# Effect of Partial Replacement of Sodium Chloride with Potassium Chloride on the Quality Traits of Beef Burgers During Refrigerated Storage

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## **Keywords**

Beef Burger, Sodium Substitution, Microbiological, Sensory, Colour. Excessive sodium intake is associated with chronic diseases such as hypertensions, renal failure, and cardiovascular disease. There is growing interest in lowering the level of sodium in foods. Accordingly, the aim of this study was to evaluate the impact of the substitution of sodium chloride with potassium chloride on the microbiological and physical properties of beef burgers during refrigerated storage at 4°C for 9 days. Microbiological parameters (aerobic bacteria, psychrotrophic bacteria, and yeasts/molds), colour traits (L\*a\*b\*), and sensory properties were assessed. The study included four treatments: the control group (0% sodium substitution), R15 (15% sodium substitution), R30 (30% sodium substitution), and R50 (50% sodium substitution). At the end of storage, L\* values of the control group significantly increased, while those of the low-sodium group slightly increased. Moreover, sodium chloride substitution with potassium chloride had no effect on the redness index (a\*). All groups showed a significant (p<0.05) decrease in a\* during storage in a consistent manner. Overall, even microbial counts gradually increased over storage, all treatments exhibited acceptable safety limits, and there were no significant differences in total aerobic, psychrotrophic, and fungal counts between treatments. Salt replacement had no effect on the sensory characteristics (perceived saltiness, juiciness, and overall acceptability), since there was no significant difference (p-value > 0.05) between the treatments. In conclusion, the study revealed that sodium chloride substitution with potassium chloride had no impact on the stability and the quality of the product during storage, and the changes observed were attributed to the effect of storage.

## 1. Introduction

It is commonly known that consuming large amounts of salt increases the risk of developing chronic illnesses,

including cardiovascular disease and hypertension (Chen et al., 2023). Accordingly, the World Health Organization advises cutting daily consumption of sodium to less than two grams per day (Salman, Kadota,

& Miura, 2024). The consumption of salt is still very high over the world and reached 9-12 g/day (Hunter, Dhaun, & Bailey, 2022).

Several approaches have been investigated to reduce sodium content in processed meat products. One of the most common approaches is replacing sodium chloride with salt replacers (such as KCl, CaCl<sub>2</sub>, and MgCl<sub>2</sub>) (Wang et al., 2023). In this context, potassium chloride is frequently utilized as a salt replacer in processed meats due to its comparable ionic characteristics and functional qualities (Carraro et al., 2012) as well as perceived saltiness (Taruno & Gordon, 2023). It has no impact on heart diseases or high blood pressure (Kim, Yu, & Shin, 2024). Furthermore, it has antibacterial effects similar to sodium chloride in food (Wang et al., 2023). According to Bower (2016), the primary adverse effects of potassium chloride are its metallic and bitter taste.

It was found that molar replacement of potassium chloride with sodium chloride showed an equivalent effect on some pathogens as the control. According to Mudalal & Petracci (2019), replacing 30% of sodium chloride with potassium chloride showed no detrimental effects on the quality of marinated meat products. It was revealed that KCl was the best salt replacer in jerked beef, and it was possible to replace 50% of the sodium chloride with a combination of KCl and CaCl<sub>2</sub>. According to previous research, it was found that substitution greater than 30-40% produced a metallic taste that negatively impacted the sensory qualities of the finished product (Horita et al., 2014).

According to Khdour et al. (2013) and Hallak et al. (2017), 27.6% of the population in Palestine over 25

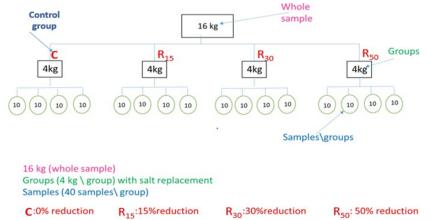
years of age has hypertension, which is considered a high prevalence. Approximately 6,000 mg of sodium is consumed daily by Palestinians (Abdeen & Qasrawi, 2010), which is 2.5 times greater than the World Health Organization recommendations (2.4 g/day). Processed meat products contributed approximately 25-30% of daily salt consumption (Wang et al., 2023).

