was also observed on day 0 in all treatments in this study. In relation to the treatments, sodium content increased slightly during storage for most treatments. Calcium content was observed to drastically reduce while potassium and magnesium maintained a fairly constant level over the storage period. The increase in sodium content could be as a result of moisture loss. This result does not agree with reports of Zanardi *et al*. (2010) who observed reduced sodium content and remarkable increase in calcium and magnesium contents of salamis during storage. Also, Gimeno *et al*. (2001) observed a decline in sodium content of chorizo, a dry-fermented Spanish sausage, with significant increase in potassium and calcium content, even though the sensory property scores were reduced.

The fatty acid composition is a major component of meat products that can be affected with changes in sodium chloride content of the product, thereby affecting the sensory and nutritional properties of the product (Wood *et al*., 2008). It can also be affected by the rate of lipid oxidation with unsaturated fatty acid been more susceptible to the oxidation changes (Dos Santos *et al*., 2017). Fatty acid composition of stored fillets, either saturated, mono- or poly-saturated, was observed to significantly reduce over storage, as well as with increased combination of chloride salts. These results suggest that lipolytic activities continued in stored fillets during storage, with more activities observed with increasing combinations of chloride salts. Similar results were observed by Dos Santos *et al*. (2017) for reduced NaCl salamis during a 30-day storage. Zanardi *et al*. (2010) however observed lower fatty acid composition in Italian salami with sodium chloride treatment compared to the treatments with other chloride salt mixture.

Experiment Four

Consumer Acceptability and Keeping Quality of Cured Smoked Chicken Fillets Developed with *Capsicum* spp. Extract and Reduced Sodium Level

Chicken fillets prepared with 0.45% bell pepper extract and 50% Sodium chloride + 25% Magnesium chloride + 25% Potassium chloride were vacuum-packed and stored for an extended duration of 90 under refrigeration and at room temperature.

The physicochemical properties of these fillets have been greatly impacted by the length of storage. For any of the treatments, the pH values of fillets did not follow a clear trend over storage. Similarly, Gadekar *et al*. (2014), discovered unpredicTable behaviour in pH values during refrigerated storage. This may be due to the lower microbial spoilage found during storage, as increased growth of spoilage species could contribute to higher protein degradation and aggregation of bacterial metabolites on protein and amino acids, increasing the pH of the meat product (Jay, 1996; Lawrie, 1998). In this study, however, the obtained pH outcome does not agree with reports by Muhlisin *et al*. (2013), who observed decreased pH values of modified-atmosphere-packed pre-cooked hamburger patties during refrigerated temperature storage. He related this reduction to the presence of salts of organic acid used in the formulation. also reported decreased pH in organic salts-enhanced beef steaks (3 percent calcium lactate). Increasing pH values for buffalo meat stored at 4^{o}C, 25^{o}C and 37^{o}C over storage were observed by Vishnuraj *et al*. (2014), even though control samples (4^{o}C) had reduced pH values compared to others. A significant but slight association between storage duration and fillet pH values was observed, indicating that variables other than storage duration had an effect on fillet pH during storage.

The water holding capacity of meat and meat products is directly related to myofibrillar proteins (Gadekar *et al*., 2014) and the decrease in fillets' water holding capacity during storage may be related to the decrease in meat proteins' water holding capacity due to denaturation and microbial activity, regardless of storage circumstance (Suvanich *et al*., 2000). Chowdhury *et al*. (2006) found that the WHC of refrigerated aerobically stored minced goat meat declined with storage time.

Meat tenderness, as measured by shear force, is an outcome of multiple ongoing meat or meat product processes. Myofibrillar protein proteolysis, which ensures structural stability of muscle fibers, determines meat tenderness (Koohmaraie *et al*., 2002). Proteolytic

deterioration weakens muscle fibers, causing meat to become tender. For fillets' shear force values, significant effects of storage duration were observed, but no consistent pattern of impact was observed. Marcinkowska-lesiak *et al*. (2016) observed no significant effect of storage on the shear force of vacuum packed or modified atmosphere-packed meat under refrigerated storage and concluded that tenderisation process which influences the shear force of meat and meat product takes time to occur, as evidenced by the significant effect of storage duration on fillets in this study.

