INFLUENCE OF PARTIAL SUBSTITUTION OF SODIUM CHLORIDE BY POTASSIUM CHLORIDE IN CABBAGE FERMENTATION

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This study aimed to evaluate the influence of NaCl substitution by KCl on the physico-chemical, microbiological and sensory characteristics of sauerkraut. The partial substitution of NaCl by KCl was effective in reducing the pH throughout the fermentation, where all samples had a final pH below 4.5. The percentage of lactic acid in sauerkraut has gradually increased until stabilization, with no significant difference between treatments from the fourth day of fermentation. It was observed that in the evaluated conditions, NaCl and KCl were effective in reducing coliforms at 35°C, where from the eighth day of fermentation, a count of < 0.3 $MPN.mL^{-1}$ was detected. The count of lactic acid bacteria increased in 3 log cycles until the eighth day and then reduced in up to 2 log cycles until the end of the process, where no significant difference was observed. In the sensory evaluation there was not significant difference for the parameters of color, flavor and texture of sauerkraut. According to these results we can conclude that the partial substitution of NaCl by KCl in the sauerkraut production has not altered the physico-chemical and microbiological characteristics, and has nonegative influence on the sensory characteristics of the product.

KEYWORDS: SAUERKRAUT, PICKLES, LACTIC FERMENTATION, SODIUM REDUCTION

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INTRODUCTION

High perishability of vegetables has encouraged the development of techniques for their conservation and increased useful life. However, for most of the population in less developed countries, preservation methods such as refrigeration and freezing are inaccessible, requiring the use of natural conservation methods. In this context, acid fermentation and salting are still the most practiced methods for preserving vegetables, reducing pathogenic micro-organisms growth and thus ensuring the product microbiological safety (Zhang et al., 2016). These processes are characterized by positively altering the food sensory characteristics and improving its nutritional value (Tamang, 2010; Nguyen et al., 2013; Gagné et al., 2015).

Among the fermented vegetables, we can highlight sauerkraut, a product obtained by cabbage lactic acid fermentation in the presence of approximately 2.0% sodium chloride (NaCl) (Peñas et al., 2010a; Xiong et al., 2014). Sauerkraut fermentation and the consequent lactic acid production is dependent on lactic acid bacteria such as *Leuconostoc mesenteroides* and *Lactobacillus plantarum* (Peñas et al., 2010a; Tamang, 2010; Xiong et al., 2014). This process promotes several physical and chemical alterations, causing the product to have desirable nutritional characteristics, which makes it a great vitamin and mineral source (Peñas et al., 2010a; Beganović et al., 2011; Rabie et al., 2011; Xiong et al., 2014).

NaCl is an important component for sauerkraut fermentation development and for its sensory properties. However, high sodium consumption is associated with the development of cardiovascular disease, osteoporosis, gastric cancer, renal diseases, asthma, obesity, and may increase the number of deaths in the population (Desmond, 2006; Brown et al., 2009; Campbell; Neal; MacGregor, 2011; Wang e Labarthe, 2011; Webster et al., 2014). High sodium intake is responsible for about 30% of hypertension cases and 63% of the number of deaths in the world, cooking salt being the main sodium source in human diet (Campbell; Neal; MacGregor, 2011; Smyth et al., 2015).

Previously conducted research has reported that the world population is consuming sodium beyond physiologically necessary quantities (Elliott; Brown, 2006). In countries like the United States, New Zealand and Canada, the sodium intake exceeds 3400 mg/day, while in Eastern Europe and Asia, consumption reaches higher values, greater than 12000 mg/day (Tsugane, 2005; Strazzullo et al., 2009). The World Health Organization (WHO) recommends a maximum intake of 2000 mg of sodium/day, equivalent to 5g of salt/day (WHO, 2012). Given these data, in 2013 a World Health Assembly was held, in which all WHO Member States signed an agreement in order to reduce the global sodium consumption by 30% by 2025 (Webster et al., 2014).

