An- Najah National University

Faculty of Graduate Studies

Effect of replacing sodium chloride by potassium chloride on the quality traits of beef burger

By

Azhar Farah

Supervised

Dr. Samer Mudalal

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This Thesis was Defended Successfully on 23/7/2020 and approved by:

Defense Committee Members	**Signature**
1. **Dr. Samer Mudalal / Supervisor**	....
2. **Dr. Fuad Alrimawi / External Examiner**	....
3. **Dr. Mohammad Altamimi / Internal Examiner**	....

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الاقرار

أنا الموقع أدناه موقع الرسالة التي تحمل العنوان:

Effect of replacing sodium chloride by potassium chloride on the quality traits of beef burger

أقر بأن ما اشتملت عليه الرسالة هو نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أي درجة أو لقب علمي أو بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provide in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's name: **اسم الطالب:** ازهار فرح عثمان الحج

Signature: **التوقيع:** ازهار

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Abstract

Sodium is one of the most important nutrients for human body functions. However, sodium dietary intake currently exceeds the nutritional recommendations around the world, and it is considered a primary cause of hypertension and cardiovascular diseases. Due to strong association between excess of sodium intake and the incidence of hypertension and other chronic diseases, several health international organization recommend to reduce sodium content in foods. Meat and meat products have significant contribution in sodium intake. Accordingly, the aim of this study was to evaluate the influence of replacing sodium chloride by potassium chloride on the quality traits of beef burger. Microbiological stability (aerobic bacteria, psychrotrophic bacteria, and yeasts/molds), chemical properties (proximate composition, TBA, metmyoglobin, pH), and physical properties (texture profile analysis, color traits (L*a*b*), and sensory traits) have been evaluated during the period of study. In total, 160 patties of beef burger were manufactured to represent four treatments: control group (0% sodium reduction), R_{15} (15% sodium reduction), R_{30} (30% sodium reduction), and R_{50} (50% sodium reduction). Salt replacement did not affect ($p>0.05$) ash, fat, protein content, textural properties, sensory parameters, microbiological stability, and cooking loss. Using potassium chloride resulted in samples with similar sensory characteristics to sodium control samples. Although there were a minimal changes on color and moisture between samples.

Keywords: beef burger, sodium chloride, replacement, quality traits.

Chapter 1

Introduction

1.1 Background

Nowadays, the consequences of high sodium dietary intake is considered one of the most important health issues in the world. High sodium dietary intake is associated with an increased risk of several chronic diseases such as high blood pressure (Hp), kidney failure and CVDs (Doyle & Glass, 2010). As recent epidemic studies showed that sodium intake reached globally to high range about 9 to 12g\day of sodium chloride, which is equal about 3.6 to 4.8g\day as sodium (Rust & Ekmekcioglu, 2016). The current sodium intake exceeded the Recommended Daily Allowance (RDA) where World Health Organization (WHO) recommended that sodium intake should not exceed 2g\day that is equal to 5g\day of sodium chloride (NaCl) (WHO 2012a). A reduction of sodium intake has positive economic and health consequences. In this context, cardiovascular disease is considered the most expensive health related disease around the world which accounted around 11% of total health expenditure. It was found that the reduction in sodium intake would prevent 8.5 million of cardiovascular-related deaths in the world over 10 years (Neal et al, 2006, Asaria et al, 2007). Both hypertensive and normotensive people can benefit from sodium reduction, but it is still more effective for hypertensive patients (Liem et al, 2011). The main source of salt (sodium chloride) is from regular diet (DSI) (Figure 1) especially from meat products, cheese, bread, and ready meals. It was found that processed and fast food contribute to around 75% of sodium intake in the diet (Liem et al,2011) (Figure 2). Therefore, reducing sodium chloride in processed meat products is considered an effective step within the plan to reduce salt consumption.

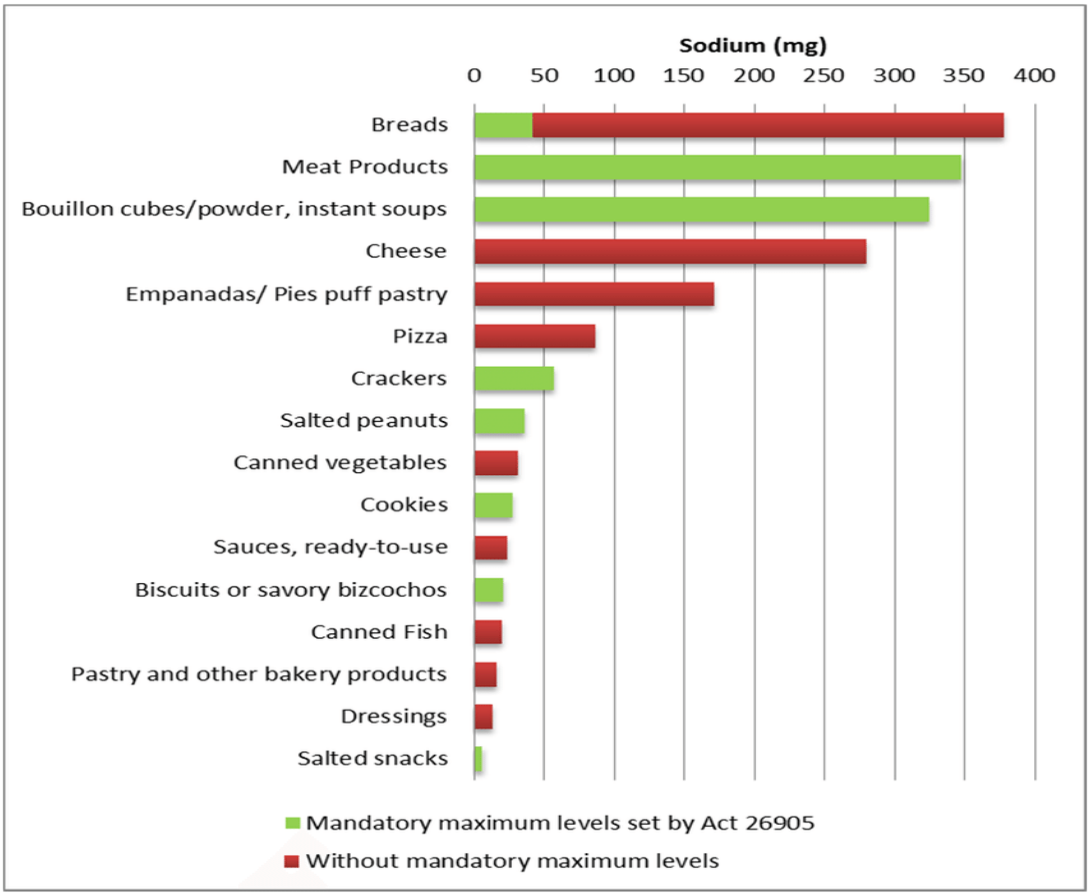

Figure 1: Dietary food sources of sodium (Elorriaga et al, 2017).

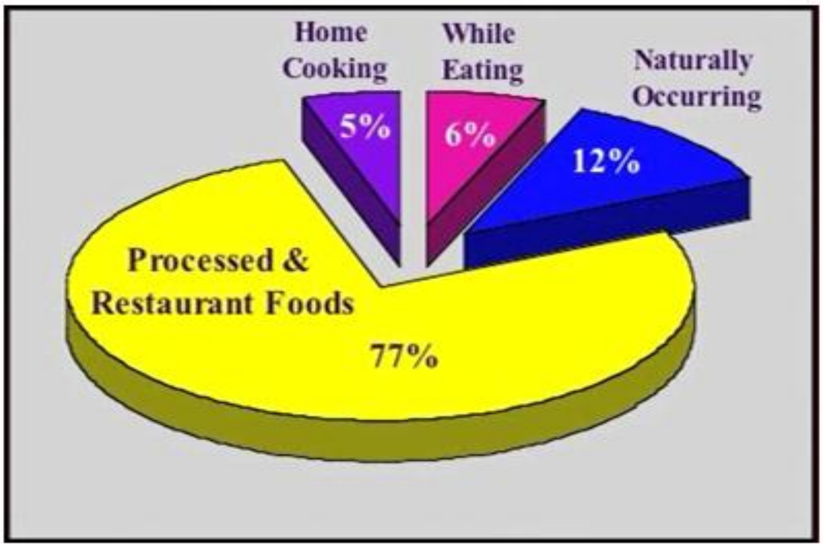

Figure 2: The percentage in which food made from salt is involved in food (Liem et al,2011)

Adding salt to meat during the manufacturing process is an important step as it helps to control the microbial level in meat products as well as improve the textural properties and water holding capacity. Moreover, sodium chloride imparts salty taste and enhance the flavor of the meat products. It was found that reduction of sodium chloride adversely affected sensory, textural, quality and yield (Bower, 2016). There are many strategies to reduce sodium chloride in meat products. It is possible by reducing the amount of added sodium chloride, or by substitute NaCl with other salts (KCl, CaCl2, and MgCl2) (Lorenzo et al. 2015a, b). Based on many studies, potassium chloride (KCl) is considered the most salt used as replacer for sodium chloride (NaCl) in strategies to reduce sodium in processed meat products. This because potassium chloride (KCl) has similar properties to sodium chloride (NaCl). Potassium chloride had no effect on development of high blood pressure and heart diseases (Castro &

Raij, 2013). The main side effect of potassium chloride is a metallic or bitter taste in high concentration (Bower, 2016).

There are many processed meat products like ham, burger, sausage and bacon etc, with a have salt concentration that ranges from 1.5-3.0%. Consumer's acceptability for meat products was negatively affected if the salt concentration reduced below 1.5% (Tobin et al, 2012x).

Beef burger is one of the most popular products of processed meat products that traditionally contains from 2.2 to 2.4% salt (Freitas et al, 2017), which represents around 50% of the recommended daily amount of sodium in 100 g of product (WHO, 2003; 2012). Although burger consumption contributes to high sodium intake, but it is still famous food and people consume it widely around the world, so it affects human’s diet directly. Thus, researches on reducing the amount of sodium in burgers are needed.

1.2 Problem statement

In Palestine, the overall prevalence of hypertension is 27.6% between population aged 25 years and over, which is considered high (Khdour et al, 2013) (Hussein et al, 2017). Palestinian individuals consume about 6000 mg of sodium per day (Pcbs, 2011) (Abdeen & Qasrawi, 2002), this means that Palestinian individual consumes two and a half times than recommended according to WHO (2.4 g\day). In addition, the prevalence of hypertension in Palestine is 27.6% (Hussein et al, 2017). Processed foods contributed to around 75% of sodium intake (Liem et al, 2011), while meat products contributed to around 20-30% of sodium daily intake (considered a main source of sodium in human diet) (Jiménez-Colmenero et al, 2001; Inguglia et al, 2017). Furthermore, researchers stated that meat and meat products are considered the second largest contributor to dietary salt after cereal products (Matthews & Strong, 2005). Therefore, one of the most effective ways to reduce sodium intake is by partial substitution of sodium by potassium in processed meat.

1.3 Research objectives

1.3.1 Overall Goal

The main goal of this research is to evaluate the possibility of sodium chloride reduction in beef burger by using potassium chloride.

1.3.2 Research Objectives

Research aims to achieve the following objectives:

- To evaluate the effect of replacing sodium chloride by potassium chloride on technological and sensory properties as well as microbial shelf life of beef burger.
- To develop a beef burger product with low sodium content with reasonable shelf life and accepted sensory traits.

1.4 Hypothesis of the research

We hypnotized that changing the amount of sodium and replacing it with potassium in beef burger will affect chemical, physical, sensory parameters to certain level, but will not significantly affect microbiological activity, because both sodium and potassium have the same effect against microbes (Bidlas & Lambert, 2008).

Chapter 2
Literature review

2.1 Salt (sodium chloride)

Sodium chloride, which is also known as common salt or table salt, is a chemical compound with chemical formula NaCl. It is a translucent, white crystals that are cubic in shape and strongly soluble in water. It is responsible for the salinity of sea and ocean water. Historically, there are two main sources of salt: seawater and or rock salt. By weight, salt consists of 40% sodium and 60% chloride (5g salt equal 2g sodium), and it provides human diet around 90% of the sodium He et al. (2012).

Salt is an important element in human life and it has been used by humans since ancient times for many uses such as food preservation and seasoning. The ability of salt to preserve food assisted in the availability of seasonal food in the off-season, and contribute to facilitate the transport of food over long distances.

In ancient times, it was difficult to obtain salt because it was high value element. There were a number of areas around the world lacking it until it was transported to them by sea navigation and trade. Today, salt can be accessed almost all over the world and it is relatively cheap and often fortified with iodine. As historical records showed that humans used salt since ancient times in small amounts around 1g/d. This amount mainly came from ingestion of meat, which considered high in salt (around 0.6g for lb.), as salt was used; since ancient times to preserve food especially meat. However, this little intake of sodium did not last, where nearly about 1000 years ago; salt consumption has increased to about 5g/d. When refrigerators were invented, salt was no longer used as a preservative, so the consumption of salt declined, but recently with increased consumption of salted processed food, salt consumption has reached to 9-12g/d, which is two times more than recommended by World Health Organization (Institute of Medicine, 2010;Ha,2014).

In our country (Palestine), sodium intake by Palestinians reached approximately 6000 mg/daily (Palestinian central bureau of statistics, 2011), which is twice the amount recommended by World Health Organization (Hussein et al, 2017), and that is considered high amount (Khdour et al, 2013).

Physically, sodium (Na+) is definitely important for life, which represents the greatest cation in extracellular fluid in the human body. In addition, it has a role in contracting and relaxing of muscles, keeping the appropriate balance of water and minerals, and also the role in conducting nerve impulses. Sodium concentration between extracellular and intracellular fluid controlled by sodium-potassium pump depending on concentration gradient. A healthy human body can absorb 100% of consumed sodium from food, particularly the absorption occurs in distal small bowel and colon. The overall sodium balance maintained by kidneys. Disposal of sodium from body occurs via urinary excretion through kidneys (Parrish, 2014; Strazzullo & Leclercq, 2014; Cepanec et al, 2017). Despite the importance and necessity of sodium to the human body, excessive sodium consumption associated with occurrence of many chronic diseases and health implication such as heart disease, high blood pressure (He & MacGregor, 2010), kidney failure (Cepanec et al, 2017), stomach cancer (Tsugane et al, 2004), decreased bone mineral density (Osteoporosis) (Devine et al, 1995), and possibly obesity. As these diseases grew and salt consumption increased, WHO recommended that the upper intake of sodium should be around 2.0-2.4 g/day which equals around 5.0-6.0g/day of salt (World Health Organization, 2012).

Salt is a famous additive in processed meat because it has diverse properties and low cost (Albarracın et al, 2011). It plays very important roles in processed meat production. It is contributes to sensory taste and safety of processed meat products. In addition, it increases cooking yield, water holding capacity, and processed meat product shelf life (Desmond, 2006; Ruusunen & Puolanne, 2005). Salt has major roles in preservation of meat (Albarracin et al, 2011). In addition, salt caused a change in microbial flora to further salt tolerant bacteria like lactic acid bacteria that improved product quality and shelf life (Blickstad & Molin, 1983).

2.2 Sodium consumption and its sources

In the past, human was used to consume a diet that consists of natural plant and animal foods containing small amounts (less than 2g/day) of sodium. Nowadays, the consumption of sodium has increased to reach to 5.7-10g/day (Campbell et al, 2012), while the body needs only 1.5g/day for adults to maintain normal physiological functions (Verkleij, 2012). World Health Organization (WHO) recently recommended that the consumption of salt should be less than 5 g/day (87 mmol sodium/day), that equals 2\3 tsp of table salt (Campbell et al, 2012). The following table shows the average sodium intake around the world.

Table 1: The average sodium intake around the world

Country	Average sodium intake
US	140–160 mmol Na/day (8.2–9.4 g NaCl/day) (Cordain et al, 2005)
United Kingdom	161 mmol Na/day (9.4 g NaCl/day) (Brown et al, 2009)
Asian countries	More than 206 mmol Na/day (12.0 g NaCl/day) (Liem et al, 2011)

The daily median of sodium consumption in Palestinian population was around 5294mg (4,646 mg for female, 5941 for male). The salt consumption for population under 40 years was 4,828 - 4,778 mg/day for females, and 6,120 - 5,364 mg/day for males, while the intake of oldest group was lower (females 4,211 mg, males 4,859 mg). In addition, the salt consumption of Gaza population was higher than in West Bank. However, all intakes higher than recommended and need to be reduced (Abdeen & Qasrawi, 2010). As sodium is very important to human body, the consumed amount is more than needed for the physiological functions (Aburto et al,2013).