One of the most well-known processed meat products is beef burger, which typically has a salt content ranging from 1.5% to 2.5% (Freitas et al., 2017). This amounts to approximately 50% of the daily recommended sodium intake per 100 grams of product (WHO, 2012). Burgers are well-known dishes that are consumed by people worldwide and in Palestine, even though they can lead to high salt intake. Studies to lower the salt content of burgers are thus necessary. Accordingly, the aim of this study to evaluate the stability of low-sodium beef burgers during refrigerated storage, considering potassium chloride as a salt substitute.

#### 2. Materials and Methods

## 2.1. Preparation of Beef Burgers

Approximately sixteen kilos of fresh beef meat were purchased from a nearby slaughterhouse in Tulkarem, Palestine. With a traditional meat grinder equipped with a disk that had holes measuring 3 mm, fresh beef flesh was minced. To generate a comparable initial microbial load, the minced beef was carefully mixed. Four portions, each weighing four kilograms, were taken from the quantity of beef to represent the four treatments (C, R15, R30, and R50) (Figure 1). According to Table 1, four treatments with increasing substitution levels of NaCl by KCl (0, 15, 30, and 50%) were tested.



**Figure 1:** An Experimental Design Showing the Four Groups. Group C, which Contains 100% Sodium Chloride (NaCl), Is The Control. Experimental Groups Using 15%, 30%, and 50% Potassium Chloride (KCl) in Replacement of NaCl are Represented by Groups R15, R30, and R50, Respectively.

The total meat mass was divided into four groups (4 kg/group). Forty meat patties belonging to each group were formed by a hand-operated forming machine, and each one had a 100 g weight.

**Table 1:** The Salts (NaCl:KCl) were Added to each Group (4 kg) to Achieve a Final Salt Concentration of 1.5% with the Target Replacement of NaCl with KCl.

Replacement Level	0%	15%	30%	50%
NaCl (g)	60	51	42	30
KCl (g)	0	9	18	30

A total of 160 beef burger patties weighing approximately 100 g were produced to represent the four groups (40 patties/group). For nine days, the patties were kept in a chilled condition (4 °C), and chemico-physical and microbiological properties were evaluated at 0, 3, 6, and 9 days.

#### 2.2. Measurement of Colour Indices

Using a portable Minolta Chroma Meter CR-400 equipped with C as the illuminance source, three colour indices (L\*, a\*, and b\*) were measured. During the research period, the colour indices of the 10 raw meat patties were measured. Prior to the measurements, the instrument was calibrated using a white ceramic tile as a reference (Mancini et al., 2022).

The colour of each beef patty was tested in triplicate in three separate regions, avoiding areas with colour abnormalities.

## 2.3. pH Measurements

Because pH has an impact on the qualitative attributes, shelf life, and colour of meat products, pH values have been measured. However, 2.5 g of beef meat was homogenized with 25 ml of distilled water for 30 seconds at a speed of 10,000 rpm. A pH meter was used to measure pH by directly inserting the probe into the solution (Jeacocke, 1977). The pH was measured in ten replications from ten patties.

# 2.4. Microbiological Analysis

The microbiological characteristics (total aerobic bacteria, psychrotrophic bacteria, and yeast and mold) were assessed in triplicate for all treatments during the studied storage time at each 3-day interval. To determine the overall growth of aerobic and psychrotrophic bacteria, 10 g of meat from each burger patty was weighed in a sterile stomach bag, and 90 ml of peptone water was added.

Using a stomacher, the contents were homogenized for 30 seconds. After preparing the proper serial dilution, the Petri dishes containing plate count agar were inoculated with 0.1 ml from each dilution using a micropipette. The Petri dishes were then incubated for 48-72 h at 37 °C and for 10 days at 4 °C in a refrigerator to count psychrotrophic bacteria. Furthermore, Petri dishes containing potato dextrose agar were cultured with samples and incubated for five days at room temperature (25 °C) to determine fungal count (Valerie et al., 2001).