To reduce sodium in food products, potassium chloride (KCl) can be used isolated or in combination with other salts, such as magnesium chloride ($MgCl_2$) and calcium chloride ($CaCl_2$) (Aliño et al., 2010) or flavor enhancers (Desmond, 2006; Campagnol et al., 2012; Santos et al., 2014; Pietrasik; Gaudette, 2014).

Among all possible salt substitutes, KCl most resembles NaCl in its microbial efficiency and technological functionality, besides providing 80% of the NaCl salting capacity (Cruz et al., 2011; Souza et al., 2013). However, NaCl cannot be entirely replaced by KCl, which in concentrations above 40% provides a residual bitter, metallic taste (Corral; Salvador; Flores, 2013; Souza et al., 2013).

To evaluate the influence of NaCl reduction on the fermentation of vegetables is important for the development of new processes that contribute to the reduction of sodium consumption. However, many studies do not evaluate the sensory acceptance of products, which may vary among different populations. The evaluation of physico-chemical and microbiological parameters during sauerkraut production, with or without sodium reduction, has been performed (Beganović et al., 2011; Xiong et al., 2012; Xiong et al. (2014). However, it is important to relate these parameters to the sensory acceptance of products resulting from processes with reduction or replacement of sodium chloride. Studies involving sensory evaluation in association with other parameters of the process have a significant contribution to research in food development.

In this context, the aim of this study was to evaluate the influence of the partial replacement of NaCl by KCl in cabbage fermentation and its influence in product acceptance.

MATERIAL AND METHODS

CABBAGE FERMENTATION

For the sauerkraut production white cabbage (*Brassica oleracea* L. var. Capitata) was used, purchased fresh in a market in the city of Rio Paranaíba, MG, Brazil. The outer leaves were discarded and the remaining leaves were washed in fresh, tap water, and cut into small strips approximately 20 mm thick. Chopped leaves were transferred to glass flasks with total capacity of 550 g, prepared with different NaCl and KCl concentrations, according to the experimental design. The flasks were then closed and incubated at 21°C in a BOD incubator for 16 days, collected and analyzed every 4 days, all analyzes being performed in triplicate.

EXPERIMENTAL DESIGN

The experiment was performed with three repetitions per treatment and conducted with a control treatment (T_1): 2.5% NaCl; Treatment 2 (T_2): 2.0% NaCl + 0.5% KCl (substitution of 20% of NaCl by KCl) and Treatment 3 (T_3): 1.5% NaCl + 1.0% KCl (substitution of 40% of NaCl by KCl).

PH AND TITRATABLE ACIDITY DETERMINATION

The pH of the brines was measured using a pH meter previously calibrated with buffer solutions pH 4.0 and 7.0.

The titratable acidity was calculated by titration using 0.1N NaOH with a 1% phenolphthalein alcoholic solution as an indicator (AOAC, 1990).

COLIFORMS AT 35°C AND 45°C

The samples were subjected to 3 successive dilutions and 1 mL aliquots were transferred to a series of nine tubes containing Lauryl Sulfate Tryptose (LST) broth and inverted Durham tubes. After incubation at 35ºC for 48h, leaves from the positive tubes (which presented gas formation), were transferred to two other tubes, both containing inverted Durham tubes. One of these tubes contained 2% Brilliant Green Lactose Bile Broth (BGLBB) and was incubated at 35ºC for 48 h to check for coliforms at 35°C. The other tube contained Escherichia coli (EC) broth and was incubated at 45 for 24h to detect *E. coli* presence. The results were determined by a specific table and were expressed as most probable number (MPN) per mL (Rice; Bridgewater; APHA, 2012).

LACTIC ACID BACTERIA

Lactic acid bacteria determination was carried out using the MRS (Man, Rogosa and Sharpe) agar culture medium, the plates incubated at 37ºC for 48 h (APHA, 2015).