Processed food provides about 70-75% of total sodium intake, 10-15% found naturally in unprocessed food and about 10-15% comes from the addition of sodium during cooking or at the table (optional), however some people use more optional sodium (Aburto et al,2013). Some national surveys reported that bread and cereals contribute to about 25% of sodium intake, meat and meat products contribute to around 18%, the contribution of cheese products was about 17%, and sauces and soups was about 10% (Aburto et al,2013).

In order to make a healthier choice, and reduce the intake of salt or sodium of processed food, British Dietetic Association developed the following food labeling handy guide:

	Low	**Medium**	**High**
Salt	0g-0.3g	0.3g-1.5g	More than 1.5g
Sodium	0g-0.1g	0.1g-0.6g	More than 0.6g

2.3 Health implications of excess sodium dietary intake

Sodium is a vital nutrient for human life. This cation ($Na+$) is responsible for crucial functions in human body. It influences the physiological process in interaction with other cations like calcium and potassium. Through intestines, our body can absorb around 98% of digested dietary sodium, and mainly excreted by kidneys with a small amount lost through perspiration. Nowadays, the intake of sodium chloride increased around the world causing increase the incidence of many chronic diseases like hypertension, cardiovascular diseases, bone disease, kidney failure, and stomach cancer, etc. (Doyle & Glass, 2010)

2.3.1 Hypertension

Excess sodium chloride intake can lead to hypertension (high blood pressure), which is abnormal elevation of blood pressure more than 140/90 mmHg (Bower CG, 2016). Hypertension is a widespread public health problem due to high prevalence and it leads to other diseases such as cardiovascular diseases like myocardial infarction and stroke, renal failure and may lead to death if not discovered early and cured properly (James et al, 2014). Blood pressure increases as a reaction to high sodium intake in order to avoid salt and fluid accumulation through increasing total excretion of salt and water (Karppanen & Mervaala, 2006). Hypertension can occur without any signs or symptoms. There are many evidences indicate that excess dietary salt intake is the main cause of hypertension. In general, there are many ways to minimize hypertension, one of them that is very efficient, but not in all cases, is the limitation intake of dietary salt (Haddy & Pamnani,1995). In addition, other lifestyle factors that may cause raise in blood pressure like inadequate potassium intake, excess body weight, low physical activity and alcohol consumption. However, reducing sodium intake led to decrease blood pressure (He & MacGregor, 2003).

The prevalence of hypertension is increasing worldwide. In 2000, the number of people who suffers from hypertension was around 972 million; 333 million in developed countries and 639 million in developing countries (26.4%), and the number in 2025 is expected to reach around 1.56 billion (increase about 50%) (Kearney et al, 2005). Aburto et al (2013) reported that about half of the world's adults have been affected currently from hypertension. The prevalence of hypertension in Palestine particularly was 27.6% (Hussein et al, 2017). Khdour et al (2013) reported that the prevalence of hypertension between Palestinian adults was high and in particular it was higher between men. Hypertension had an economic impact worldwide. Hypertension considered a leading cause of cardiovascular disease. Both diseases cause economic load on nation in which high blood pressure costs around $51.2 billion\year, and cardiovascular diseases cost around $316.1 billion\year in 2012-2013 (Wang et al, 2018).

2.3.2 Cardiovascular Diseases (CVDs)

CVDs are one of the non-communicable diseases that leads to death around the world. It is accounted around 30% of deaths around the world in 2005 (Gaziano et al, 2010) There are many risk factors for CVDs such as high blood pressure, insulin resistance, high blood lipids and obesity (metabolic syndrome), but the main risk factor is high blood pressure. Excess of sodium intake reduces the relaxation of smooth muscles in the endothelium of arteries. This reaction happens to response to shear stress of flowing blood. High salt intake in salt-sensitive people caused less excretion of sodium in urine, this increased blood pressure. This causes increase in cardiac output, and a continued raise in systemic vascular resistance (Karppanen & Mervaala, 2006). However, there is a weak correlation between sodium intake and cardiovascular diseases, because cardiovascular diseases progress over many years and there are many dietary and lifestyle factors that affect it (Doyle & Glass, 2010). Asaria et al (2007) reported that 8.5 million cardiovascular-related deaths worldwide will be prevented with a 15% reduction in human sodium intake.

2.3.3. Bone Diseases

There are many studies that indicated the effect of high sodium intake on bone health. Both sodium and calcium share the same transport mechanism in kidneys. At tubular renal level, reabsorption of calcium equals the reabsorption of sodium. There is a direct effect of sodium on renal calcium reabsorption as it increases calcium excretion (Carbone et al, 2003), which leads in combination with low calcium intake to bone loss by raising the parathyroid hormone (PTH) (Lin et al, 2003). About 20-60 mg of calcium excreted in urine for every 2300 mg of sodium ingested (Doyle, 2008). Teucher et al (2008) stated that there was an increase in calcium excretion in a postmenopausal women diet that contained 11.2g of salt /day in comparison with diet that contained 3.9g of salt/day. Bone health is also affected by other factors such as dietary factors, gender, age, menopausal status…etc (Doyle & Glass, 2010).

2.4 Roles of salt in meat and meat products

Sodium chloride is widely used to develop of many desirable sensory and technological characteristics in meat products. It plays an essential function in the quality and safety of meat products (Inguglia et al, 2017). The role of sodium chloride depends on the type of meat product. For example, in dry fermented meat products (e.g. Salami, Coburger), sodium chloride is needed to prohibit microbial spoilage by reducing free water in the product which reduces growth of microorganisms or prevent it. On another hand, in cooked sausages and minced meat products, the major role of salt is to solubilize myofibrillar proteins in order to optimize emulsification of fat with water. Therefore, salt reduction may reduce proteins solubilization, water holding capacity and gel strength of meat product (Verkleij, 2012). Sodium chloride also helps in building the texture of meat product by extracting myofibrillar proteins (Bombrun et al, 2104), enhance the salty flavor and other flavors of the product (Aaslyng et al, 2014). Also sodium chloride increased moisture retention in raw meat through cooking (Horita et al., 2014). In addition, it increased shelf life of meat product by delaying the growth rate of microbial population in the product (Stanley et al, 2017)

2.4.1 Role of salt in flavor and texture

One of the basic tastes is saltiness, this taste sensed by the tongue. The saltiest flavor of all sodium compounds is sodium chloride (Doyle & Glass, 2010). Salty taste in food comes from sodium ions to chloride ions (Verkleij, 2012). In addition to the role of salt in adding salty taste to food, it also enhances other flavors. Although the effect of salt to enhance other flavors is not understood, but it may be related to enhancing the taste of sweetened ingredients and lowering the taste of bitter ingredients (Verkleij, 2012) Adding sodium compounds like sodium chloride and monosodium glutamate to food enhances the flavor of other components in food. It was found that it was impossible to replace salt flavor without using sodium, even with its replacement with other alternatives such as potassium chloride (Bower, 2016). This explained that only sodium had a compound taste primarily salty to human (McCaughey, 2007), while calcium and potassium had other flavors sometimes (bitter, or metallic) beside saltiness (Doyle & Glass, 2010). The transduction mechanism of sodium is specific on taste receptor cells. Receptor cells include epithelial sodium channels (ENaCs) that is responsible for the uniqueness of sodium as an incentive to taste salt (Chandrashekar et al, 2010). Epithelial sodium channels (ENaCs) have two types: one for high sodium concentration, and it is activated by multiple cations which is responsible for the dislike of extreme sodium concentration. The other type is responsible for the pleasantness of salty taste which is activated at low sodium levels (Liem et al, 2011). Also, sodium chloride affects the texture of food by interacting with a major ingredient in food. For an example, the addition of sodium chloride to meat helps in reducing the fluid loss during heat processing of meat products and increases the tenderness of meat by improving water binding capacity. In the presence of salt, actin and myosin swell and allow to bind more water and fat to form heat stable emulsion of meat. The binding of these myosin proteins together improves the texture characteristics of processed meat (Man, 2007; Bower, 2016). Salt concentration in processed meat product usually ranges from 1.5-3.0% (Desmond, 2006), but if the concentration was below 1.5%, it can have a negative effect on the products acceptability by consumers (Bower, 2016). Studies showed that salty flavor in meat products increases the consumer acceptability, therefore it is difficult to replace its flavor by another ingredients. Fat also plays a role in flavor of meat products. Tobin et al (2012) reported that the consumer was more satisfied in low fat and more salty frankfurters' product. In turkey breast muscle, sensory properties were significantly got better when it was tumbled with phosphate and salt (Froning & Sackett, 1985). Therefore, it is easy to notice the reduction of sodium chloride in processed meat product by consumers. Accordingly, the reduction process should be gradually followed (Ruusunen & Puolanne, 2005).

2.4.2 Role of salt in extraction of myofibrillar proteins

Myofibrillar proteins are group of meat proteins that are responsible for capturing fat and binding water. Adding salt to solubilize myofibrillar proteins was the best method to bind water and fat in sausage (Lonergan et al, 2019). Sodium chloride is responsible in some meat products for the solubilization of myofibrillar proteins. The solubilization is essential because myofibrillar proteins are responsible for the emulsifying and gelling capacity in meat products (Verkleij, 2012). Tobin et al (2012x & y) reported that there was a direct relationship between salt concentration and myofibrillar protein extraction (increasing salt concentration leads to increase in myofibrillar protein extraction). However, the salt concentration should be at limited concentration (2-3%) due to flavor.

2.4.3 Role of salt in water holding capacity

Water holding capacity is the ability of food matrix to retain water in three-dimensional structures (Chantrapornchai & McClements, 2002). Different theories were built to study the ways of how salt affects water binding in meat product (Bower, 2016). According to Ruusunen & Puolanne (2005), chloride ion builds a strong interaction with meat proteins, leading to increase the negative charge. These negative charges lead to rise repulsion forces between the proteins which lead to muscle swelling. This increases the space between proteins and allowing more water to interact with the polar side chain of amino acid. This interaction of water with polar portion of proteins attract additional layers of water molecules. While the other part (non-polar side) of proteins, pushes water molecules and forming arch-like shape around themselves. The presence of these two forces combined to make tension giving water a near-solid structure that is very beneficial for both to bind water more tightly during processing and cooking. Therefore, salt plays a role in cooking yield and aids in the ability of meat to hold water. Tobin et al (2012y) found that in beef patties there was an inverse relationship between salt concentration and cooking loss, as cooking loss increased with decreasing salt concentration and increased with decreasing salt concentration. Horita et al. (2014) found a similar result in frankfurters. This is a result of the ability of salt to raise water retention and protein solubility (Bower, 2016)

2.4.4 Role of salt in preservation

Salt increases the osmotic pressure out of the cells which draw water from inside the microbial cell, this translocation of water affects adversely vital microbial metabolic reactions. In addition, salt prevents the growth of spoilage organism by allowing the growth of lactic acid bacteria (Albarracín et al, 2011). Water activity (a_w) of fresh meat is about 0.99, which is appropriate to support the growth of many microbes and pathogens (Doyle & Glass, 2010). The increase in the concentration of salt creates changes in cellular metabolism because of its osmotic effect, which affects microorganisms at different concentrations. The effect of salt on microbes relates to its ability to reduce water activity (a_w). Whereas, adding salt ions to the media around the cells of bacteria causes water to flow out through their semipermeable membranes. Therefore, as cell components need suitable amount of cytoplasmic water, cell try maintain homeostasis by active accumulation of ions or uptake or synthesis of compatible solutes. This causes cell spend energy, and leading to reduces the growth rate of microbes, and at the end prevent the growth. The ability of microorganisms to tolerate salt varies between species in optimal conditions. Nevertheless, it can be reduced by nutrient availability, sub optimal pH, the availability of other antimicrobial agents, and temperature (Stringer & Pin, 2005). However, it can decrease the nutritional value of preserved foods because water-soluble components such as minerals and vitamins can be reduced (Albarracín et al, 2011). Salt can also reduce the growth of gram-positive bacteria like *Pseudomonas* sp, *Acinetobacter* spp and *Enterobacteriaceae* that do not negatively affect the quality of the product, and need more time to reach the levels of spoilage (Bower, 2016). A study conducted on frankfurters resulted in high growth rate of natural flora when salt percent decreased to 1.5% in the products (Whiting et al, 1984). Blikstad and Molin (1983) stated that the growth of salt sensitive microorganisms like *Enterobacteriaceae* and *Pseudomonas spp* decreased or even stopped. Nevertheless, appropriate concentration of salt depends on the species of microorganisms, while there are some organisms sensitive to salt like *Campylobacters,* and others can be found in salt concentration more than 20% like *S. aureus* with the control of the other conditions of growth (Doyle & Glass, 2010). However, salt didn’t inhibit the growth of microbes completely, but it reduced the growth rate. Salting is inadequate as a preservation method alone, and it needs a combination with other preservation methods such as osmotic dehydration, drying, appropriate packaging and severe refrigeration. Therefore, it is possible in this way to prevent growth of pathogenic microbes and toxins production, consequently extend the product self-life (Albarracín et al, 2011). Reddy & Marth (1990) reported that sodium chloride worked in conjunction with sodium nitrite to retard *C. botulinum* growth.

2.5 Sodium chloride in process meat products

World Health Organization (WHO) recommended to reduce sodium chloride intake to less than 6g\d (around 2300 mg/d, and around 1500 mg/d for those more than 51 years and who suffer from high blood pressure , diabetes, chronic kidney disease), in order to reduce the elevation of blood pressure and the risk for cardiovascular diseases. WHO pursues to achieve this aim by developing many actions and strategies such as public education, dietary counseling, and food labeling (by regulation of sodium amounts in food industry) in order to encourage consumers to pick low sodium choice (Cobb et al, 2012). In addition, European commission developed many strategies in 2008 to reduce dietary salt intake and reached WHO recommendations (no more than 5g\day), by developing initiative to increase public awareness and also by developing an action to food industries targeting meat, ready meals, cheeses and bakery products (Zanardi et al, 2010). Actually, the consumed amount of salt currently can be obtained from many food sources, about 77% comes from packaged and processed food and the other 23% can be found naturally in food or added while cooking or on the table as shown in Figure 3 (Corriher, 2008).

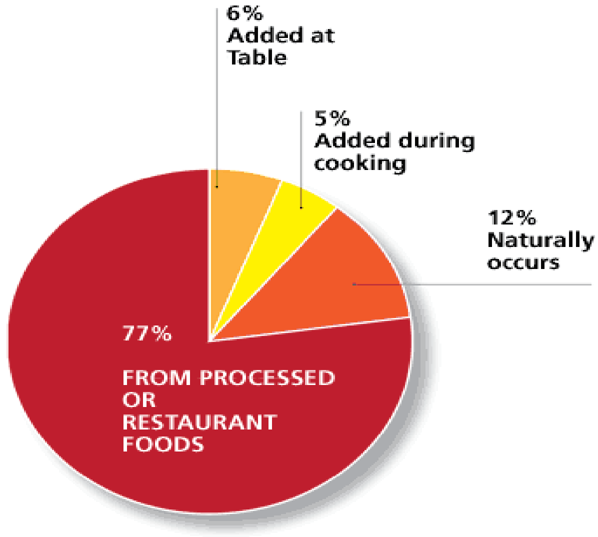

Figure 3: Percentage of salt contained in different sources of food (CHCHE,2011)

Reducing salt in packaged and processed foods is an important step to reduce the amount of consumed salt, because it contributes to the largest proportion of consumption as shown in Figure 3. However, reduction process should be done gradually by food manufacturers in their products (Gillespie et al, 2009), because there are a number of complications that occur as a result of this reduction due the major roles of salt in taste and sometimes in the preservation of the food products (Dötsch et al, 2009).

Recently, total meat production and consumption has increased. Meat includes poultry, cattle, sheep, and goat. Meat production has tripled over the last four decades and raised 20% in the last 10 years around the world (figure 4). The global production of meat increased rapidly since 1961. Asia was considered as the big meat producer which accounted around 40-45% of total meat production, despite there was increase in production in other countries, but the largest share was Asia (Ritchie & Roser, 2019).