# 2.5. Sensory Evaluation

Four beef patties from each group were cooked for 15 minutes at 200°C in an electric oven for sensory evaluation. The patties were cooked, then allowed to cool to room temperature before being divided into eight equal pieces. In order to evaluate three sensory qualities (perceived saltiness, juiciness, and overall acceptability), fifty students (both male and female, ages 18 to 25) from An-Najah National University in Tulkarm were recruited.

Each sample was evaluated by the participants using the following seven-point rating system:

- The range of perceived saltiness is 1 (very bland, unsalted) to 7 (very salty).
- Juiciness scores range from 1 (very dry) to 7 (very juicy).
- Total acceptability ranges from 1 (very undesirable) to 7 (extremely acceptable).

# 2.6. Statistical Analysis

Before applying ANOVA, the Shapiro-Wilk test was used to determine whether the data distribution was normal, and Levene's test was used to confirm the homogeneity of variances. The findings were statistically analysed with a two-way analysis of variance (ANOVA) to determine the replacement effects (0, 15, 30, and 50%) and storage time (0, 3, 6 and 9 d), as well as the interaction effect using a statistical analysis system (SPSS). Duncan's multiple range test was used to classify the order of the mean values of measured traits at significance level (p<0.05). Moreover, 95% confidence intervals were considered during statistical analysis for all tested parameters.

#### 3. Results and Discussion

# 3.1. Effect of Sodium Chloride Replacement on Colour Traits

The impact of substituting potassium chloride with sodium chloride on the lightness index (L\*) for each

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group over the course of nine days of storage is displayed in Figure 2A. At day 0, all groups showed similar lightness. Moderately significant difference between the groups started to appear on day 3, followed by clear significant differences on day 6. On the last day of storage, groups R15 and R50 had moderate values, whereas the control group had significantly (p<0.05) greater L\* values than did group R30. There was often no interaction between storage time and treatment. The lightness (expressed as L\*) raised at the end of storage in the control group, while the L\* in the other groups slightly increased compared to that on the first day.

According to Stanley, Bower, & Sullivan (2017), pork sausage patties with varying salt reduction levels did not significantly differ in lightness. Furthermore, a prior study has shown that pork patties were unaffected by the substitution of sodium chloride with potassium chloride. According to Raseta et al. (2018), the acceptability of pork burger color was not affected by the partial substitution of potassium chloride with sodium chloride.

Freitas et al. (2017) revealed that the L\* values of low-sodium products were considerably lower than those of the control treatment. These outcomes were explained by the fact that sodium chloride has a stronger oxidative impact than potassium chloride. This study agreed with our findings.

Regarding a\*-values (Figure 2B), group R15 had a considerably (p<0.05) greater a\* value than did the other groups at day 0 and day 3 of storage. This may be attributed to initial color variations in each batch. Practically, it was difficult to obtain a totally uniform ground beef mass. On day 6, group R30 had a\* values that were significantly (p<0.05) greater than those of the control group, whereas the values of the other groups were moderate. When comparing the values of a\* at the end of storage to the first day (day 0), there was a significant (p<0.05) decrease in all groups. Our results demonstrated that redness value in all groups gradually reduced during storage in a consistent manner, indicating that the impact of storage rather than the effect of treatment is responsible for these changes.

From day one to day nine, all groups exhibited a shift in red colour, changing from a purplish-red colour on the first day to a brown colour on day nine. The variations in the colour of product may be explained by the addition of salt, which causes the amount of myoglobin to decrease. Myoglobin is the main pigment that gives fresh meat its red hue. This pigment may oxidize over time and during

storage to produce the brownish component known as metmyoglobin (MMb). Consumers sometimes interpret this change as an indication of diminished freshness or spoiling, as it causes noticeable discoloration. The amount of myoglobin in meat products is known to become unstable when NaCl is added, increasing heat denaturation. An increase in oxidative conditions and denaturing myoglobin may lessen the redness of cooked food (Jeong, 2017); this might also account for the decline in the a\* value (Bae et al., 2018). Olivera et al. (2013) reported that as the storage duration increased, the a\* values decreased.