Lipid oxidation of stored meat products occurs due to oxidative changes in the meat product's unsaturated fatty acids throughout storage. It was observed that the lipid oxidation rate of the fillets increased steadily over storage, peaking at 45, after which it stabilized until the end of storage. This increase may have been as a result of the production of volatile metabolites during storage (Gadekar *et al*., 2014). However, irrespective of the storage status, the values observed were low and this can be due to the antioxidant activity of the bell pepper extract used. The behaviour can be due to its ability to quench free radicals, scavenge oxygen, reduce globin denaturation or function as a reducing agent (Lawrie, 1979). Oxygen, a significant precursor to lipid oxidation, may also have been removed during the storage process as a result of the packaging medium used. Lara *et al*. (2011) found that cooked pork patties processed in modified atmosphere packaging oxidized at a low rate. Wójciak *et al*. (2011) observed high lipid oxidation of pork meat product during chilled storage while samples treated with red pepper extract had decreased lipid oxidation rate. Samouris *et al*. (2007) discovered an increase in the rate of lipid degradation in raw and cooked turkey breast meat stored under refrigeration.

The degradation of protein in meat products, represented by the formation of volatile basic nitrogen compounds, is correlated with the activity of microorganisms' amino acid decarboxylase (Lin and Lin, 2002). The large increase observed for fillets stored under refrigeration may be that protein degradation in refrigerated fillets over storage increased relative to those stored at room temperature, resulting in high volatile basic nitrogen values being observed. The volatile basic nitrogen values of the fillets, with increasing microbial load and spoilage, may also have been increased with the extended storage time. This outcome is similar to reports by Chen *et al*. (2004) that also observed increased formation of volatile basic nitrogen values during prolonged storage of pork jerky.

Meat colour is an important parameter in meat evaluation, and it is the means through which consumers assess its freshness and quality. Maintaining the colour parameters of meat for the longest time possible during storage is therefore desirable. It was found that the lightness and redness of cured smoked fillets stored under various conditions significantly decreased over storage. This reduction may be attributed to progressive myoglobin oxidation and metmyoglobin accumulation (Mancini and Hunt, 2005). Wójciak *et al*. (2011) also observed declining lightness values under chilled storage over storage for minced pork meat. This however does not agree with reports by Muhlisin *et al*. (2013) for pre-cooked hamburger patties during storage. They observed an increase in lightness of the patties during the storage period while redness slightly reduced. According to the findings of this study, the increase in fillets' yellowness during storage may be as a result of meat pigmentation changes during storage. Storage over a long period, regardless of the colour-impacting agent used, contributes to increased metmyoglobin synthesis. Similar findings were obtained by Marcinkowska-lesiak *et al*. (2016) in which chicken meat was stored under refrigerated conditions in vacuum and modified atmosphere package. Saucier *et al*. (2000) also reported substantial increases in the yellowness of poultry meat. This rise was due to the continuing process of meat spoilage and oxidative damage occurring in poultry meat irrespective of oxygen-free packaging or storage conditions.

Microbial load of fillets significantly increased over storage with fillets stored under refrigeration having overall lower values compared to those stored at room temperature. This higher values observed for fillets stored at room temperature for both total anaerobic and lactic acid bacteria counts could be related to temperature of storage, which allowed for increase in microbial count over storage. Microbes proliferate under favourable conditions and the temperature of storage favoured their growth, irrespective of the storage package used. Also, the microflora dominant in the fillets could be mesophilic in nature, which allowed for better thriving in moderate temperature ranging from 20^{o}C to 45^{o}C (Schiraldi and De Rosa, 2014). This is however not certain, as the assessment of microbial diversity in the fillets was not carried in this study but can be explored in further studies. Initial microbial load, packaging gas permeability, and storage temperature are all factors that can affect the shelf life of packaged meat and meat products (McMullen and Stiles, 1991). Similar increases in the growth of lactic acid bacteria during refrigerated vacuum storage and subsequent aerobic storage of minced goat meat have been observed by Babji and Murthy (2000). However, even though an increase over storage was observed, Babji *et al*. (2000) noted no

substantial impact of storage condition on lactic acid bacteria counts of refrigerated minced goat meat. In pre-cooked hamburger patties during storage, Mushlisin *et al*. (2013) also found increased counts of anaerobic bacteria, regardless of the storage environment or storage package used. In refrigerated vacuum-packed buffalo, with increased storage time, Sahoo and Anjaneyulu (1997) reported increases in both lactic acid bacteria and total anaerobic bacteria counts. It is also noteworthy that microbial counts recorded were below the maximum permissible limit of $7\log_{10}$ cfu/g (ICMSF, 2002) during the storage period. This may be due to one or a combination of the antimicrobial activity of the extract of bell pepper, the combination of salt used, the product's moisture content and the packaging medium used.