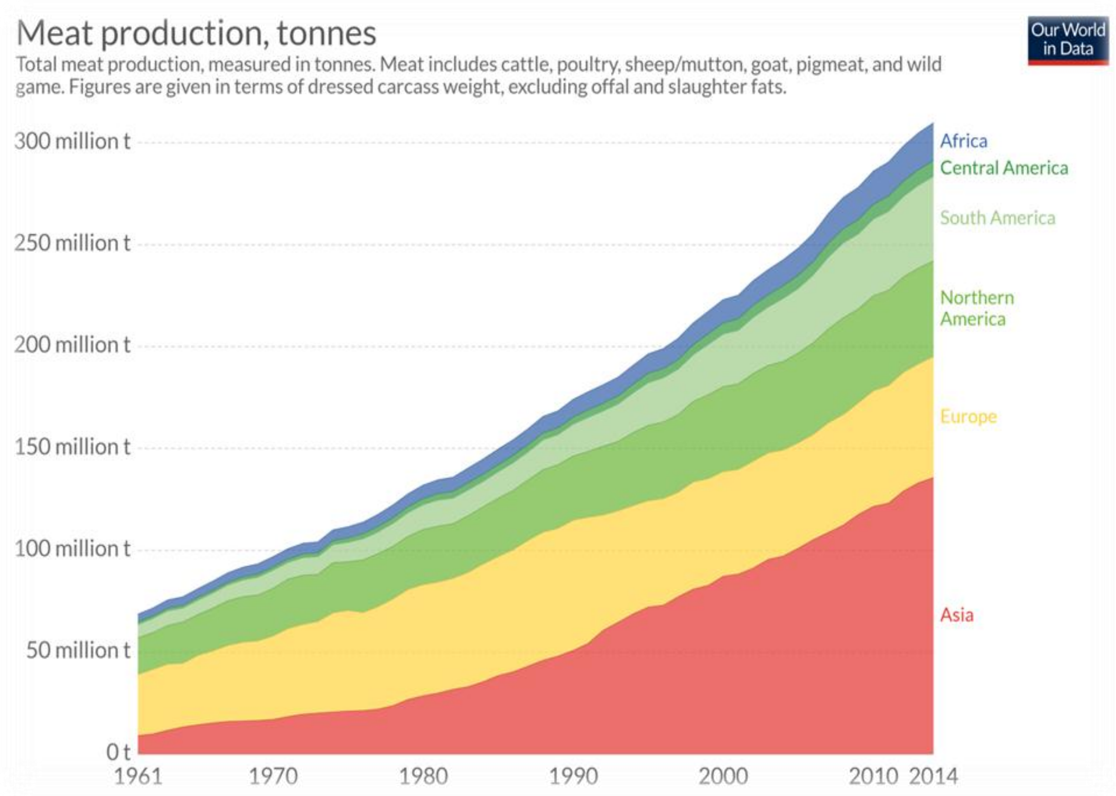

Figure 4: The changes in production and consumption of meat over the times in global context (Ritchie & Roser,2019).

Sodium chloride is considered one the most important ingredient for meat industries; because it plays a significant role in the texture, flavor and shelf life of the product. In addition, salt provides a salty taste and enhance other flavors to the product. Adding sodium chloride to raw meat products increases its shelf life in which it delays the growth rate of microbial population (pathogenic and spoilage organisms) by reducing water activity of the product (Stanley et al ,2017). During processing and manufacturing of meat products, the presence of sodium chloride affects the texture of the product by improving binding ability of fat and water that helps in the formation of eligible gel texture and also enhances moisture retention during cooking to improve the texture of the product (Ruusunen & Puolanne, 2005, Stanley et al, 2017).

2.6 Strategies to reduce sodium chloride in processed meat

Due to the correlation between excessive sodium intake and chronic diseases like high blood pressure and cardiovascular diseases, there is growing demand on low sodium food products. Processed meat products are usually contained a high percentage of sodium chloride. Accordingly, there is need to formulate low-sodium meat products, where meat industries launched several processed meat products with low content of sodium. Although the reduction of sodium has a number of obstacles due to crucial roles of salt in meat, but several strategies were successful in reducing salt, taking into consideration different aspects of quality traits. These ways include: 1) reducing the amount of sodium chloride (NaCl), 2) substituting all or part of sodium chloride (NaCl) with other chloride salts {$MgCl_2$ (magnesium chloride), $CaCl_2$ (calcium chloride), and KCl (potassium chloride)}, 3) substituting all or part of sodium chloride (NaCl) with other non-chloride salts like phosphate, 4) combination of all ways. Potassium, magnesium, and calcium have a favorable effect against high blood pressure, CVDs, and stroke, but the effect of potassium is stronger than magnesium and calcium (Karppanen & Mervaala, 2006).

In order to apply these ways, researchers did many studies to evaluate the effect of replacement or substitution of sodium chloride on the quality and overall acceptance of meat products. In meat industry, potassium chloride was widely used as an alternative of sodium chloride, because it is chemical properties, ionic strength, and it is similarity in molecular structure to sodium chloride (Horita et al., 2014). However, substitution should not be in excessive amount because it affects the taste of meat by promoting a production of metallic and better taste (Vidal et al, 2019) thus affecting the overall acceptance of final product (Stanley et al, 2017). Replacement of sodium chloride by potassium chloride can be carry out on the molar or weight bases. Stanley et al (2017) have studied meat products where sodium chloride (NaCl) has been replaced with potassium chloride salt based on both by weight or molar basis.

2.6.1 Substitution of sodium chloride in processed meat products

Researchers developed many salty alternatives to replace sodium chloride that enhance the salty flavor and, in some cases, replicate the function of sodium chloride without affecting sodium content (Chapman, 2012). Salt replacer, which are other minerals such as modified potassium chloride, potassium chloride, calcium chloride, magnesium sulphate, and ammonium chloride can replace sodium chloride to certain levels. These salt replacers may produce an undesirable flavor to food if exceeded certain levels (Chapman, 2012).

2.6.1.1 Potassium Chloride

Potassium chloride is a salt that naturally occurred and extracted from sea salts and rock. Biologically, it is a very important nutrient, in which it is needed for keeping the volume of total fluid in the body, normal cell function, and acid and electrolyte balance (Young, 2001). Aburto et al (2013) reported that dietary potassium intake around the world decreased. This because the trend of consumers turned form the consuming high potassium diet like fruit and vegetables into consuming a diet high in processed food that are less in potassium content. Low potassium and high sodium intakes have been associated with increased incidence of high blood pressure, cardiovascular diseases, renal failure, etc. Binia et al (2015) and Aburto et al (2013) founded that dietary intake of potassium chloride led to reduce the risk of high blood pressure, which reflected the effect of sodium chloride in body. WHO reported that high intake of potassium could be a protective step against these diseases (WHO, 2003). Studies suggested that the optimum amount of potassium for reducing blood pressure and decreasing the risk for stroke is 90-120 mmol\day, whereas consumers intake of potassium is less than 70-80 mmol\day (Disclaimer, 1988). So, if a person consumes less than 2 g\day of sodium and 90 mmol\day of potassium, the consumption ratio will be one to one roughly, and this ratio is suitable for health (WHO, 2003). The daily intake of potassium for Palestinian population from food sources is around 2290 mg\day (2101 mg\day for females, 2479 mg\day for males), which is less than recommended amount (3500 mg\day), but it is considered adequate (Abdeen & Qasrawi, 2010).

e usual daily median intake of Potassium from all food sources for the Palestinian population was 2,290 mg (males 2,479 mg, females 2,101 mg). There were apparent trends in intakes across age groups.

However, these intakes appear to be adequate in comparison to the Reference Nutrient Intake of 3,500 mg/day (UK DRV).

It is extremely unlikely that any problem of inadequate intake exists in Palestine In meat industry, the use of potassium chloride instead of sodium chloride is a common strategy to reduce sodium chloride in meat products. Potassium chloride is considered the most direct and successfully replacer of sodium chloride. It is commonly used as a partial substation of sodium chloride because they share the similarity in molecular composition, and both have the same ionic force properties (Carraro et al, 2012). It also stimulated the same receptors as sodium chloride, which assisted in keeping the salty flavor (Chapman, 2012). However, the use of potassium chloride should be in a limited concentration, because at high concentration negatively affected the sensory properties of final product by reducing salty taste and providing a bitter and metallic taste. In addition, potassium chloride had antimicrobial effect in foods in a similar way to sodium chloride (Doyle, 2008). The replacement process is done either by weight or molecular weight. The molecular weight of potassium chloride is 74.55 g and sodium chloride is 58.44 g, so 27.5% more potassium chloride should be used to obtain the same molecular weight between sodium and potassium chloride. While the same weight of potassium and sodium chloride can be used in case of weight-based replacement. Bidlas & Lambert, (2008) stated that replacement of sodium chloride by potassium chloride depending on molar basis exhibited equal effect against some pathogens as control. Mudalal & Petracci (2019) stated that replacing sodium chloride with 30% potassium chloride did not negatively affect any quality traits of marinated turkey breast meat. Paulsen et al, (2014) examined the possibility to substitute 30-40% of sodium chloride by potassium chloride in fermented sausages. It was found that replacement sodium chloride by potassium chloride in concentration higher than 30-40% imparted a metallic taste, which affected adversely the sensory properties of final product (Horita et al, 2014). Aliño et al (2009) showed that it was possible to get a dry-cured loin with 50% sodium chloride replacement by potassium chloride without any change on physico-chemical characteristics. However, Santos et al (2014) found that substituting of 50 and 75% of sodium chloride by potassium chloride showed an effect on the sensory properties of fermented cooked sausages products, but the addition of some ingredients such as monosodium glutamate with taurine, lysine, disodium guanylate, and disodium inosinate masked the undesirable effect. Frye et al (1986) studied the effect of replacing 50% of sodium chloride by potassium chloride in tumbled ham product, and found that replacement in this ratio had acceptable water binding characteristics and sensory properties. However, Brandsma (2007) revealed that the maximum level of replacement of sodium chloride by potassium chloride was 30% in the majority of food products, because higher level of potassium chloride imparted a metallic flavor to food products.

Buren et al (2016) investigated the effect of using potassium chloride as an alternative of sodium chloride on different food products. The results showed that replacement sodium chloride by potassium chloride increased the intake of potassium, and this step will help people to reach WHO recommendation for potassium intake. Replacement at 20% increased potassium intake by 453 mg/day, while replacement by 50% increased potassium intake by 674 mg/day, and 100% replacement of sodium chloride increased potassium intake by 733 mg/day. Full replacement of sodium chloride my increase potassium intake to harmful level to some groups of population who suffer from impaired potassium excretion. These groups include people who suffer from chronic kidney disease, sever heart failure, adrenal insufficiency, end stage renal disease, and diabetes.

2.6.1.2 Modified Potassium Chloride

Researchers developed a modified potassium chloride, by modifying crystals size of potassium chloride. This form of potassium showed the same functional properties as pure potassium but exhibited less noticeable taste (metallic\ bitter taste that associated with the addition of potassium chloride to food) (Chapman, 2012). Potassium chloride is somewhat weaker ionically than sodium chloride. Therefore, the ability of potassium chloride to increase the yield or reduce water activity will be affected. On the other hand, the ability of modified potassium chloride to reduce water activity or increase the yields was similar or better than sodium chloride (Nu-Tek, 2010). Pasin et al (2008) studied the effect of replacing salt in pork sausage patties with modified potassium chloride. The results showed that replacing salt with 75% of modified potassium chloride resulted in acceptable sensory properties. So, modified potassium chloride was suggested as a good replacer to sodium chloride instead of potassium chloride. (Chapman, 2012).

2.6.1.3 Other Salt Replacer

Researchers studied the effect of other mineral salt replacer on meat properties. Cáceres et al (2006) reported that it was healthy to use calcium chloride (CaCl2) as a partial alternative of sodium chloride because this may impart additional calcium to human diet, although it increased the hardness of sausage, and showed a little decrease in sausage lightness. Bower (2016) revealed that there was a little effect of CaCl on flavor but it decreased emulsion stability and protein solubility.

2.7 Effect of sodium dietary intake reduction on health

Many studies reported that reducing sodium intake led to reduction in blood pressure and CVDs between adults. So, the current recommendations for public health is to reduce sodium consumption from (9-12g\day) to (5-6g\day). World Health Organization recommended adults to reduce the intake to 5g\day or less. In US and UK, 6g\day or less was recommended (He & MacGregor, 2003). Studies found that reduction of sodium intake by 3g\day led to a fall in blood pressure of 1.8 to 3.5\0.8 to 1.8 mm Hg (systolic\diastolic) in normotensive, and 3.6 to 5.6\1.9 to 3.2 mm Hg in hypertensives (He & MacGregor, 2003). Moreover, the studies predicted that this reduction will reduce ischemic heart disease (IHD) by 10% and strokes by 13%. The effect of reduction will be doubled with 6g\day reduction and triplet with 9g\day reduction (He & MacGregor, 2003). Appel and Foti (2018) showed that it was difficult to achieve sodium reduction, and the maximum reduction level can be reached was 400 mg\day even by elimination salt from table or cooking.

2.8 Effect of sodium reduction on meat quality

2.8.1 Effect of salt reduction on palatability and consumer acceptance

Taste of food is a very important factor in choosing food. Salty taste is a preferred taste between humans (Chandrashekar et al, 2006). There was a reduction in consumer acceptance for products with a large reduction in sodium content (Liem et al, 2011). Studies showed that a small reduction in sodium content was more effective because consumer can't detect the gradual reduction of sodium. Nevertheless, continual small reduction in sodium content will reach a point where consumer will able to detect the reduction of sodium in the product. Liem et al (2011) reported that it was possible to do a large reduction in sodium content in food product, but the reduction should be replaced in order to decrease the negative effect of reduction on taste.

2.8.2 Effect of salt reduction on texture and other quality characteristics

Texture and other quality characteristics (fat, moisture content, color, and pH) and processing conditions were affected by reducing sodium content in meat products (Dötsch et al, 2009). As sodium chloride is responsible for water holding capacity and the binding ability of protein and fat in meat as well as salty taste. So, reducing sodium chloride in meat batters needed to be replaced by other sodium replacer, to compensate the salty taste and other functions that may lose during replacing of sodium chloride (Doyle & Glass, 2010).

2.8.3 Effect of salt reduction on preservation and microbial safety

Sodium chloride can control and reduce the growth of pathogens and spoilage organisms in some food by reducing water activity of food, and making the product safe for human consumption. For instance, in processed meat products, sodium chloride reduces and limits the growth of *Clostridium Botulinum* and its toxin. Also, sodium chloride can act in cooperation with other sodium salts against spoilage and pathogenic microorganisms. For example, sodium chloride, sodium diacetate and sodium lactate work against the growth of *Listeria monocytogenes* and *lactic acid* bacteria in ready-to-eat meats. In addition, they work against growth of *Clostridium Botulinum* and it is toxins in processed meat and chesses (Taormina, 2010). There are many theories that explains the importance of sodium chloride against microbial growth and increasing shelf life of different types of foods. One of these theories assumes the role of sodium chloride to resist the microbial growth by osmosis process (draw out water from both cell of microbes and food) (Doyle and Glass 2010). Another theory is due to the toxicity of chlorine ion to microorganisms (Taormina ,2010). Low sodium food product needs more care to be taken through storage and cooking temperature, and also in packaging. Additionally, another preservatives and preservation methods are needed to be added to sodium-reduced product to increase safety and self-life of the product (Liem et al, 2011).

Chapter 3
Material and Methods

3.1 Samples Collection and Preparation

About 16 kg of topside fresh beef meat has been purchased from a local slaughter house in Tulkarem city (Palestine). The meat has been trimmed from fat, visible connective tissues, and cartilages. Fresh beef meat has been minced by using conventional meat grinder fitted with 3 mm holes disk. The minced meat has been thoroughly mixed in order to obtain similar initial microbial load. The mass of meat was divided into four parts (each part 4 kg) representing four treatments (C, R_{15}, R_{30}, and R_{50}) (Figure 5).In each treatment, sodium chloride and potassium chloride were added to obtain 0, 15, 30, and 50% replacement of sodium chloride by potassium chloride according to Table 2 with starting point as 1.5% NaCl.

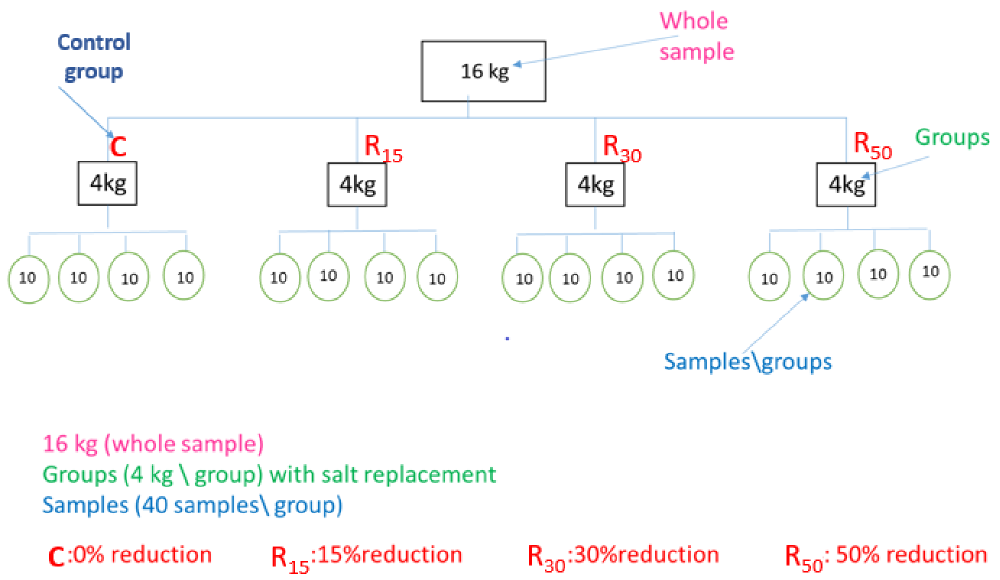

Figure 5: The experimental design for the study considering the distribution of samples in each group and the total number of the samples.