In contrast, roasted chicken breast treated with sodium chloride showed an increase in myoglobin content when salt content increased from 0 to 2% (Jeong, 2017). Regardless of the processing conditions, cooked turkey breast containing 2% salt showed greater myoglobin denaturation and a lower myoglobin content than the control group (without salt) (Bae et al., 2018).

According to Jeong (2017), the pre-salting time of meat products containing 0 or 1% sodium chloride had no significant impact on the a\* values. Min, Cordray, & Ahn (2010) revealed a considerable increase in redness by the addition of salt. The addition of sodium chloride may cause myofibrillar proteins to become more soluble, increasing the possibility that they will react with heme pigments and produce a more intense pink color.

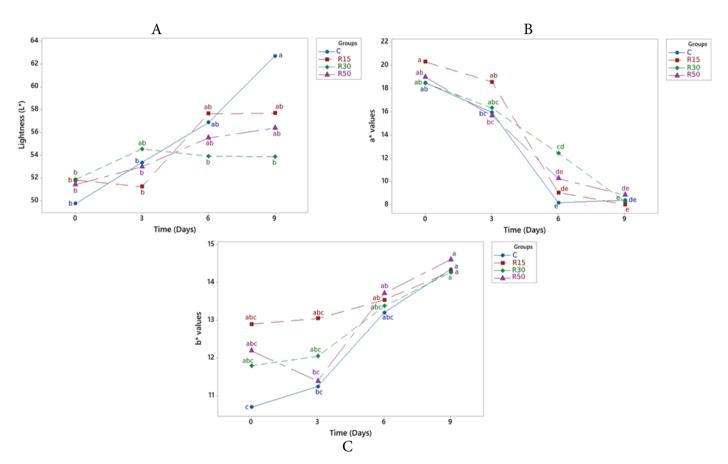
The impact of the substitution of sodium chloride with potassium chloride on b\*-values is shown in Figure 2C. During the first three days of storage, there were moderate differences in b\* values between the groups. Moreover, there was a progressive increase in b\* values in all groups, without significant differences between groups. All groups showed similar patterns of changes in b\* values during storage.

When the control group was compared to the other groups on the first day of storage, the difference in the b\* value was significant (p<0.05). There were somewhat significant differences between the groups on day 3 and day 6. When comparing the groups' yellowness values on the last day of storage, there was a significant (p<0.05) increase compared to that on the first day. The control group showed the largest variations in yellowness values between the first and last days of storage.

Meat yellowness is determined by the quantity of intramuscular fat tissue and by a variety of pigments, which can intensify or lessen the yellow-blue hue of meat (Lukanov et al., 2018). According to Stanley et al. (2017), the yellowness index of the potassium chloride group was greater than that of the sodium chloride group when an equivalent molar substitution was made. Similarly, prior studies demonstrated that the colour characteristics of reduced-sodium beef products (in which potassium chloride was used as a salt replacer) had no significant differences compared to the control. On the other hand, several studies have shown that, when compared to controls, pork patties with high levels of potassium chloride had reduced redness and yellowness indices.

The pooled effect of replacing NaCl with KCl (excluding

the effect of storage) on the colour traits of beef burgers showed that burgers get darker ( $\downarrow$ L\*), less red ( $\downarrow$ a\*), and more yellow ( $\uparrow$ b\*). This may be attributed to disruption of ionic strength when KCl is substituted with NaCl, which lowers antioxidant effectiveness and water retention. As a result of the oxidative processes (lipid/protein oxidation, myoglobin breakdown) are accelerated. The noticeable hue shifts show how these effects are amplified at higher KCl levels (e.g., R50). These results are consistent with research showing how important NaCl is for beef products' color stability and antioxidant protection (Kim et al., 2021; Pateiro et al., 2021).



**Figure 2:** Changes in the L\* Values (A), a\* Values (B), and b\* Values (C) of Beef Burgers During the Storage Period (9 days). Group C: Zero Substitution; Group R15: Substitution of 15% of NaCl by KCl; Group R30: Substitution of 30% of NaCl by KCl; Group R50: Substitution of 50% of NaCl by KCl. Different Letters are Significantly Different (P <0.05). N= 10, the Number of Samples Selected from each Group to Measure Color Traits.