Sensory quality of fillets significantly reduced over storage with fillets stored at room temperature having higher deterioration than those stored under refrigeration. The reduced quality observed could be as a result of spoilage that occur in fillets as a result of microbial growth, lipid oxidation and build-up of volatile basic nitrogen in fillets during storage. The lower quality observed for fillets stored at room temperature could be attributed to the temperature of storage, which could influence faster deterioration compared to refrigerated storage. Microbial growth was visible on some surfaces of the fillets, off odour and sulphuric odour was recorded for fillets and overall quality reduced from excellent to slightly accepTable by the end of the storage period. Similar results were also reported by Babji *et al*. (2000). Sulphide and putrefactive odour was observed in refrigerated vacuum and aerobically packed minced goat meat at 40 days and 28 days of storage respectively, with acceptability maintained up to day 28 for vacuum-packed goat meat.

Mineral composition of fillets was not significantly affected by storage condition, however, a reduction was observed over storage for both treatments. A reduction in the mineral composition of fillets observed over storage could be as a result of increased moisture content observed in the fillets regardless of storage condition, at the end of the storage period.

A decrease in the fillets' fatty acid composition during the period of storage was observed. However, significant effects of storage status on fillet fatty acids except for acetic acid on day 0 and behenic acid on day 90 of storage were not observed. Both acids are saturated fatty acids, so they could probably not have influenced the development of lipid oxidation in the product, as low levels of lipid oxidation were reported in this study. This is due to the fact that variations in fatty acid composition are significant indirect indicators of lipid oxidation in stored meat and meat products (Orkusz and Michalczuk, 2020). The sensitivity of meat

products to lipid oxidation is also primarily determined by the level of unsaturation of their fatty acids (Wazir *et al*., 2019).

The emergence of functional foods has been as a result of increased consumer awareness of the health and nutritional effects of their foods and increased demand for healthy meals. Meat has been manipulated as a functional food by modifications and the introduction of ingredients that have the potential to positively affect consumers' health (Hathwar *et al*., 2012). Examples of such modifications are the use of natural antioxidant and antimicrobial sources in meat product development, and sodium reduction in meat products. the consumer acceptability of cured smoked chicken fillets prepared with bell pepper extract and reduced sodium level showed that a large percentage (90.50%) of the respondents (n = 84) were willing to pay/buy the product if sighted on shelf. According to the respondents, this is because of its attractive and hygienic packaging, the form of presentation, that is its ability to serve both as ready-to-eat and ready-to-cook meat product, its accepTable taste and juiciness, its composition in terms of the addition of natural antioxidant, antimicrobial and colourant from red pepper extract, in replacement of the synthetic compounds, as well as its reduced sodium content, its relative low cost compared to other low moisture meat products. This showed that consumers accept the developed meat product. It also indicates a positive market opportunity for the developed chicken fillets, as well as valuable insight into further functional meat product development. Aaslyng *et al*. (2014) suggested that, while their sensory characteristics might vary from the traditional product, customers are likely to regard meat products with reduced salt content as suiTable products. de Andrade *et al*. (2018) reported that sheep meat Coppa, a revolutionary meat product produced with reduced salt content, has high consumer acceptability. Hung *et al*. (2016) also stated that customers expressed favourable attitudes and purchasing intentions towards the production of novel processed meat products with natural compounds and decreased levels of nitrite. Their buying intentions were related to the preference for natural additives over chemical additives and to the overall health benefits obtained from the product's use.

Chapter 6 CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Contributions to Knowledge

i. Extract of bell pepper can be used in curing mixture for meat products because of its superior antioxidant, antimicrobial and colour imparting properties
ii. Incorporating bell pepper extract up to 0.45% inclusion rate in curing solution for the development of cured-smoked chicken fillets gave comparable effects on quality as nitrite-cured chicken fillets
iii. The use of a salt combination of 50% Sodium chloride + 25% Potassium chloride + 25% Magnesium chloride in curing solution for chicken fillets reduced ingoing sodium level while ensuring acceptable product quality
iv. Cured-smoked chicken fillets with natural additive and reduced ingoing sodium levels was acceptable to consumers

6.2 Conclusion

Capsicum annuum (Bell pepper) showed higher antioxidant and antimicrobial activities, in comparison with other selected peppers while also having an acceptable colour imparting property

Increasing inclusion levels of bell pepper extract positively influenced the quality of chicken fillets during storage

0.45% inclusion level of bell pepper extract had comparable effects with the positive control (nitrite) with respect to quality indices measured during storage of cured-smoked chicken fillets