The distribution of whole samples in four groups with a 4 kg (40 samples) for each group. The quantity in each group was distributed roughly evenly over 4 periods (10 samples\period as shown in Figure 2), and each group had a specific sodium-potassium composition.

Table 2: Amount of salt (NaCl:KCl) added to each group and the level of sodium chloride replacement by with potassium chloride.

Salt conc	**0 reduction**	**15% reduction**	**30% reduction**	**50% reduction**
NaCl	60 g	51 g	42 g	30 g
KCl	0 g	9 g	18 g	30 g

After each group was mixed with its own salt composition, under aseptic conditions, burgers were formed using a manual burgers machine. 160 beef burger patties (the weight of each patty was about 100 g) were formed. In each treatment or group, there were 40 patties. The meat patties in each treatment were stored at refrigerated condition (4 ^{o}C) for nine days. In each group, different quality traits (physico-chemical and sensory traits as well as microbiological analysis) were evaluated at day 0, 3, 6, 9. Four samples were used for chemical analysis, four samples for sensory analysis, and two samples for microbiological analysis (Figure 6).

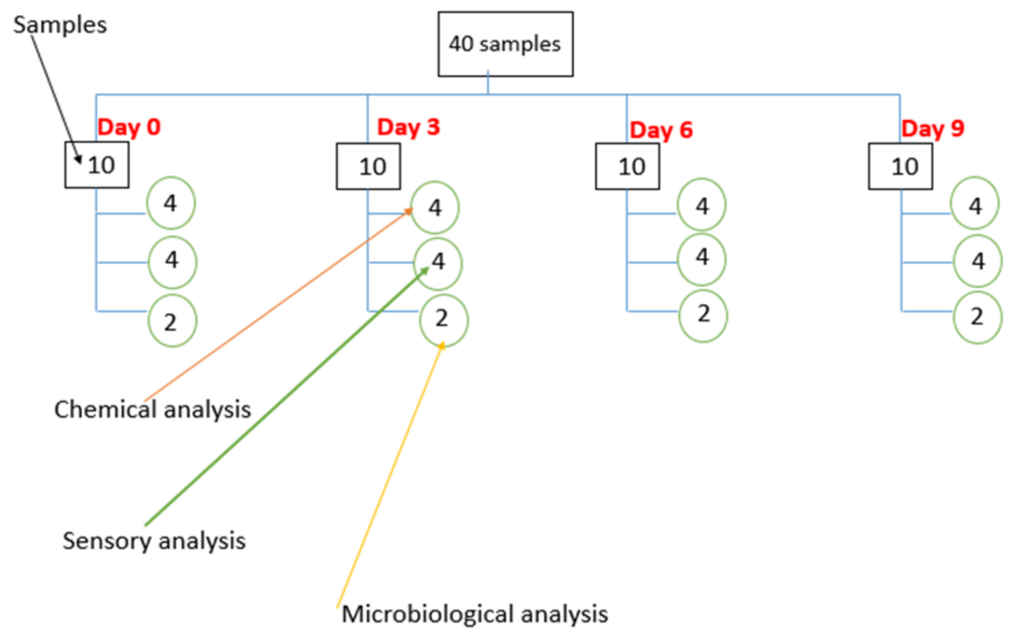

Figure 6: Distribution of samples within the groups during storage period showing quality parameters for investigation (chemical, microbiological and sensory).

3.2 Quality Traits Analysis

Four samples of beef burgers from each group during study period (0, 3, 6, and 9 days) were dedicated to proximate chemical analysis (fat content, moisture content, protein content, and ash content).

Determination of fat content: Determination of fat content in meat is very important in all stages of meat production, processing and for consumers. In our study, samples were taken from all groups, before and after cooking, to study the effect of replacing sodium chloride by potassium chloride on fat retention.

There are many methods to determine the amount of fat in meat. In our study, the determination of fat content was carried out by performing Soxhlet method, in which it depends on extracting fat from samples by using solvent (petroleum ether), then measuring the weight of fat recovered. The sample was placed in porous thimble that allows the solvent to cover the entire sample completely. During extraction, thimble was used to hold the sample and it worked as a filter that allows the solvent to be recycled continuously on the sample, this expanded the contact time between the solvent and the sample, ensuring sufficient time to dissolve all the fat in the sample.

The fat analysis procedure was described as follows:

1. About 1.5-2 grams of finely minced meat were weighted accurately at analytical balance and put in accurately weighted pre-dried extraction thimble (meat should be minced for solvent to penetrate the sample accurately).
2. An accurate weight was taken for porous thimble with sample inside it (package). Then they placed it in Ankom, in which it was rinsed by 300 ml of petroleum ether for 90 min.
3. After that, packages (porous thimble+ sample) were put in acetone and then accurately measured.
4. Then packages were put in oven at 110 ^{o}C for 15 min to dry, and then they were weighted accurately.

   Crud fat percentage content was calculated as follows:

$$Fat\ \% = \frac{Weight\ of\ fat}{Weight\ of\ sample} * 100\%$$

Determination of moisture content: The moisture content was measured by placing 5 grams of finally chopped meat in aluminum pans, which were weighted (X2) after drying for 1 hour at 103 ^{o}C and then cooled in desiccators (X1). Then all samples were dried in conventional oven for 16 hours at 105 ^{o}C After that all samples were cooled in desiccators then weighted (X3). Moisture content was calculated by the following equation:

Moisture (%) = (initial weight (x2) - dry weight (x3-x1))
/
*(initial weight(x2)) *100*

Determination of protein content: A procedure reported by Kjeldahl method (AOAC, 1990) was used to determine the protein content of samples which depends on determining the total nitrogen content that includes (ammonia, amine, urea nitrogen fraction, and urea). In order to determine protein content, samples pass through three phases: digestion of sample, distillation and titration of liberated ammonia. The results will give the amount of nitrogen, and by multiplying it with specific coefficient, will estimate the percent of protein in samples.

As a first step of calculating protein content, samples pass through digestion phase, in which about 0.5 gram of sample was accurately weighted and added in to Kjeldahl tube with 15 ml of sulfuric acid, catalyst and boiling chips. The temperature of digestion was 400/420°C for 3 h; this degree was gradually achieved during the process of digestion from 80 to 420°C at the rate of 50°C/15 min. This process finishes when the color of tubes content completely changed to clear color. After that, the samples were subjected to distillation phase. In this phase, about 50 ml of distilled water and 65 ml of sodium hydroxide (solution with 30% of NaOH) were added in order to neutralize the effect of the acid solution used in the mineralization phase, in which NH4+ converts to NH3 followed by boiling and condensation of NH3 in receiving solution (boric acid) that captures ammonia. Finally, in titration phase, distilled ammonia was titrated by using 0.2 N hydrochloric acid (HCl). After that, we calculated protein content by multiplying the concentration of nitrogen through converting factor 6.25 (conversion factor of meat and meat products) as in the following equations:

$$\text{Nitrogen \%} = \frac{(\text{ml of acid for sample} - \text{ml of acid for blank}) \times \text{N of acid} \times 1.4007}{\text{Weight of sample (grams)}}$$

Protein % = Nitrogen% conversion factor*

Determination of ash content: Ash is residue remaining after water and organic matter removed from food by heating in the presence of oxidizing agents. The results of ash content measure the total amounts of minerals present within food, in which these minerals are not destroyed by heating.

Ash determination methods vary like dry ashing, wet ashing and low temperature plasma dry ashing. Dry ashing method was used in our research. It depends on using a high temperature muffle furnace capable of maintaining temperatures of between 500 and 600 °C. In this method, water and other volatile substances evaporate and organic substances are burned in the presence of the oxygen in air to CO_2, H_2O and N_2. Most minerals are converted to oxides, sulfates, phosphates, chlorides or silicates.

At first, we weighted the sample dishes accurately, and then we heated them in a muffle furnace at 525°C for one hour, and then cooled them in desiccators. About 5 g of finely minced meat was accurately weighted in the ashing dish and then dried in an air oven at 102°C for two hours. The sample was charred in a muffle furnace at 200°C, and then followed by charring the step at 525°C for four hours. After that, the samples have been cooled to 200°C and moved to desiccators for cooling at room temperature. Then we weighted the sample after ashing to determine the concentration of ash present. Ash content can be calculated by using the following equation:

$$Ash\ (wet\ basis)\ \% = \frac{M\ ash}{M\ wet} * 100$$

Where (M ash) refers to the mass of the ashed sample, and (M wet) refers to the original mass of the wet samples.

In addition, four samples were used for sensory test, cooking loss, textural profile analysis, color, pH test, and two samples in two replicates for microbiological test (total bacteria count, anaerobic bacteria, psychrotrophic bacteria, yeast and mold). The color, pH, and microbiological tests were carried out at each day of study (0, 3, 6, and 9). Sensory analysis and textural profile analysis were done for cooked samples only during study period.

3.3 Measurements of color indexes

Three parameters (L*, a*, b*) for color measurement based on CIE system (Commission Internationale de l'Eclairage) by using handheld Minolta Chroma Meter CR-400. The color traits were measured for raw and cooked samples during study period. Three parameters represent differences in lights as shown in Figure 7. Handheld Minolta Chroma Meter CR-400 was calibrated with a reference white ceramic tile before measurements.

Reflectance colorimeter (Minolta Chroma Meter CR-400) with C as illuminant source was used to carry out the measurements. The colorimeter was calibrated with a reference white ceramic tile (Y = 93.9, x = 0.3130 and y = 0.3190) before measurement. Avoiding areas with color defects, color (CIE L* = lightness, a* = redness, and b* = yellowness) was measured in triplicate on each meat patties in three different areas.

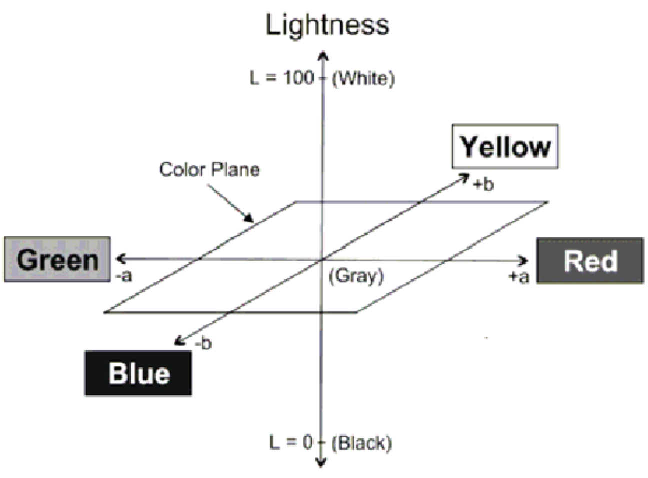

Figure 7: Three-dimensional representation of (L*, a*, b*) system, where L* represent the difference between light (L=100) and dark (L=0), a* represent the difference between green (-a*) and red (+a*), and b* represent the difference between yellow (+b*) and blue.

3.4 pH Measurements

pH is a very important parameter in our study because it affects quality traits, shelf life, and changes the color of meat product. In our study, pH values were determined by direct insertion of pH meter in the solution that consists of 2.5 grams of meat homogenized with 25 ml of distilled water for 30 s at speed 10000 rpm.

3.5 Microbiological analysis

Two beef burger samples per group (considered as replications) were used to evaluate the microbiological population (total bacterial count, anaerobic bacteria, psychrotrophic bacteria, yeast and mold) of the treatments during the storage period (day 0, 3, 6, and 9) (Image 1). To estimate total aerobic, anaerobic, and psychrotrophic bacteria growth, 10 g of meat from each slice of burger were weighted under sterile condition into a sterile plastic bag, and then 90ml of peptone water (slime) was added. The contents were homogenized for 30 sec by using a stomacher mixer. Then appropriate log dilution (serial dilution) were prepared, and by using micropipette, 0.1 ml was added to petridishes (plate count agar PCA medium) and incubated for 48-72 h at 37 ^{o}C and at refrigerator temperature 4 ^{o}C for 10 days to count possible number of psychrotrophic bacteria. In addition, petridishes of potato dextrose agar were incubated at room temperature 25 ^{o}C for 5 days to enumerate viable counts of yeast and molds.

Image 1: Cultivate microbial content in beef burger samples

3.6 Sensory analysis

Four beef burger samples per group were selected to cook in electrical oven (200 ^{o}C for 15 min), and allowed to equilibrate to room temperature for sensory test. The final cooked beef burger samples were cut into small similar pieces (8 pieces\ sample). Sensory test was performed in nutrition lab of faculty of agriculture and veterinary medicine in Tulkarm city-Palestine. Fifty untrained assessors (students from both sexes, within age ranges from 18-25 years, from both An-Najah and Khadouri PTUK universities in Tulkarm), were recruited to evaluate three parameters of beef samples. Parameters including saltiness, juiciness, and over all acceptance of the samples. The plate of each assessor has contained four different warm pieces (piece from each group). The assessors were asked to evaluate and score each sample for saltiness ranging from 1 (extremely bland (unsalted)) to .7 (extremely salted). Juiciness ranging from 1 (extremely dry) to 7 (extremely tender), and level of acceptance that ranging from 1 (extremely unaccepted) to 7 (extremely accepted) by using the following questionnaire:

Questionnaire

<u>استبيان التقييم الحسي للبرجر</u>

الاسم:

الجنس: • ذكر • أنثى

العمر:

هل أنت مستهلك للبرجر بشكل منتظم: • نعم •لا

<u>التعليمات</u>

في الصندوق المقابل لكل عينة، ضع علامة عند الخيار المناسب حسب رأيك:

Samples	LEVEL OF ACCEPTANCES						
	Extremely unaccepted 1	Very unaccepted 2	Slightly unaccepted 3	Neither accepted Nor unaccepted 4	Moderate accepted 5	Very accepted 6	Extremely accepted 7
A	☐	☐	☐	☐	☐	☐	☐
B	☐	☐	☐	☐	☐	☐	☐
C	☐	☐	☐	☐	☐	☐	☐
D	☐	☐	☐	☐	☐	☐	☐

Samples	LEVEL OF JUICINESS						
	Extremely dry 1	Very dry 2	Slightly dry 3	Neither dry Nor juicy 4	Moderate juicy 5	Very juicy 6	Extremely tender 7
A	☐	☐	☐	☐	☐	☐	☐
B	☐	☐	☐	☐	☐	☐	☐
C	☐	☐	☐	☐	☐	☐	☐
D	☐	☐	☐	☐	☐	☐	☐

Samples	LEVEL OF SALTNESS						
	Extremely bland(unsalted) 1	Very bland 2	Slightly bland 3	Neither salty Nor bland 4	Moderate salty 5	Very salty 6	Extremely salty 7
A	☐	☐	☐	☐	☐	☐	☐
B	☐	☐	☐	☐	☐	☐	☐
C	☐	☐	☐	☐	☐	☐	☐
D	☐	☐	☐	☐	☐	☐	☐

3.7 Cooking Loss

Eight samples from each treatment were taken and weighted before and after cooking. The samples were placed in an oven at a temperature 200 ^{o}C for 15 minutes. During cooking each sample was turned on the other side at minute 7:30. Four samples of all groups were placed in the oven with each other in the distribution, to ensure equal heat distribution of four groups (Figure 8), which ensures the distribution of heat evenly on all treatments.