# 3.2. Effect of Sodium Chloride Replacement on pH Values

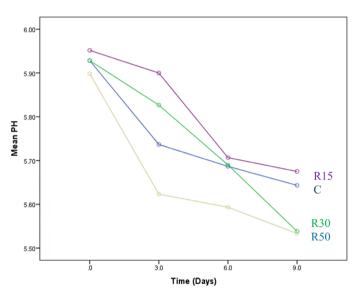
The average pH values of beef burger samples in all groups gradually dropped over the course of nine days of refrigeration. Initially (day 0), the pH levels of all groups were very comparable, ranging from 5.9 to 6.0. Nevertheless, a significant drop in pH was observed

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for all groups as storage time increased. A greater pH decline was observed in the group with increased potassium chloride (KCl) substitution (shown by the line with the steepest drop) than in the control group (100% sodium chloride). This trend implies that the partial substitution of KCl for sodium chloride would have affected metabolic processes or microbial activity, potentially accelerating acidification.

Bower (2016) found that the pH of meat samples was reduced during the storage time, and this change was attributed to the proliferation of lactic acid bacteria (LAB) accompanied by the production of lactic acid. Additionally, Chikthimmah et al. (2001) showed that the level of NaCl in the product was a crucial parameter for either stimulating or inhibiting the growth of LAB.

Li et al. (2016) showed that control fermented and cooked sausages had pH values that were significantly lower than those of sausages that contained 50% potassium chloride instead of sodium chloride. This outcome may be ascribed to the suppressive effect of KCl replacement on the proliferation of coliforms, which metabolize basic nitrogen compounds and induce pH fluctuations.



**Figure 3:** The values of pH for Beef Burgers in all Groups During Refrigerated Storage (9 d. Group C: Zero Substitution; Group R15: Substitution of 15% of NaCl by KCl; Group R30: Substitution of 30% of NaCl by KCl; Group R50: Substitution of 50% of NaCl by KCl. Different Letters are Significantly Different (P <0.05). N= 10, the Number of Samples Selected from each Group to Measure pH.

There are different mechanisms explaining the effect of sodium reduction on the pH of meat products. Firstly, through the disruption of microbial cell membranes and the reduction of water activity, sodium chloride prevents the development of microorganisms. This antibacterial activity may be weakened by partial substitution with KCl (e.g., R50), which would permit the growth of lactic acid bacteria (LAB). LAB immediately lowers pH by converting carbohydrates to lactic acid. Secondly, low-salt conditions increase the activity of proteolytic enzymes, such as cathepsins, which break down muscle proteins into peptides and free amino acids. The system may become even more acidic due to these breakdown

products. Because KCl has a lower ionic strength than NaCl, it might not be able to effectively suppress enzyme activity, which would speed up proteolysis in R50 burgers (Desmond & Vasilopoulos, 2019).

# 3.3. Effect of Sodium Chloride Replacement on Microbiological Properties

Several microbiological analyses (total aerobic bacteria (Figure 4 A), psychrotrophic bacteria (Figure 4B), and yeast and mold (Figure 4 C)) were used to investigate the impact of NaCl substitution with KCl.

The R50 group exhibited a significantly (P<0.05) greater aerobic bacterial count than the control group, while the  $R_{15}$  and  $R_{30}$  groups had intermediate values on Day 0. These differences in initial microbial load may be attributed to the microbiological homogeneity of raw materials but not to the effects of the treatments.

Even if there were minor initial changes in microbial counts between groups on day 0, the validity of the comparisons was unaffected; these fluctuations were within a reasonable microbiological range. Furthermore, reducing initial microbial variation and improving the repeatability of results might be achieved in future research by implementing a more rigorous randomization method during sample preparation.

Microbiological assessment has been carried out immediately after preparation of the products where it was impossible to reach an osmotic balance between cations and anions. In addition, the pH of beef meat is low, which is not favourable for bacterial growth (Bower, 2016). From day 3 to day 6, there was a moderately significant increase in the aerobic bacterial load in all groups compared to day 0. At the end of storage, all groups had a significantly greater aerobic bacterial load than on the first day of storage.