SENSORY ANALYSIS

To perform the sensory analysis, fifty untrained tasters were used, of both sexes and aged between 18 and 30 years of age. Differences in color, flavor and texture attributes between each treatment were evaluated according to the difference from the control (Meilgaard; Carr; Civille, 2006). The samples of treatments T_1, T_2 e T_3 were randomly presented to the judges in plastic cups, with a standardized amount of 10 g and coded with three digits. Together with the coded samples, another sample marked with the letter C (referring to T_1) was also served, besides mineral water for use between tastings. After the samples presentation the judges were asked to evaluate each of the test samples compared to the control sample, comparing them according to the specific attribute and using a scale (0 = no difference from C to 6 = very large difference from C).

The analysis was conducted after approval of the Ethics Committee of the Federal University of Viçosa, order number 974653.

STATISTICAL ANALYSIS

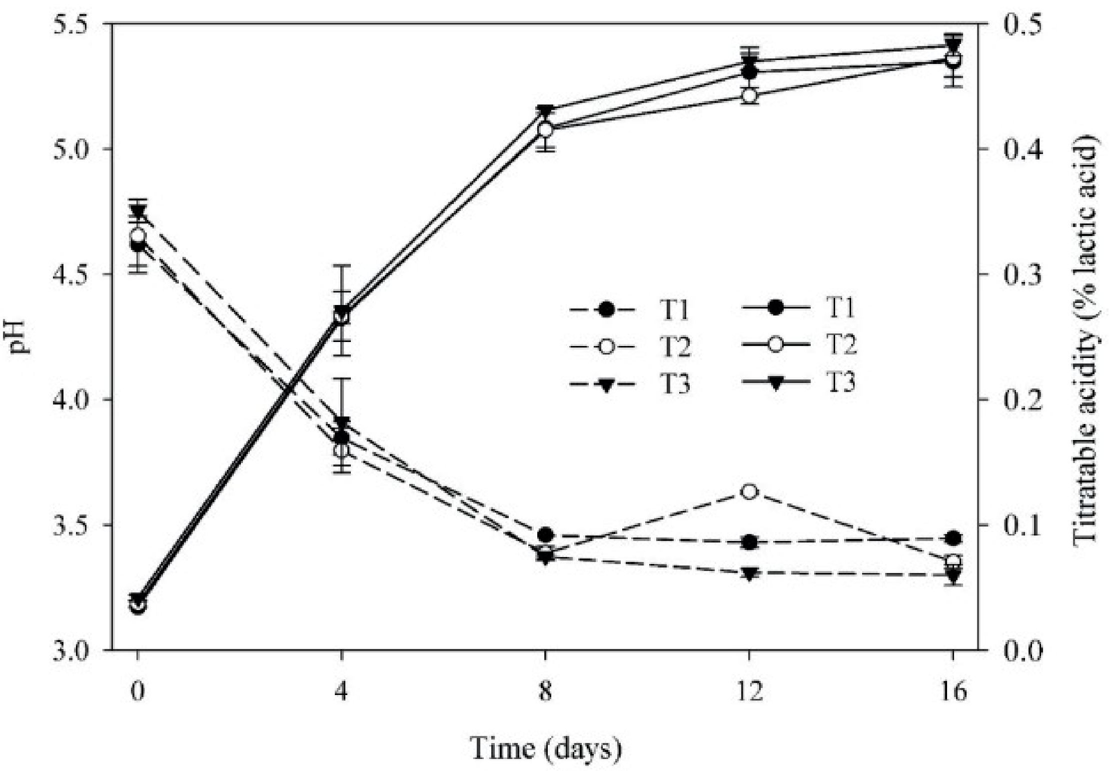
For physicochemical and microbiological analysis evaluation, statistical analysis using analysis of variance (ANOVA) and Tukey mean test at 5% level of significance were carried out. For the sensory analysis, the results were evaluated by analysis of variance (ANOVA) and the means were compared by the Dunnett's test at a 5% significance level.