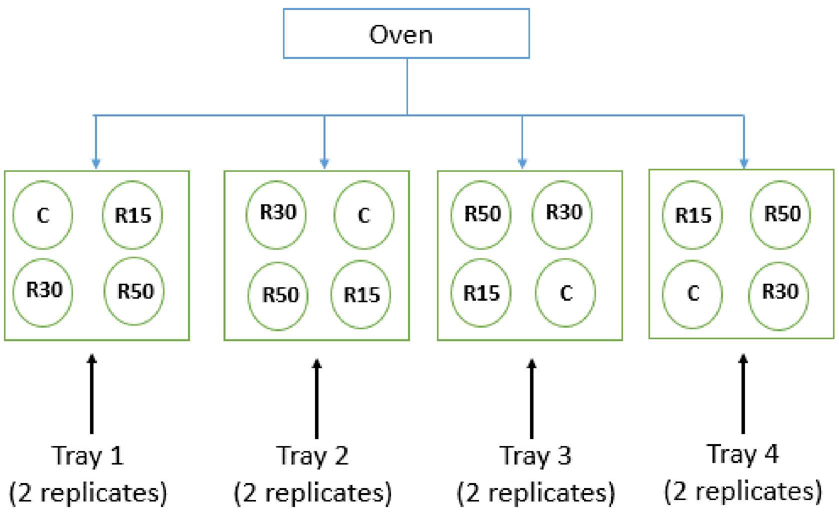

Figure 8: The distribution of samples in oven in order to cook it, each tray replicated twice to cook eight samples from each tretment. C: control group (zero sodium chloride replacement by potassium chloride, R_{15}: 15% replacement of sodium chloride by potassium chloride, R_{30}: 30% replacement of sodium chloride by potassium chloride, R_{50}: 50% replacement of sodium chloride by potassium chloride.

Cooking loss was measured by using the following equation:

$$\%\ cook\ loss = \frac{(Raw\ weight - cooked\ weight) \times 100}{(Raw\ weight).}$$

3.8 Textural Profile Analysis (TPA)

Textural profile analysis is a common double compression test for defining the textural properties of food. Many parameters are measured using texture analyzer (Brookfield texture analyzer CT500) such as Hardness, Cohesiveness, Springiness, Gumminess, Chewiness, and Cohesiveness. In our study, we have examined the previous parameters by using texture analyzer. The samples were cut in a cylindrical shape, and their weight and length were taken before placing them on texture analyzer and compressed twice (Image 2 & 3).

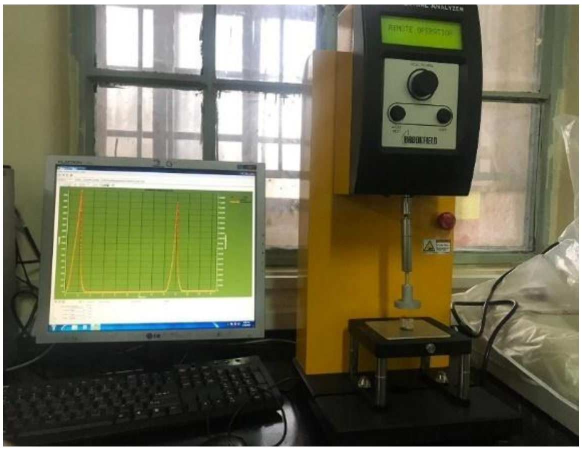

Image 2: Textural Analyzer

Image 3: Samples that have been prepared to be examined on Textural Analyzer

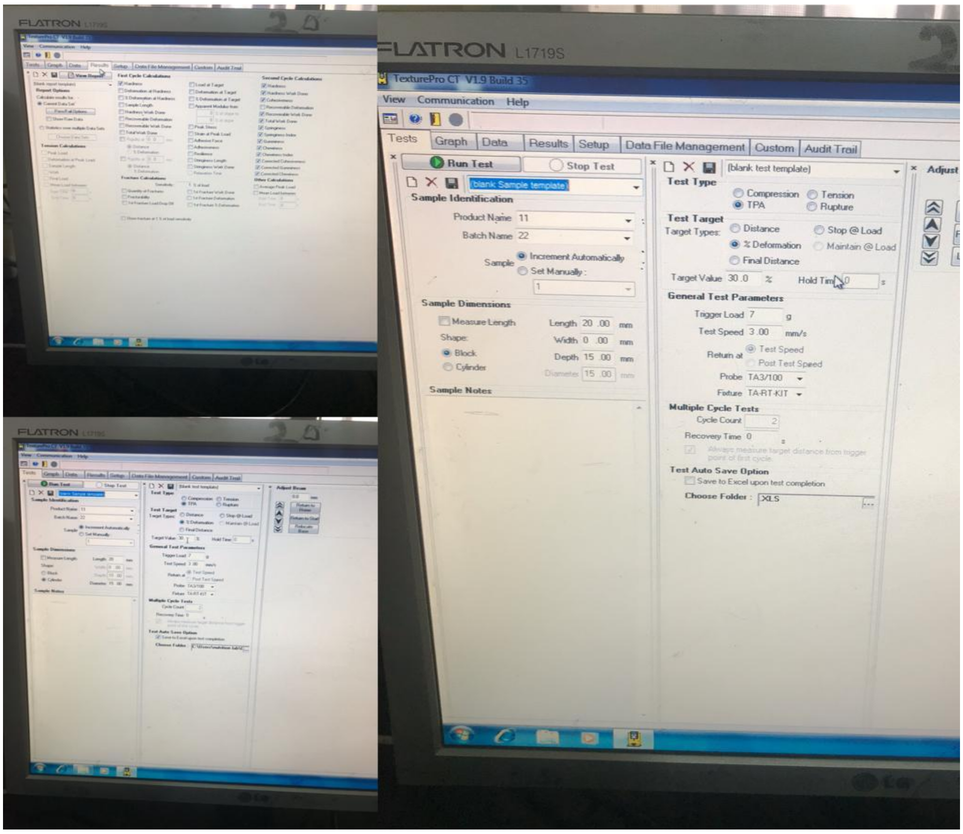

Image 4: Parameters to be checked on Textural Analyzer

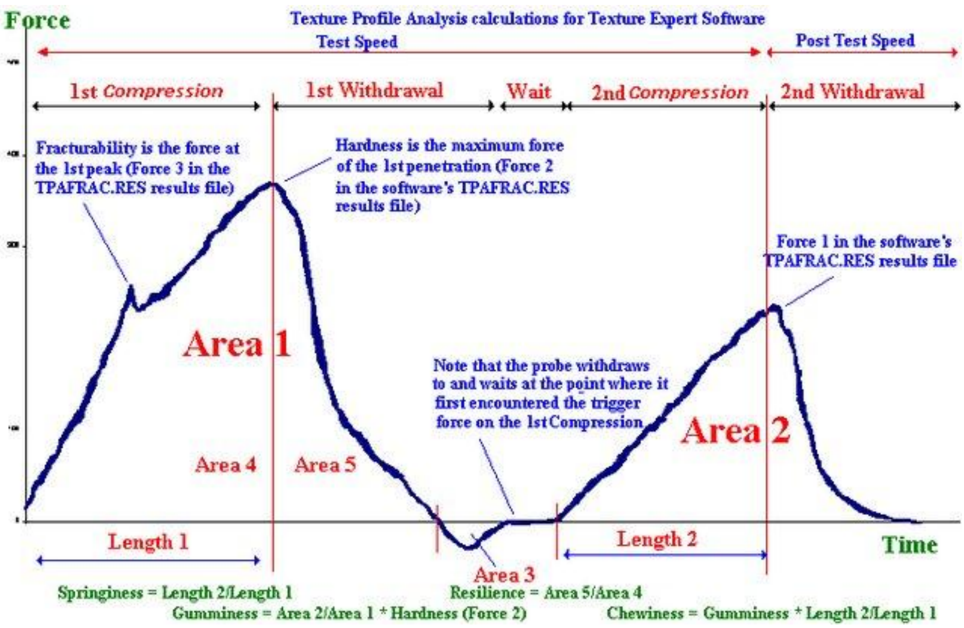

Figure 9: Texture Profile Analysis calculations for Texture Expert Software Test Speed (Nii et al., 2015)

Samples were compressed to 40% of the initial height by using a 50 kg loading cell connected to a 50 mm DIA cylinder aluminum probe using brookfield texture analyser (CT-500, USA). The test speed of the probe was 4 mm/sec, while the pre- and post-test speeds were both 3 mm/sec. The compression value was recorded as the maximum force needed to compress 40% of the initial height of the sample and expressed in g. Hardness is defined the peak force of the first compression of the product. The hardness does not necessarily occur at the point of deepest compression, although it typically does for most products.

Cohesiveness is how well the product withstands a second deformation relative to how it behaved under the first deformation. It is measured as the area of work during the second compression divided by the area of work during the first compression. (Refer to Area 2/Area 1 in the below graph). Springiness is how well a product physically springs back after it has been deformed during the first compression. The spring back is measured at the down stroke of the second compression, so the wait time between two strokes can be relatively important. In some cases, an excessively long wait time will allow a product to spring back more than it might under the conditions being researched. Springiness is measured in several ways, but more typically, by the distance of the detected height of the product on the second compression (Length 2 on the below graph), as divided by the original compression distance (Length 1). The original definition of springiness used the

Length 2 only, and the units were in mm or other units of distance. Many TPA users compress their products a % strain, and for those applications a pure distance value (rather than a ratio) is too heavily influenced by the height of the sample. By expressing springiness as a ratio of its original height, comparisons can be made between a more broad set of samples and products.

Gumminess only applies to semi-solid products and it is equal to Hardness *Cohesiveness (which is Area 2/Area1). Gumminess is mutually exclusive with chewiness for a product would not be both a semi-solid and a solid at the same time. Gumminess is definite as the force necessary to disintegrate a semisolid food until the condition that permits to swallow it. Chewiness only applies for solid products. Chewiness is mutually exclusive with Gumminess for a product would not be both a solid and a semi-solid at the same time. Chewiness indicates the force needed to disintegrate a solid food until the condition that permits to swallow it. It is calculated multiplying hardness × cohesiveness × springiness.

3.9 Statistical analysis

Data were statistically analyzed with two-way analysis of variance (ANOVA) to determine the effect of reducing sodium chloride and replacing it with potassium chloride using statistical analysis system (SPSS) and effect of storage time. Duncan's multiple range test was used to determine differences between mean values. Statistical significance was considered when p-value was less than 0.05.

Chapter 4
Results and Discussion

4.1 Color and proximate Composition (Ash, protein, Moisture, and Fat) Analysis

The effect of sodium chloride replacement by potassium chloride on the color indexes was shown in Table 3.

In general, there was a moderate significant effect for replacement sodium chloride by potassium chloride on color indexes. In this context, control group exhibited significantly lower lightness (L*) values (48.96 vs. 51.05, $p<0.05$) than group R_{30} respectively while group R_{15} and R_{50} showed moderate values. For a* value, control group had significantly lower value (10.60 vs. 12.45, $p<0.05$) than group R_{15} while group R_{30} and R_{50} had moderate values. On another hand, group R_{15} showed higher value for b*value when compared to group R_{50} (14.64 vs. 13.34, $p<0.05$) while other groups showed intermediate values. In general, color indexes are affected by different factors such as pH, heat treatment; atmosphere composition, etc. Even if, there were some significant differences in color indexes but these differences were very small. In general, the major factor that has effects on color indexes is pH. In our study, there were no significant differences in pH values. Accordingly, the differences in color indexes may be attributed to differences in compositions.

Table 3: The effect of sodium chloride replacement by potassium chloride on color indexes (L*, a*, b*) for all groups a-b Means within a column followed by different superscript letters differ significantly (P < 0.05).

groups	L*-value	a *-value	b *-value
	Mean ± SD	Mean ± SD	Mean ± SD
C	48.96 ^{b}	10.60 ^{b}	13.53 ^{ab}
R_{15}	49.90 ^{ab}	12.46 ^{a}	14.64 ^{a}
R_{30}	51.04 ^{a}	11.26 ^{ab}	13.72 ^{ab}
R_{50}	50.71 ^{ab}	11.53 ^{ab}	13.35 ^{b}
P-value	<0.05	<0.05	<0.05

C: control group (zero sodium chloride replacement), R_{15}: 15% of sodium chloride replacement by potassium chloride. R_{30}: 30% of sodium chloride replacement by potassium chloride, R_{50}:50% sodium chloride replacement by potassium chloride.

The effect of sodium chloride replacement by potassium chloride on color, ash, moisture, fat, and protein content for samples before and after cooking has been shown in Table 4. There was a significant (p-value<0.05) effect of both sodium chloride replacement and cooking process on color (L*, a*, b*) parameters and moisture contents for all groups during storage period.

For L* parameter, R_{30} group exhibited significantly higher value (51.05 vs. 48.95, p-value<0.05) than control group respectively, while groups R_{50} and R_{15} values showed moderate values. For a* values, group R_{15} showed significantly higher values (12.46 vs. 10.60, p-value<0.05) than control group, while group R_{30} and R_{50} showed intermediate values. Our results were in agreement with some previous studies. Jeong (2017) reported a significantly increase in a* value in breast turkey with the addition of 2.0%

salt if compared to those without added salt. This change was attributed to sodium chloride addition may increase solubilization of myofibrillar proteins which increased the probabilities of oxygen reaction with heme pigments, thus resulting in more pink color formation. Cooking process reduced redness of meat because of increasing degradation and denaturation of myoglobin and increase oxidative conditions during cooking (Trout, 1989, Min et al, 2010). b* values in-group R_{15} was significantly higher (14.64 vs 13.34, p-value<0.05) than R_{50} values, while other groups showed intermediate values. For moisture, R_{50} group showed highly significant values (62.30 vs. 61.23, p-value <0.05) than R_{15}, whereas other groups showed moderate values. The effect of replacement and cooking did not have any significant effect (p-value>0.05) on protein, fat and ash content.

Table 4: The effect of sodium chloride replacement by potassium chloride and coooking process on color indexes (L*,a*,b*) and proximate composition (Ash, Protein, Moisture, and Fat) content for all groups a-b Means within a column followed by different superscript

Groups	**C**	**R_{15}**	**R_{30}**	**R_{50}**	**p-value**
parameters	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
L *	48.95 ^{b}	49.87 ^{ab}	51.05 ^{a}	50.72 ^{ab}	<0.05
a *	10.60 ^{b}	12.46 ^{a}	11.25 ^{ab}	11.53 ^{ab}	<0.05
b *	13.53 ^{ab}	14.64 ^{a}	13.72 ^{ab}	13.34 ^{b}	<0.05
ash	2.80	2.89	2.75	2.91	0.47
Moisture	61.42 ^{ab}	61.22 ^{b}	62.00 ^{ab}	62.30 ^{a}	<0.05
fat	11.24	11.12	11.01	10.77	0.51
Protein	26.16	26.58	25.76	25.84	0.82

C: control group (zero sodium chloride replacement), R_{15}: 15% of sodium chloride replacement by potassium chloride. R_{30}: 30% of sodium chloride replacement by potassium chloride, R_{50}:50% sodium chloride replacement by potassium chloride.

The effect of cooking process on color index (L*, a*, b*) and proximate composition (Ash, Protein, Moisture, and Fat) for all groups was shown in Table 5.

As shown in Table 5, cooking affected significantly (p-values<0.05) color, ash, moisture, fat, and protein values. Lightness (L*), and redness (a*) values were significantly decreased (p<0.05) after cooking, while yellowness (b*) values were significantly (p-value<0.05) increased. Oz and Celik (2015) also found a reduction in both lightness and redness values and increase in yellowness in meat after cooking. Gok et al. (2008)

interpreted the reduction of L* values due to the browning reactions that are induced by cooking which changed brightness in meat. Moreover, cooking process causes a denaturation of myoglobin (a bright red color pigment in meat) and formation of metmyoglobin which lead to formation of brown color, this expressed by decreasing in a* values and increasing in b* values. (Gok et al, 2018). Heat leads to form grayish-brown pigment that caused by the degradation of both pigments in meat muscle (myoglobin and hemoglobin) (Oz and Celik, 2015). In respect to proximate composition, ash, fat, and protein values significantly (p-value<0.05) increased after cooking, while moisture significantly (p-value<0.05) decreased after cooking. This can be explained as cooking process that leads to reduce water content of meat causing increase in dry matter (Oz & Celik, 2015).

Table 5:The effect of cooking process on color index (L*, a*, b*) and proximate composition (Ash, Protein, Moisture, and Fat) for all groups. a-b Means within a column followed by different superscript letters differ significantly (P < 0.05).

Parameters \samples	Before cooking	After cooking	p-value
L *	56.51^{a}	43.78^{b}	<0.05
a *	13.77^{a}	10.15^{b}	<0.05
b *	13.50^{b}	14.13^{a}	<0.05
ash	2.67^{b}	3.01^{a}	<0.05
Moisture	69.18^{a}	54.29^{b}	<0.05
fat	9.09^{b}	12.98^{a}	<0.05
Protein	22.51^{b}	29.66^{a}	<0.05

Figures 10-12 show the interaction effect between sodium chloride replacement and cooking process on CIE parameters, moisture, and fat in all groups. For lightness (L*), cooking process and salt replacement reduce lightness value (p-value<0.05) for all groups and samples became darker in color due to the effect of heat to produce a brown-pigment for meat by degradation of meat pigments. There was no significant interaction effect on redness (a*) and yellowness (b*).