Overall, the results showed that there was no significant change in the growth of aerobic bacteria between the groups throughout the course of the storage period; nevertheless, bacterial count in each group gradually increased during storage, eventually reaching 5.5-6 logs.

In general, our results were in agreement with previous studies. According to Doyle & Glass (2010), there was no discernible impact on the aerobic plate count when raw pork sausage was formulated by using different replacement levels of sodium chloride with potassium chloride. Nevertheless, our results did not coincide with those of some earlier studies. In this regard, Blesa et al. (2008) reported that reducing the

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sodium chloride concentration in dry-cured ham had an impact on aerobic bacterial development.

The psychrotrophic bacteria count was substantially higher in the R50 and R30 groups than in the control and R15 groups on the first day of storage, as a result of the substitution of the salt with KCl. This outcome may be linked to the initial variations in the microbial burden between the meat batches. Over the course of the storage period, the number of psychrotrophic bacteria increased gradually and significantly in each group. During storage, all groups showed comparable patterns of bacterial growth.

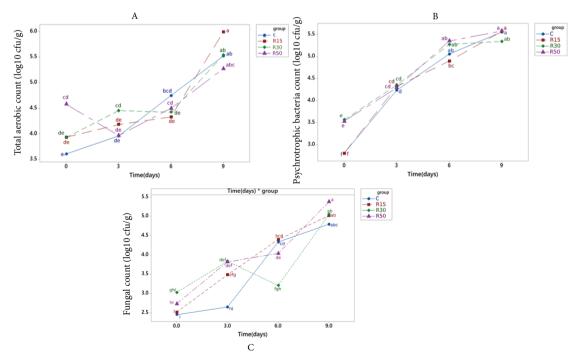
In summary, the growth of psychrotrophic bacteria was not significantly affected by substituting KCl for NaCl in any of the groups.

An investigation was carried out on dry-cured ham to assess the impact of substituting sodium chloride with potassium, calcium, and magnesium salts. According to Blesa et al. (2008), there were no significant differences in the microbial counts between the treatments under study. Nevertheless, Gelabert et al. (2003) showed that there was a minor impact on microbiological stability when KCl, glycine, and K-lactate were partially substituted for NaCl. On the other hand, it was found that dry-cured bacon that included 50% KCl and 50% NaCl had increased

microbial counts (Lorenzo et al., 2015).

The initial counts of yeast and molds in the control and R15 groups were lower than the R30 and R50 groups on the first day of storage. On the third day, the control group (C) had considerably (p<0.05) lower counts of molds and yeasts than did the other groups (R15, R30, and R50). Group R30 was found to have considerably (p<0.05) fewer yeasts and molds than the other groups (C, R15, and R50) on day 6. Except on days 3 and 6, all treatments showed comparable patterns.

A broad temperature range of 5 to 35 °C and a diverse pH range of approximately 2 to 9 are suitable for the growth of several yeasts and molds. Additionally, Tapia, Alzamora, & Chirife (2020) showed that some genera of fungi may develop at low water activity levels (aw  $\leq$ 0.85). Even though potassium chloride exhibited more antibacterial action than sodium chloride, the number of yeasts and molds increased gradually during the course of storage (Samapundo et al., 2010). However, replacement levels up to 50% proved ineffective. Reducing the salt content in fermented beef products without compromising their safety and microbiological stability throughout the manufacturing and storage phases can be achieved by partially substituting potassium chloride for sodium chloride, as suggested by Carraro et al. (2012).



**Figure 4:** Total Aerobic Count (A), Psychrotrophic Bacterial Count (B), and Yeasts and Molds Count (C) of Beef Burgers from all Groups During Refrigerated Storage (9 days). Group C: zero Substitution; Group R15: Substitution of 15% of NaCl by KCl; Group R30: Substitution of 30% of NaCl by KCl; Group R50: Substitution of 50% of NaCl by KCl. Different Letters are Significantly Different (P <0.05). N= 3, the Number of Samples Selected from each Group to Evaluate Microbiological Properties.