The combination of chloride salts in treatment 7 (50%NaCl + 25%KCl + 25%$MgCl_2$) maintained protein and microbial quality of chicken fillets during the storage period

Refrigeration increased lipid oxidation of stored chicken fillets but reduced protein degradation and microbial spoilage

Storage conditions did not alter the colour properties of the product

Overall sensory quality of chicken fillets stored at refrigerated temperature was acceptable up to day 74 while fillets stored at room temperature were acceptable up to day 69

The majority of respondents were willing to buy/pay for the product if sighted on shelf, as a result of its acceptable sensory qualities and ability to serve as a ready-to-eat/cook meat product

6.3 Recommendation

- Further molecular studies should be carried out on determining the microbial diversity in cured smoked chicken fillets as affected by red pepper extract, chloride salt combinations and storage condition.
- Assessment of other levels of inclusion of the chloride salts should be carried out for further refinement of best inclusion level in chicken fillets.
- Other meat products can also be developed and assessed using the test ingredients used in this study.
- Further meat product development studies should be carried out using the breed of chicken used in this study, the *Funaab Alpha* breed, because of its high meat yield potential.

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APPENDIX

University of Ibadan
Department of Animal Science

Consumer Assessment of Cured-Smoked Chicken Fillets Prepared with Red Pepper Extract and Chloride Salt Combinations

Kindly fill in the required information. Thanks.

Consumers' demography

1. Sex: (a) Male ( ) (b) Female ( )
2. Age (years): 11 – 20 ( ); 21 – 30 ( ); 31 – 40 ( ); 41 – 50 ( ); 51 – 60 ( ); Above 60 ( )
3. Marital status: (a) Single ( ) (b) Married ( ) (c) Divorced ( ) (d) Others: ……..
4. Educational qualification: (a) Primary ( ); (b) Secondary ( ); (c) Tertiary - Undergraduate ( ); Post-graduate ( ); (d) Others: …….
5. Income (monthly): #0 - #5000 ( ); #5001 – #10,000 ( ); #10,001 - #50,000 ( ); #50,001 - #100,000.00 ( ); Above #100,000.00 ( )

Consumers' consumption pattern of meat products

6. Do you consume processed meat products? Yes ( ); No ( )
7. Which types do you consume? (Kindly tick all that is appropriate)
   Suya ( ); Kilishi ( ); Asun ( ); Kundi ( ); Corned beef ( ); Hot dog-sausage ( ); Minced meat ( ); Others ……..
8. How often do you consume such? Frequently ( ); Occasionally ( ); Never ( )

Sensory evaluation of freshly prepared cured-smoked chicken fillets

Instruction: Please tick your level of preference for each trait of the given sample

Score	Colour	Flavour	Texture	Tenderness	Juiciness	Overall Acceptability
1	**Extremely dark**	**Not perceptible**	**Extremely coarse**	**Extremely tough**	**Extremely dry**	**Dislike extremely**
2	**Just dark**	**Just perceptible**	**Very coarse**	**Very tough**	**Very dry**	**Dislike very much**
3	**Moderately dark**	**Moderately perceptible**	**Moderately coarse**	**Moderately tough**	**Moderately dry**	**Dislike moderately**
4	**Slightly dark**	**Slightly perceptible**	**Slightly coarse**	**Slightly tough**	**Slightly dry**	**Dislike slightly**
5	**Intermediate**	**Intermediate**	**Intermediate**	**Intermediate**	**Intermediate**	**Intermediate**
6	**Slightly light**	**Slightly intense**	**Slightly fine**	**Slightly tender**	**Slightly juicy**	**Like slightly**
7	**Moderately light**	**Strongly intense**	**Moderately fine**	**Moderately tender**	**Moderately juicy**	**Like moderately**
8	**Very light**	**Slightly intense**	**Very fine**	**Very tender**	**Very juicy**	**Like very much**
9	**Extremely light**	**Extremely intense**	**Extremely fine**	**Extremely tender**	**Extremely juicy**	**Like extremely**

Consumers' willingness to buy/pay for product

9. Would you be willing to buy/pay for this product if sighted on shelf? Yes ( ); No ( )
   If Yes, why?
   ……..…….
   If No, why?
   ……..……..
10. If product cost (per kilogram) is within the following range, would you purchase?
    a. #1200.00 - #1500.00 Yes ( ); No ( )
    b. #1500.00 - #1800.00 Yes ( ); No ( )
    c. #1800.00 - #2100.00 Yes ( ); No ( )
    d. #2100.00 - #2500.00 Yes ( ); No ( )