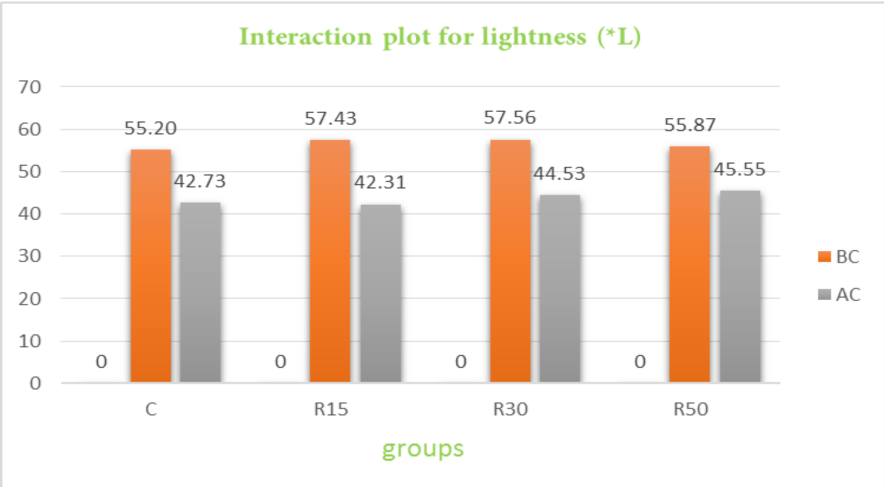

Figure 10: The effect of cooking process and salt reduction and replacement on lightness values of all grorups. C: control group (zero sodium chloride replacement), R_{15}: 15%replacement of sodium chloride by potasium chloride, R_{30}: 30% of sodium chloride replacement by potassium chloride, R_{50}: 50% of sodium chloride replacement by potassium chloride. BC: befor cooking (raw samples) values, AC: after cooking (cooked samples) values.

The interaction effect of cooking process and salt replacement increased fat values significantly (p-value<0.05) for all groups as shown in Figure 11. This increase in fat values may be attributed to the loss of free water during the cooking process (Gerber et al, 2009). Stanley et al (2017) found that there was no significant effect of salt reduction and replacement by potassium chloride (pork patties composition) in both raw and cooked amples in fat content.

Figure 11: The effect of cooking process and salt reduction and replacement on fat values of all grorups. C: control group (zero sodium chloride replacement), R_{15}: 15%replacement of sodium chloride by potasium chloride, R_{30}: 30% of sodium chloride replacemen. R_{50}: 50 % of sodium chloride replacement by potassium chloride. BC: befor cooking (raw samples) values, AC: after cooking (cooked samples) values.

The effect of cooking process and salt replacement on moisture values of all groups was shown in Figure 12. Moisture values significantly (p-value<0.05) decreased after salt replacement and cooking. This was as expected that cooking process reduced moisture of samples (Oz & Celik, 2015). Oz et al (2016) found that cooking process reduced moisture content in beef steaks. On another hand, Freitas et al. (2017) and Stanley et al. (2017) found that no significant differences were shown in moisture in both raw and cooked burger samples in all groups of study. Stanley et al (2017) also found that there was no significant difference in moisture content between raw and cooked samples of pork patties, and stated that there was no effect of salt reduction and replacement on composition during cooking. The decrease in moisture content can be explained due to protein denaturation because of cooking, and loss ability of meat proteins to hold water (Voller et al, 1996). Carraro et el (2012) showed that the differences in moisture content between different treatments of bologna sausages were due to the variation between original composition of raw material not due to the effect of sodium chloride replacement by potassium chloride.

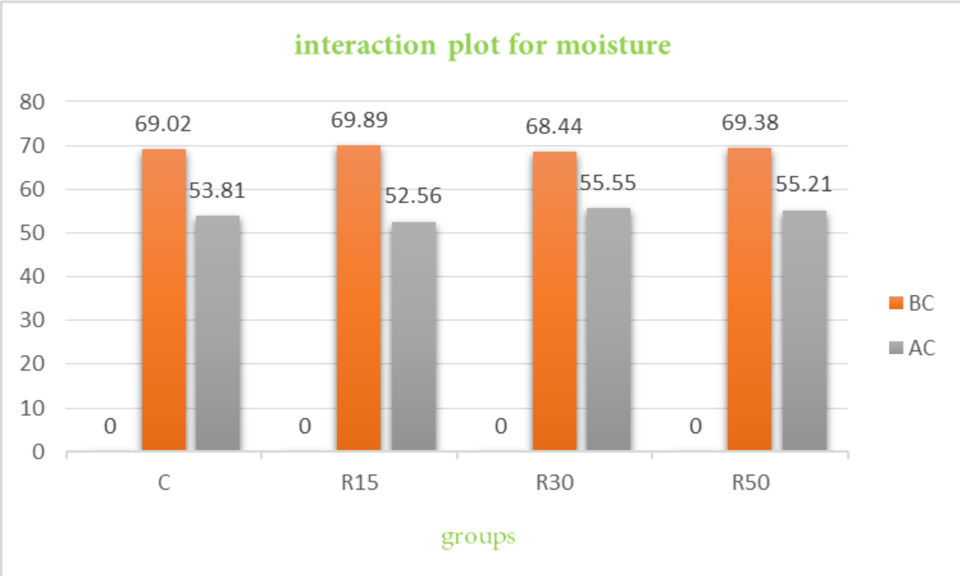

Figure 12: The effect of cooking process and salt reduction and replacement on moisture values of all grorups. C: control group (zero sodium chloride replacement), R_{15}: 15%replacement of sodium chloride by potasium chloride, R_{30}: 30% of sodium chloride replaceme, R_{50}: 50% replacement of sodium chloride by potassium chloride. BC: before cooking (raw samples) values, AC: after cooking (cooked samples) values.

4.2 Effect of sodium chloride replacement on color parameters

Different color parameters were measured for all groups during storage period. Parameters were measured by using chronometer that depends on CIE system (Commission Internationale de l'Eclairage. Color measurements were taken from samples of all groups in both raw and cooked cases during the study period. The effects of sodium chloride replacement by potassium chloride on color indexes (L* a* b*) during storage (0, 3. 6, 9 days) are shown in Figures 13-15.

4.2.1 Effect of sodium replacements on L*-values

Figure 13 showed the effect of replacement of sodium chloride by potassium chloride on lightness index (L*) value for all groups during 9 days of storage. At initial day of storage, there were no significant differences between groups. In day 3, there was a moderate significant difference between groups (C). In day 6, there were moderate significant differences between groups. In the last day of storage, Control group exhibited significantly ($p<0.05$) higher L* values than group R_{30}, whereas group R_{15} and R_{50} showed moderate values. In general, there was no interaction effect between treatment and time. Moreover, there was gradual slight increase in lightness index for all groups during storage but this increase was higher in control group if it is compared between the first day of beef burger production and the end day of storage.

Stanley et al. (2017) observed that there were no significant differences in lightness values and in objective color measures in pork sausage patties with different levels of sodium reduction. In addition, Zhao and Claus (2013) stated that there were no differences in redness and lightness of raw pork with 50% replacement of sodium chloride with modified potassium chloride. Moreover, substitution of sodium chloride with standard potassium chloride had no effect on pork patties (Moon et al, 2008). Raseta et al (2018) reported that partially replacement of sodium chloride by potassium chloride did not affect color acceptability of pork burgers. It was L* values were significantly lower in replaced treatments compared to control treatment (Freitas et al, 2015) study. These results were attributed to the greater oxidative effect of sodium chloride in comparison with potassium chloride.

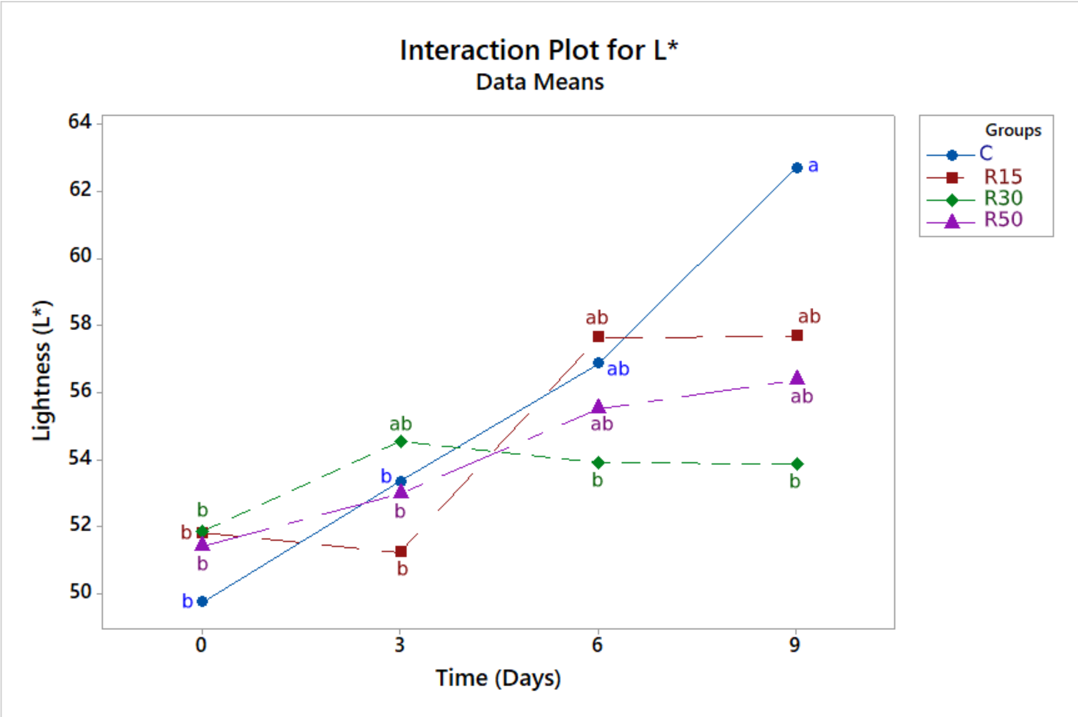

Figure 13: Changes in lightness (L*) values of beef burger from all groups during storage period (9 days). C: control group (zero sodium chloride replacement), R_{15}: 15% replacement of sodium chloride by potassium chloride, R_{30}: 30% replacement of sodium chloride by potassium chloride, R_{50}: 50% replacement of sodium chloride by potassium chloride.

4.2.2 Effect of sodium replacement on a*-values

Figure 14 shows the effect of replacement of sodium chloride by potassium chloride on redness index (a*) values for all groups during 9 days of storage. At day 0 and day 3 of storage, R_{15} showed significantly moderate ($p<0.05$) higher a* value than other groups. These differences can be attributed to initial differences in the color indexes in each batch. It is difficult to obtain completely homogenous ground meat mass. In day 6, R_{30} exhibited significantly ($p<0.05$) higher a* values than control group, while other groups showed intermediate values. At the last day of storage, there was a significant ($p<0.05$) decrease in a*(redness) values in all groups compared with initial day of storage (day 0). Our findings showed that all groups exhibited gradual reduction in redness value during storage in the same pattern and this means that these changes can be attributed to the effect of storage not to the effect of treatment. The change was convergent between groups, reducing the red pigment in all groups from day one to day nine, in which color changed from purplish-red color at the first day to brown color in both raw and cooked samples in all groups. These changes may be attributed due to the addition of salt to meat caused decreases in meat-myoglobin which reduce activity allowing more MMb (responsible for brown pigment) to accumulate, and salt promotes heat denaturation, or breakdown of myoglobin (beef decreased in redness as salt increased). The addition of NaCl is known to destabilize myoglobin in meat products upon cooking, leading to greater heat denaturation. The addition of more than 3% NaCl may reduce the redness of cooked products by promoting oxidative conditions and denaturing myoglobin (Jeong, 2017) and this could explain the decrease in a* value (Bae et al, 2018). In this context, it was found that a* values decreased with increasing storage time (Olivera et al, 2013). Jeong (2017) found that myoglobin content was decreased as salt (NaCl) concentration increased from 0 to 2% except cooked chicken breast treated with 3% sodium chloride. Claus and Jeong (2018) and Bae et al. (2018) stated that cooked ground turkey breast treated with 2% sodium chloride had less myoglobin content and high myoglobin denaturation compared to treatments without sodium chloride, regardless of the processing conditions. The addition of sodium chloride destabilizes myoglobin content due to heat denaturation in cooked meat products.

Jeong (2017) found that a* values was not significantly affected by pre-salting period of products containing 0 or 1% sodium chloride. Ahn and Maurer (1989) found that redness value in turkey breast treated with 2% salt was significantly increased if compared to turkey without salt. They interpreted these results as adding sodium chloride might lead to solubilize myofibrillar proteins so increasing the chances to react with heme pigments, will cause more pink color development. However, Trout (1989) found that sodium chloride more than 3% reduced redness in ground turkey products, possibly because of increasing degradation of myoglobin throughout cooking. Min et al. (2010) reported that adding sodium chloride significantly reduced myoglobin content in raw beef loin through storage. Generally, sodium chloride will reduce the heat stability of myoglobin and hemoglobin in meat due to the chloride ion, this increased heat denaturation.

Parameter a*expresses the redness of meat. The internal color of first cut of uncooked beef is defined as a deep, purplish-red color. The innate pigment existing is deoxymyoglobin (DMb, not bound to oxygen). When fresh, beef is cut and exposed to oxygen, it binds to heme iron (in reduced state) found in myoglobin to form oxymyoglobin (OMb). This causes the appearance of the bright cherry red color. When iron oxidized and can't bind oxygen, beef turns to brown color (metmyoglobin, MMb). Cooked beef meat converts any state of myoglobin (OMb, DMb, MMb) to hemichrome (denatured globin and oxidized heme iron) pigment which is tan color. Myoglobin is a compound that plays the largest role for determining the color of beef in both raw and cooked state. (Claus J.R, 2007)

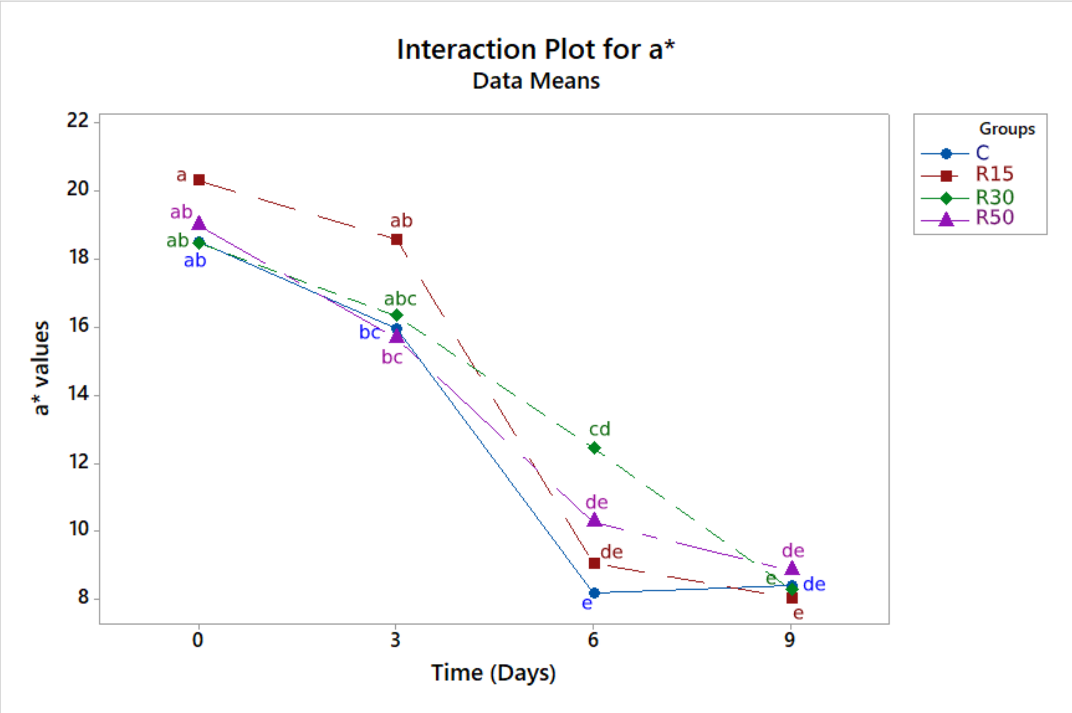

Figure 14: Changes in a*values of beef burger from all groups during storage period (9 days). C: control group (zero sodium chloride replacement), R_{15}: 15%replacement of sodium chloride by potassium chloride, R_{30}: 30% of sodium chloride replacement, R_{50}: 50% replacement of sodium chloride by potassium chloride**.**

4.2.3 Effect of sodium replacement on b*-values

Figure 15 shows the effect of replacement of sodium chloride by potassium chloride on yellowness index (b*). b*- value measures yellowness index of meat. Yellowness of meat influenced by the amount of intramuscular fat tissue and multiple pigments, which could enhance or diminish the color intensity in the yellow-blue spectrum (Lukanov et al, 2015).