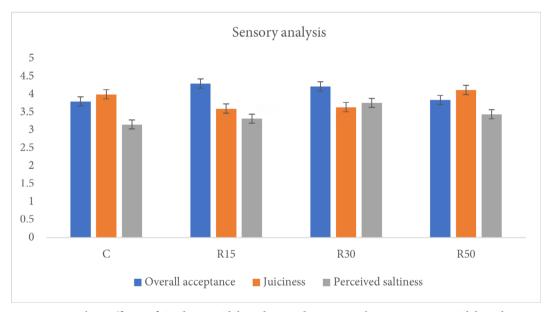
Overall, all groups with different replacement levels of sodium chloride with potassium chloride kept their microbial counts below the permissible limits for food safety during the time of refrigeration. In particular, until day 9, the levels of psychrotrophic bacteria and total bacterial counts were all within permissible limits (less than  $\log_{10}$  cfu/g) (Roberts et al., 2005). This suggests that throughout the assessed storage time, the microbiological safety of beef patties was unaffected by the partial replacement of potassium chloride for sodium chloride.

# 3.4. Effect of Sodium Chloride Replacement on Sensory Characteristics

Figure 5 displays the findings of the sensory examination conducted on the beef burger samples in the four groups after KCl was substituted for NaCl. The sensory traits (perceived saltiness, juiciness, and overall acceptability) did not significantly vary (p value> 0.05) between groups, indicating that salt substitution had no influence on these parameters. A high potassium chloride

concentration has been linked to adverse effects on meat flavour, according to several studies. Horita et al. (2014) showed that replacing more than 30% of NaCl with KCl resulted in a harsh and metallic taste. Li et al. (2016) revealed that saltiness was the most significant sensory feature of dry-cured bacon. According to a prior study, it could be possible to reduce NaCl in dry-cured bacon by 40% without affecting sensory traits. However, Gelabert et al. (2003) showed that the taste of fermented sausages remained unaffected when KCl was substituted for NaCl by more than 50%.

According to a prior study, the saltiness of rabbit lion meat dramatically decreased when 50% of the NaCl was substituted with KCl. Depending on the type of food, there may be variations in how sodium chloride replacement can affect quality traits (Li et al., 2016). In a recent study, Mudalal et al. (2025) found that it was possible to add carrot pulp and replace 30% of the NaCl with KCl without affecting the sensory characteristics of the hybrid burger.



**Figure 5:** The Effect of Sodium Chloride Replacement by Potassium Chloride on Sensory Parameters (overall acceptance, juiciness, and perceived saltiness). Group C: zero Substitution; Group R15: Substitution of 15% of NaCl by KCl; Group R30: Substitution of 30% of NaCl by KCl; Group R50: Substitution of 50% of NaCl by KCl. N= 50, the Number of Participants Involved in Sensory Analysis.

## 4. Conclusions

Potassium chloride may replace sodium chloride in beef patties up to 50%. This shows great promise for lowering dietary salt consumption without sacrificing microbiological stability or sensory perception. In terms of total aerobic bacteria, psychrotrophic bacteria, and yeast/mold counts, all treatment groups showed microbial profiles similar to the control throughout a

9-day period of refrigeration. This demonstrates that KCl may retain antimicrobial activity comparable to that of NaCl, which is essential for product safety.

Higher replacement levels (50%) of sodium chloride with potassium chloride levels showed significant effect on some colour traits. But over time, these alterations were constant for every group, suggesting that storage time (rather than KCl substitution) is the main cause

of color deterioration. The action of lactic acid bacteria is probably what caused the pH to drop consistently in all groups during storage.

Further investigations are necessary to evaluate the strategies to minimize the bitter taste of KCl as well as increase the shelf life stability under commercial conditions. Development of new products should concentrate on methods, such as taste enhancers or natural masking agents (lactate salts, amino acids, yeast extracts, or mushroom-derived extracts), to minimize the bitter taste induced by elevated potassium chloride levels. Furthermore, more investigation is advised to examine the impact of potassium chloride replacement in various meat matrices and throughout prolonged storage times.

# 4.1. Acknowledgement

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#### 4.2. Conflicts of Interest

The authors declare no conflicts of interest.

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