At the first day of storage, Control group exhibited moderately significant ($p<0.05$) lower b*-value if compared with other groups. At day 3 and 6, there were moderate significant differences between groups. At the last day of storage, there was no significant difference between groups in yellowness values, but there were significant ($p<0.05$) differences if compared with first day. The highest differences in yellowness value between the first day and the last day of storage were in control group while other group exhibited moderate differences.

Stanley et al (2017) found that replacing sodium chloride by equal molar of potassium chloride resulted in higher yellowness index than control group. Zhao & Claus, (2013) found that replacing 50% of sodium chloride by modified potassium chloride-based salt had no effect on lightness or redness of raw pork meat. Similarly, Moon et al. (2008) showed that there were no significant differences in color parameters between control and low sodium formulated meat products (sodium chloride was replaced by potassium chloride). In contrast, there are many studies reported that substitution of sodium chloride with standard potassium chloride at high level resulted in lower redness and yellowness indexes for pork patties if compared with control (Cheng, Wang, & Ockerman, 2007).

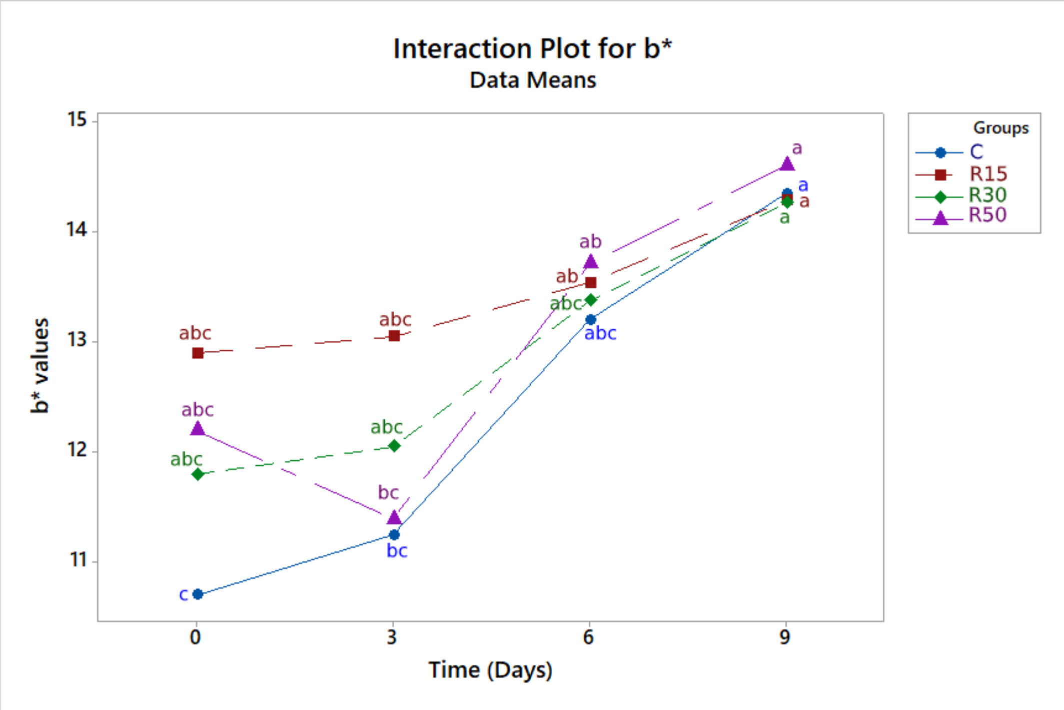

Figure 15: Changes in b*values of beef burger from all groups during storage period (9 days).C: control group (zero sodium chloride replacement), R_{15}: 15%replacement of sodium chloride by potassium chloride, R_{30}: 30% of sodium chloride replacement, R_{50}: 50% replacement of sodium chloride by potassium chloride.

4.3 Effect of sodium chloride replacement on pH values

The effect of sodium chloride replacement by potassium chloride at different levels on pH- values during refrigerated storage (4 °C) was shown in Figure.16. At the first day of storage, there were moderate significant in pH- values between groups. At day 3, R_{50} group exhibited significantly ($p<0.05$) lower pH value than group R_{15} and R_{30}, whereas control group exhibited intermediate values. In addition, at day 6, there was a mild difference between groups. At the last day of storage (day 9), group R_{30} showed a significantly ($p<0.05$) lower moderate pH- values when compared with other groups. In general, there was gradual drop down in pH in all groups during storage. Lyon et al, (1984) found that addition of salt to meat significantly raised the pH. In our study, pH values decreased in the last day of storage. This may be because of pH samples affected by storage time, and through this time there was growth of lactic acid bacteria (LAB) that produced lactic acid that reduced meat pH (Bower et al, 2017). NaCl and KCL work as food preservatives. Therefore, their presence will reduce the growth of bacteria and thus increase the pH value (Chikthimmah et al, 2001). Moreover, Chikthimmah et al (2001) reported that percentage of NaCl was a very important parameter to inhibit or stimulate the growth of LAB, it was observed that if sodium chloride in the range 1-2.5% will stimulate the growth of bacteria, but if it is higher than 5%, it will inhibit microbial growth (in our study the salt percentage was 1.5%).

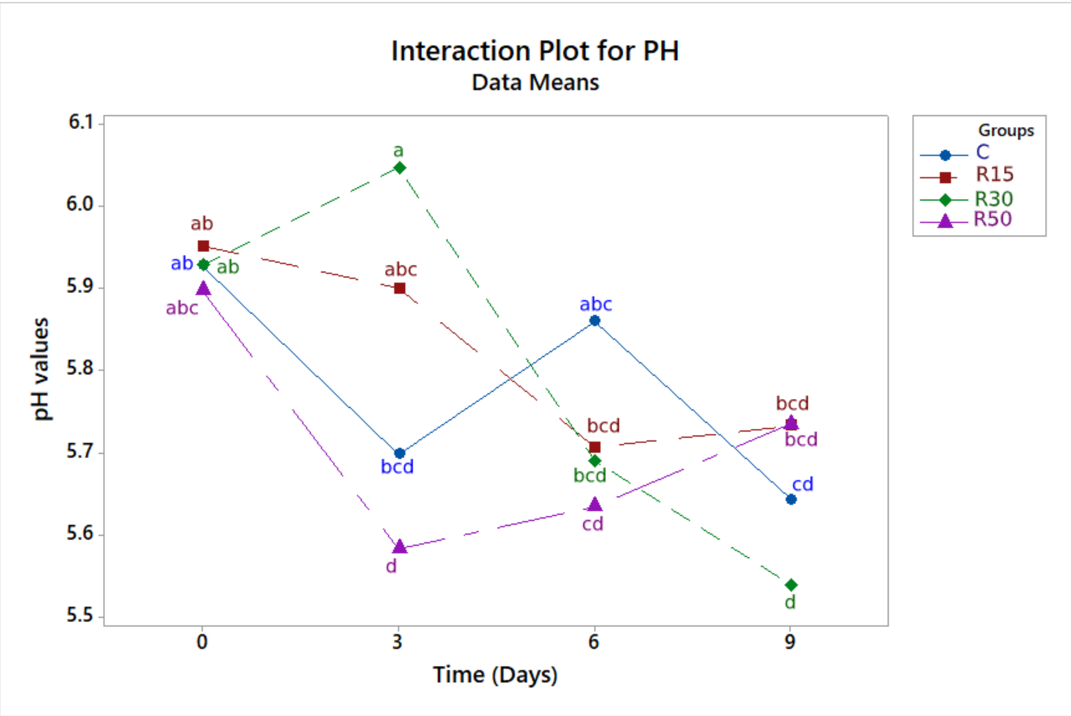

Figure 16: pH measurements of beef burger from all groups during refrigerated storage (9 days). C: control group (zero sodium chloride replacement by potassium chloride), R_{15}: 15% sodium chloride replacement by potassium chloride, R_{30}: 30% sodium chloride replacement by potassium chloride, R_{50}: 50% sodium chloride replacement by potassium chloride.

The ultimate pH of meat is highly dependent upon the amount of glycogen present in the muscle. Suwattitanun &Wattanachant, (2014) reported that pH value of roasted beef increased when the storage time increased. This was attributed to microbial excretion of di-aminase that attacked free amino acid and then produced ammonia or it may be increased by proteolysis degradation and accumulation of metabolites of bacterial action on meat (Olivera et al., 2013). It was found that there was a relationship between the pH and the color of meat, higher pH imparts darker color for meat. This is because when pH of meat is more than isoelectric point of the myofibrillar proteins, water molecules are tightly bound, causing more light absorbed by muscles, and meat looks darker in color (Allen et al., 1997).

Li et al (2016) found that control fermented cooked sausages (100% sodium chloride) had significantly lower pH values than sausages with 50% sodium chloride replaced by potassium chloride. This result may be attributed to the inhibitory action of sodium chloride replacement by KCl towards the growth of coliforms, which metabolized basic nitrogen compounds leading to pH changes in dry-cured bacon (the change in pH through processing may be related to the accumulation of non-protein nitrogen and amino compounds produced during proteolysis in dry-crud bacons).

4.4 Effect of sodium chloride replacement on microbial levels

4.4.1 Total Aerobic Bacterial Analysis

Different microbiological tests (Total aerobic, anaerobic bacteria, psychrotrophic bacteria, and yeast and mold) have been carried out to evaluate the effect of sodium chloride replacement by potassium chloride on microbiological stability. The effect of sodium chloride replacement by potassium chloride (Control treatment: 100% NaCl (C), R_{15}: 85% NaCl, R_{30}: 70% NaCl and R_{50}: 50% NaCl) on total aerobic count was shown in Figure 17.

At the first day of storage, group (R_{50}) exhibited significantly ($P<0.05$) higher aerobic bacterial count than control group, while R_{15} and R_{30} groups exhibited intermediate values. This result may be attributed to the differences in initial microbial count in the origin meat but not due to the effect of treatments. In the first day of beef burger production, it is impossible to reach the osmotic balance in cations and anions. In addition, pH of beef meat is low, which is not favorable for bacterial growth (Bower, 2016). In Day 3 and Day 6, there was a mild significant increase in aerobic count in all treatments if compared to Day 0 but at the same time, there were no significant differences between groups. At the end of storage (Day 9), all groups exhibited significantly higher aerobic count if compared to the first day of storage (Day 0). As a general result, there were no significant differences in the bacterial counts between groups during the whole storage period but there was a gradual increase in bacterial count in each group during storage, which reached up to 2.2 log. In conclusion, our finding showed that there is possibility to replace sodium chloride by potassium chloride up to 50% without affecting the aerobic bacterial count. Our findings were in agreement with Ibanez et al, (1997) who found that there were no significant differences in total aerobic count in dry fermented sausage samples treated with different replacement of NaCl with KCl. Terrell et al (1983) found that reducing NaCl by 50% did not show any effect on the growth of Micrococcus and Moraxella. It was found that raw pork sausage prepared by replacing sodium chloride with KCl\MgCl2 (70:30) at different levels (25, 50, 75 or 100%) did not show any significant effect on aerobic plate count (Terrell et al. 1982, Doyle, 2008)

On another hand, there was no agreement between our finding and some of previous studies. In this context, Blesa et al. (2008) found that reduced sodium dry-cured ham exhibited an effect on aerobic bacterial growth.

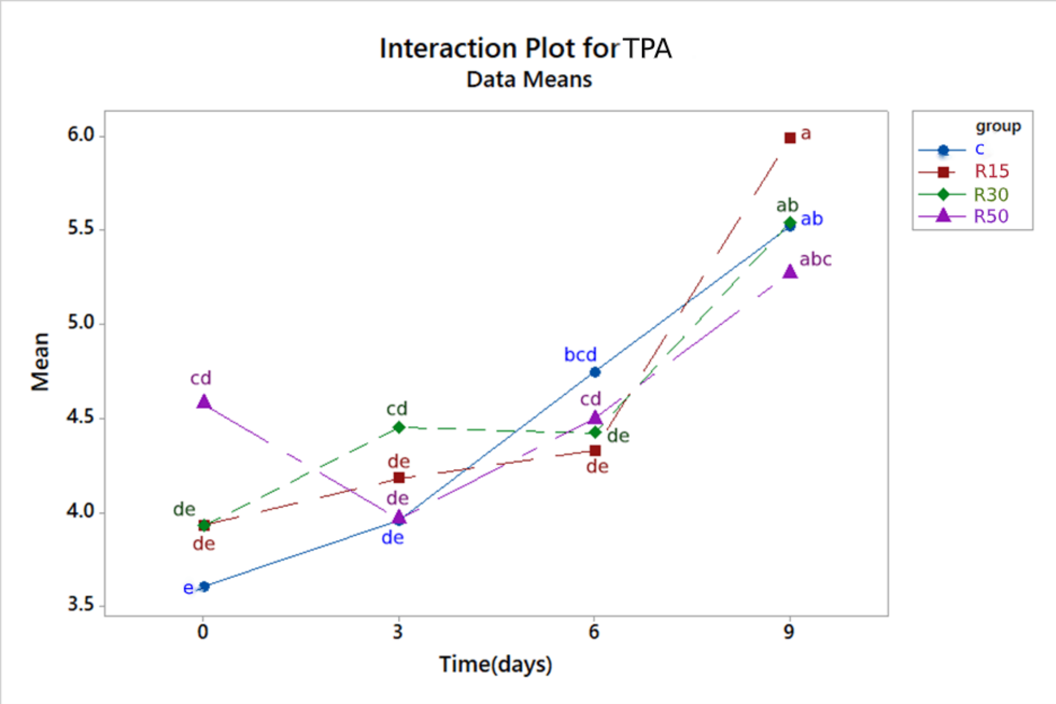

Figure 17: Total aerobic bacterial count (log cfu) of beef burger from all groups during refrigerated storage (9 days). C: control group (zero sodium chloride replacement), R_{15}: 15% of sodium chloride replacement by potassium chloride, R_{30}: 30% of sodium chloride replacement by potassium chloride, R_{50}: 50% of sodium chloride replacement by potassium chloride.

4.4.2 Psychrotrophic Bacteria analysis

The effect of substitution of sodium chloride by potassium chloride on psychrotropic bacteria count was shown in Figure 18. At first day of storage, R_{50} and R_{30} groups showed significantly ($p<0.05$) higher psychrotrophic count than control and R_{15}. This result may be referred to initial differences in microbial load between meat batches. In general, there was a gradual significant increase in psychrotrophic count in all groups during storage period (9 days). On the other hand, there were no significant differences in bacterial growth between groups at the same time during whole period of storage. Therefore, all groups exhibited similar pattern in bacterial growth during storage. In conclusion, replacement of sodium chloride by potassium chloride in all groups did not show any significant effect on psychrotrophic bacterial growth. Marvast (2011) also found no significant effect of replacing sodium chloride by potassium chloride in both beef and camel meat. In a study conducted on dry-cured ham to evaluate the effect of partial replacement of NaCl by other salts (potassium, calcium, and magnesium). Blesa et al (2008) found that no significant differences observed between studied treatment in microbial counts. Nevertheless, Gelabert et al (2003) found that the partial replacement of NaCl by KCl, glycine and K-Lactate had a slight effect on microbiological stability. However, higher microbial counts were observed in dry-cured bacon in batch that contains NaCl: KCl (50%:50%) (Lorenzo et al, 2015).

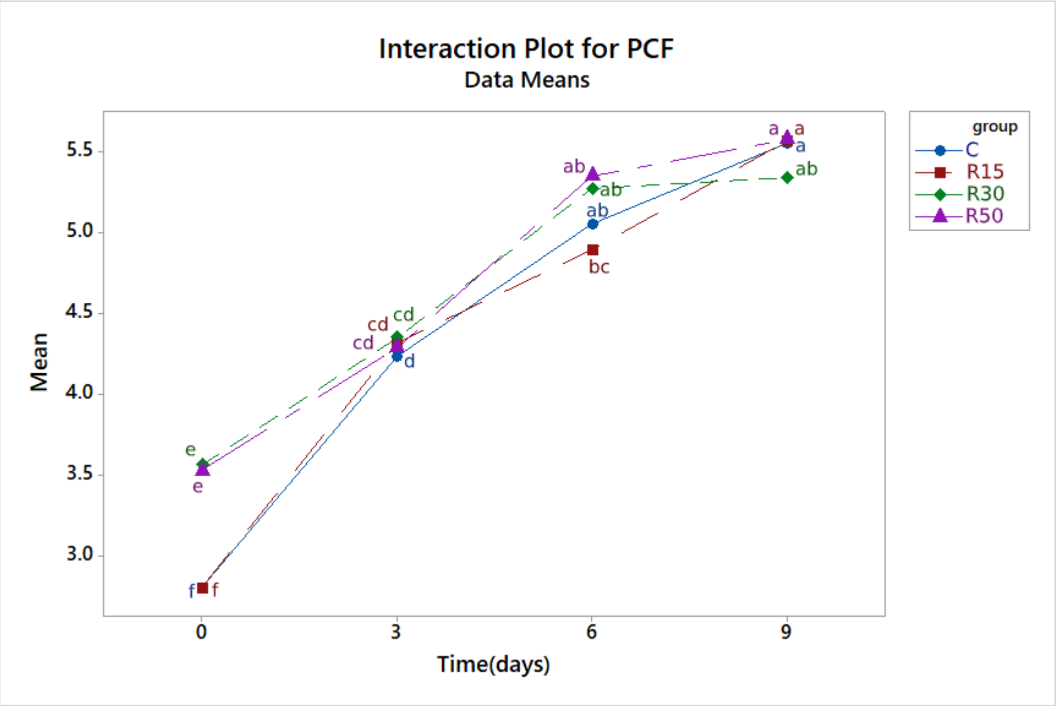

Figure 18: Psychrotrophic bacterial count (log cfu) of beef burger from all groups during refrigerated storage (9 days). C: control group (100% sodium chloride), R_{15}: 15% of sodium chloride replacement by potassium chloride, R_{30}: 30% of sodium chloride replacement by potassium chloride, R_{50}: 50% of sodium chloride replacement by potassium chloride.

4.4.3 Yeasts and Molds analysis (Y & M)

The effect of substitution of sodium chloride by potassium chloride on yeasts and molds count was shown in Figure 19. Yeasts and molds are considered as spoilage organisms. Moreover, some of mold may produce mycotoxins that are harmful for human and in the same time, they resist destruction during food cooking or processing. Several yeasts and molds can grow over a wide temperature range (5 to 35°C) and a varied pH range (about pH 2-9). In addition, some genera have ability to grow at low water activities ($a_w \leq 0.85$) (McL & Sborough, 2017). In the first day of storage (day 0), the initial count of yeast and molds in control and R15 groups was lower than groups R_{30} and R_{50}. At day 3, control group (C) exhibited significantly ($p<0.05$) lower count of yeasts and molds in comparison with other groups (R_{15}, R_{30}, and R_{50}). At day 6, it was found that group R_{30} had significantly ($p<0.05$) lower yeasts and molds counts than other groups (C, R_{15}, and R_{50}). All treatments exhibited similar trends in yeasts and molds growth during whole period of storage except in day 3 and 6. There was gradual increase in the count of yeasts and molds during storage, even that sodium chloride had higher antimicrobial activity than potassium chloride but the replacement levels up to 50% did not show any effect (Samapundo et al, 2010). According to Carraro et al (2012), using potassium chloride as a partial replacement of sodium chloride was a good substitute to reduce sodium content in fermented meat products without affecting the safety and microbiological stability of the products through manufacturing and storage stages. According to Gelabertet et al (2003) and Gimeno et al (1998), partial replacement of sodium chloride by potassium chloride was a good alternative to reduce sodium content without affecting the microbiological safety and stability of fermented meat products during manufacturing and storage phases. A study conducted by Sallam and Samejima (2004) to evaluate the effect of using Trisodium Phosphate (TSP) and sodium chloride on the shelf life of refrigerated tray-packaged chicken breast, it was found that using (TSP) alone (or being combined with sodium chloride) had no significant effect on yeast and molds count. Trisodium Phosphate (TSP) significantly reduced the growth of Enterobacteriaceae, and psychrotrophic bacteria counts and prolonged shelf life of refrigerated chicken breast to more than 12 days.

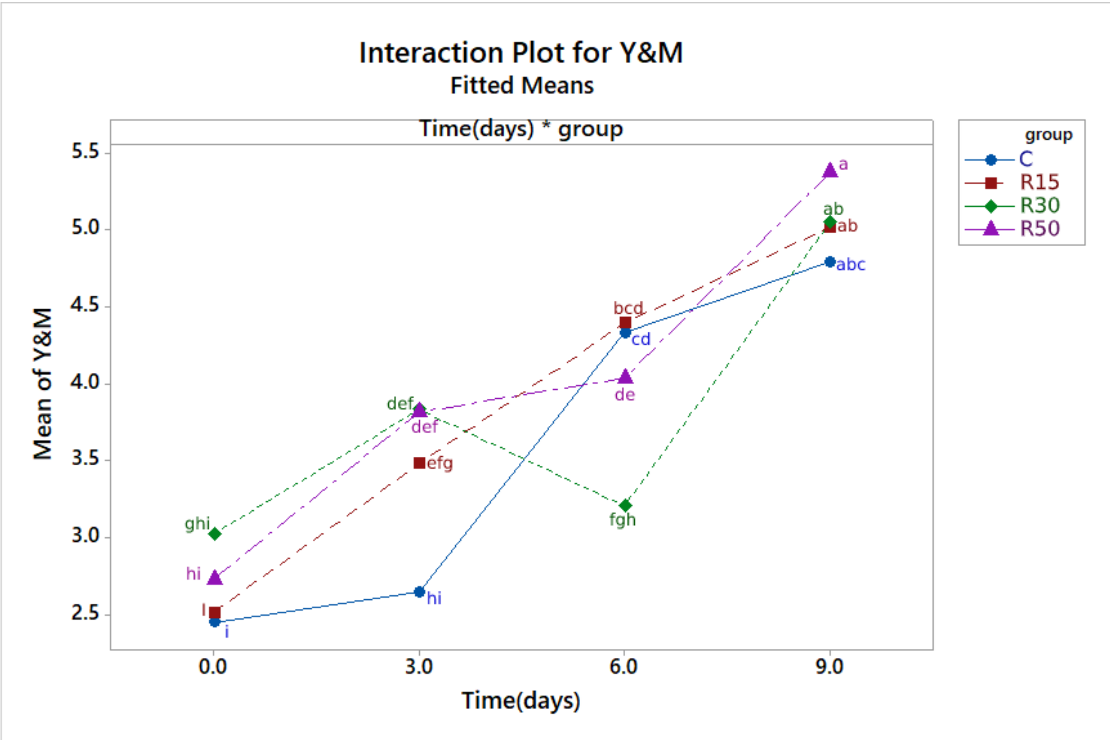

Figure 19: Yeasts and Molds count (log cfu) of beef burger from all groups during refrigerated storage (9 days). C: control group (zero sodium chloride replacement), R_{15}: 15% of sodium chloride replacement by potassium chloride, R_{30}: 30% of sodium chloride replacement by potassium chloride, R_{50}: 50% of sodium chloride replacement by potassium chloride.

4.5 Effect of sodium chloride replacement on sensory parameters

The results of the sensory analysis of replacing sodium chloride by potassium chloride in beef burger samples in four groups are shown in Table 6.

There was no significant difference (p-value >0.05) between control group and any of the other groups in sensory parameters (saltiness, juiciness, and over all acceptance), which means there was no effect of salt replacement on sensory parameters. Many studies indicated a negative effect between high level of potassium chloride and taste of meat. In this context, it was found that substitution sodium chloride by potassium chloride more than 30% produced bitter and metallic taste (Horita et al., 2014). Li et al. (2016) found that the most important sensory attribute in dry-cured bacon was saltiness, which is defined as the taste that promoted by sodium chloride. Because of the fact that the saltiness of other salts is not as pure as that of sodium chloride. It was found that in dry-cured bacon, the substitution of sodium chloride by potassium chloride led to less salty taste. Wu et al. (2014) stated that it was potential to decrease sodium chloride by 40% without undesirable flavor in dry-cured bacon. But Gelabert et al (2003) found that substitution of sodium chloride by more than 50% of potassium chloride did not affect the flavor of fermented sausages. Soglia et al. (2014) suggested that replacement of 50% sodium chloride by potassium chloride significantly reduced saltiness of rabbit lion meat. These differences in the effect of sodium chloride reduction and replacement on meat may be dependent on the type of food (Li et al., 2016).

Table 6: Results of the effect of sodium chloride replacment on sensory parameters.

Groups\	C	R_{15}	R_{30}	R_{50}	p-value
parameters	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Over all acceptance	3.80	4.30	4.22	3.84	0.46
juiciness	4.00	3.60	3.64	4.12	0.31
saltiness	3.16	3.32	3.76	3.44	0.31

C: control group (zero sodium chloride replacement), R_{15}: 15% of sodium chloride replacement by potassium chloride. R_{30}: 30% of sodium chloride replacement by potassium chloride, R_{50}:50% sodium chloride replacement by potassium chloride.

4.6 Effect of sodium chloride replacement on textural profile analysis (TPA) parameters

Texture is determined primarily by the myofibrillar and connective tissue components of muscle. It is defined as a psychological response to a physical and chemical stimulus produced by chewing. Components of texture that can be considered by the assessor during chewing include the primary ease of penetration by the teeth on the first bite, easy cutting of meat into fibers, resistance to chewing, and power and time until the meat is ready for swallowing. Parameters of textural profile analysis (hardness I, hardness Ⅱ, cohesiveness, springiness, chewiness and gumminess) were measured for all groups of meat (control, R_{15}, R_{30}, R_{50}). The effect of sodium chloride reduction and replacement by potassium chloride on TPA parameters was shown in Table 7.

Our finding showed that there were no significant differences in chewiness and springiness between groups (p-value< 0.05). For hardness, control group exhibited significantly (p-value<0.05) higher value than other groups (R_{15}, R_{30}, R_{50}), which means a reduction in hardness. Many studies also found a decrease in hardness. Alino et al (2009) found a decrease in hardness of dry-cured bacon with 70% potassium chloride substitutes, and reported a negative relationship between moisture content of dry-cured meat product and hardness. Wu and Zhang (2014) found that replacing sodium chloride by 70% resulted in increasing of juiciness and bitterness and decreasing in hardness and saltiness of dry-cured bacons. Li et al (2016), interpreted the reduction of harness in cured meat due to reduction of sodium chloride to excess of proteolysis due to the intense action of proteases, leading to softness texture. Moreover, NaCl reduction in some cured meat products was able to cause an excess of proteolysis because of the intense action of proteases, leading to softness of texture.

According to Horita et al. (2014), substitution of sodium chloride by 50% of potassium chloride resulted in high hardness values compared to control group (100% NaCl), while replacement by 25% did not affect the hardness of Frankfurt sausages. This was attributed to reduction in water binding capacity which led to increase of hardness after cooking (Horita et al., 2014). For cohesiveness parameter, group R_{50} exhibited significantly higher value than group R_{15} (p-value<0.05, 0.65 vs. 0.61) respectively, while control and R_{30} groups showed intermediate values. Control group showed higher significant value (p-value<0.05) than other groups (R_{15}, R_{30}, R_{50}) in gumminess parameter. For total work cycle 2, all groups showed moderate values.

Table 7: Results of the effect of sodium chloride replacement by potassium chloride on TPA parameters.

Trait	Treatments				p-value
	Control	R_{15}	R_{30}	R_{50}	
Hardness Cycle1	4816[a]	3938[b]	3423[b]	3299[b]	<0.05
Hardness Cycle 2	4363[a]	3393[b]	3099[b]	3113[b]	<0.05
Hardness work Cycle 2	70.99[a]	58.12[b]	46.74[c]	47.47[c]	<0.05
Cohesiveness	0.62[ab]	0.61[b]	0.63[ab]	0.65[a]	<0.05
Gumminess	2976[a]	2352[b]	2146[b]	2165[b]	<0.05
Chewiness	149.20	112.73	219.3	204.2	0.102
Chewiness Index	2542.1[a]	1991[b]	1928.6[b]	1975[b]	<0.05
Corrected Gumminess	2525.3[a]	1985.1[ab]	1818.7[b]	1651[b]	<0.05
Total Work Cycle 2	94.52[a]	77.07[ab]	64.58[bc]	55.00[c]	<0.05
Springiness	5.11	5.84	10.50	9.66	0.07

Same letters within the same column do not differ significantly ($p < 0.05$) according to Tukey's test.

Control: 100% NaCl, R_{15}: 85% NaCl + 15% KCl, R_{30}: 70% NaCl + 30% KCl, R_{50}: 50% NaCl + 50% KCl.

4.7 Effect of sodium chloride replacement on cooking loss values

The effect of replacing sodium chloride by potassium chloride on cooking loss for beef burger samples in four groups was shown in Table 8.

As shown in Table 7, there was no significant difference between groups in cooking loss. Cooking loss depends on both cooking method and temperature, and meat properties (fat, water, protein content and pH) (Gerber et al, 2009). Rodriguez-Estrada et al. (1997) stated that in general, cooking of meat caused a reduction in weight because of water loss through cooking of meat. Lopez et al (2012) found a linear relationship between sodium chloride concentration and cooking loss, as sodium chloride concentration increased from (0-1.0%), cooking loss decreased in broiler breast samples. This was explained by increasing sodium chloride concentration from (0-1.0%) which led to increase the moisture retention in chicken breast meat through cooking. Sodium chloride free meat samples had a high cooking loss. This is because without salt in meat during cooking, a low ionic strength and there is an inability to solubilize myofibrillar proteins, this will reduce water binding capacity of meat proteins (Lopez et al, 2012).

Table 8. Results of the effect of sodium chloride reduction and replacement on cooking loss of samples.

groups	mean
C	37.16
R_{15}	39.37
R_{30}	36.52
R_{50}	35.68
P-value	0.089

C: control group (zero sodium chloride replacement), R_{15}: 15% of sodium chloride replacement by potassium chloride. R_{30}: 30% of sodium chloride replacement by potassium chloride, R_{50}:50% sodium chloride replacement by potassium chloride.

Conclusion

Many studies showed that excess salt sodium consumption was associated with considered hypertension and cardiovascular diseases. As the primary approach for reducing salt in meat products has been carried out by using of salt replacers. In general, our findings showed that potassium chloride was effective as sodium chloride replacers. The findings of our study suggested the possibility of 50% replacement of sodium chloride by potassium chloride without a change in microbiological safety and physicochemical quality of beef burger. Saltiness, juiciness and over all acceptance of beef burger samples were not affected by reduction and replacement and the taste of burgers from all groups was acceptable. The cooking process affected the samples color, ash, moisture, fat, and protein content, also color acceptability of beef burger samples was affected by substitution process.

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جامعة النجاح الوطنية

كلية الدراسات العليا

تأثير استبدال كلوريد الصوديوم بكلوريد البوتاسيوم على صفات جودة برغر اللحم البقري

إعداد

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إشراف

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قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في التغذية وتكنولوجيا الغذاء، بكلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس– فلسطين.

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الملخص

الصوديوم هو أحد أهم العناصر الغذائية لوظائف جسم الانسان. ومع ذلك فان كمية الصوديوم التي يتم تناولها من الغذاء حاليا تتجاوز توصيات منظمة الصحة العالمية، يعتبر زيادة استهلاك الصوديوم سببا رئيسيا لارتفاع ضغط الدم وأمراض القلب والأوعية الدموية. بسبب الارتباط القوي بين تناول كمية مرتفعة من الصوديوم وحدوث ارتفاع ضغط الدم والأمراض المزمنة الاخرى، توصي العديد من المنظمات الصحية الدولية بتقليل محتوى الصوديوم في الأطعمة المصنعة. منتجات اللحوم المصنعة لها مساهمة كبيرة في زيادة استهلاك الصوديوم، وبناء على ذلك كان الهدف من هذه الدراسة هو تقييم تأثير استبدال كلوريد الصوديوم بكلوريد البوتاسيوم على صفات جودة برغر اللحم البقري (عدد الميكروبات، الخصائص الكيميائية والفيزيائية بالإضافة الى الخصائص الحسية). في هذه الدراسة تم استخدام 160 قطعة من برجر اللحم البقري موزعة على أربع مجموعات التي تمثل أربع مستويات من نسب استبدال كلوريد الصوديوم بكلوريد البوتاسيوم وهي 0%،15%،30% و50% بالتوالي. اظهرت الدراسة ان جميع نسب الاستبدال لم يكن لها أي تأثير على الخصائص الكيميائية والحسية والجرثومية. في الخلاصة تشير الدراسية الى امكانية تقليل محتوى الصوديوم في المنتج الى نسبة قد تصل الى 50% باستخدام كلوريد البوتاسيوم كبديل.