RESULTS AND DISCUSSION

PH AND TITRATABLE ACIDITY DETERMINATION

The treatments initial pH ranged from 4.62 to 4.75, where the final pH of treatments T2 and T3 was 3.35 and 3.45, respectively, being significantly different. For T1, the final pH was 3.30, which did not differ significantly from T2 and showed a significant difference from T3. The pH reduction was influenced by the acidity increase, which at the end of the process varied from 0.47% to 0.48% of lactic acid, with no significant difference between treatments (Figure 1).

FIGURE 1. PH AND TITRATABLE ACIDITY EVOLUTION DURING CABBAGE FERMENTATION.



The pH had a marked reduction up to the eighth day of fermentation, followed by stabilization until the end of the process. It was verified that even with a significant difference between the final pH of T1 and T3, the highest final pH was 3.45. These results agree with the study by Johanningsmeier et al. (2005), which evaluated different *L. mesenteroides* starter cultures in sauerkraut production and obtained a final pH of less than 4 for all the treatments during 14 days of fermentation. Xiong et al. (2012) observed a pH variation from 6.02 to 3.25, which resulted in 0.57% titratable acidity in 5.5 days of sauerkraut fermentation. In similar work, Beganović et al. (2011) evaluated sauerkraut production using *L. plantarum* L4 and *L. mesenteroides* LMG 7954 and obtained a final pH below 4 at the end of the process.

The pH of the medium influence the bacteria metabolic activity; low pH favors the lactic acid bacteria and inhibits undesirable microorganism growth (Hutkins; Nannen, 1993; Cotter; Hill, 2003, Montet; Ray; Zakhia-Rozis, 2014). The pH reduction during sauerkraut fermentation occurs mainly due to the action of acidifying bacteria, which produce lactic acid from sugars, providing its characteristic flavor, color and aroma (Kabak; Dobson, 2011).

Changes in pH and acidity during sauerkraut fermentation occur mainly due to the different activities of *L. mesenteroides*, *Pediococcus* and *L. plantarum* microorganisms, naturally found in fresh cabbage. At the beginning of the fermentation, the heterofermentative stage, lactic acid bacteria such as *L. mesenteroides* is predominant, leading to lactic acid and acetic acid, ethanol, mannitol and CO_2 production, also occurring rapid pH reduction and undesirable microorganism inhibition. In the second stage of the process, called homofermentative, the replacement of these starter bacteria occurs by acid-tolerant lactic acid bacteria such as *L. plantarum*, which produces lactic acid almost exclusively. At the end of this stage the pH reduces to approximately 3.5 (Gardner et al., 2001; Tamang, 2010; Peñas et al., 2010b). In this context, the fermentation can be used as a method of food preservation. The acids produced, besides inhibiting undesirable micro-organisms growth, present antioxidant activity, preventing vegetable rancidity and browning (Kabak; Dobson, 2011; Montet; Ray; Zakhia-Rozis, 2014).

COLIFORMS AT 35 °C AND 45 °C

The coliform count at 35°C was high at the beginning of fermentation for all treatments, which may have occurred because the vegetables are grown in direct contact with soil and water, environments conducive to vegetable contamination by these bacteria. From the eighth day of fermentation, the presence of these microorganisms was not detected (Table 1), suggesting that the reduction in counts can be related to the significant pH reduction observed from this fermentation time on, wherein the pH presented values lower than 3.5 (Figure 1).

TABLE 1. COLIFORM COUNT AT 35°C AND 45°C DURING CABBAGE FERMENTATION FOR THE DIFFERENT TREATMENTS.

Time (days)	Coliforms at 35ºC (MPN mL^{-1})			Coliforms at 45ºC (MPN mL^{-1})		
	T1	T2	T3	T1	T2	T3
0	46	> 110	> 110	< 0.3	< 0.3	< 0.3
4	9.3	27	> 110	< 0.3	< 0.3	< 0.3
8	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
12	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
16	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3

In order for *Enterobacteriaceae* family microorganisms to develop, a minimum pH of 3.8 is required, the tolerance to the pH being influenced by the nature of the acidulant, in which organic acids, such as lactic acid, are more effective in inhibiting development compared to mineral acids

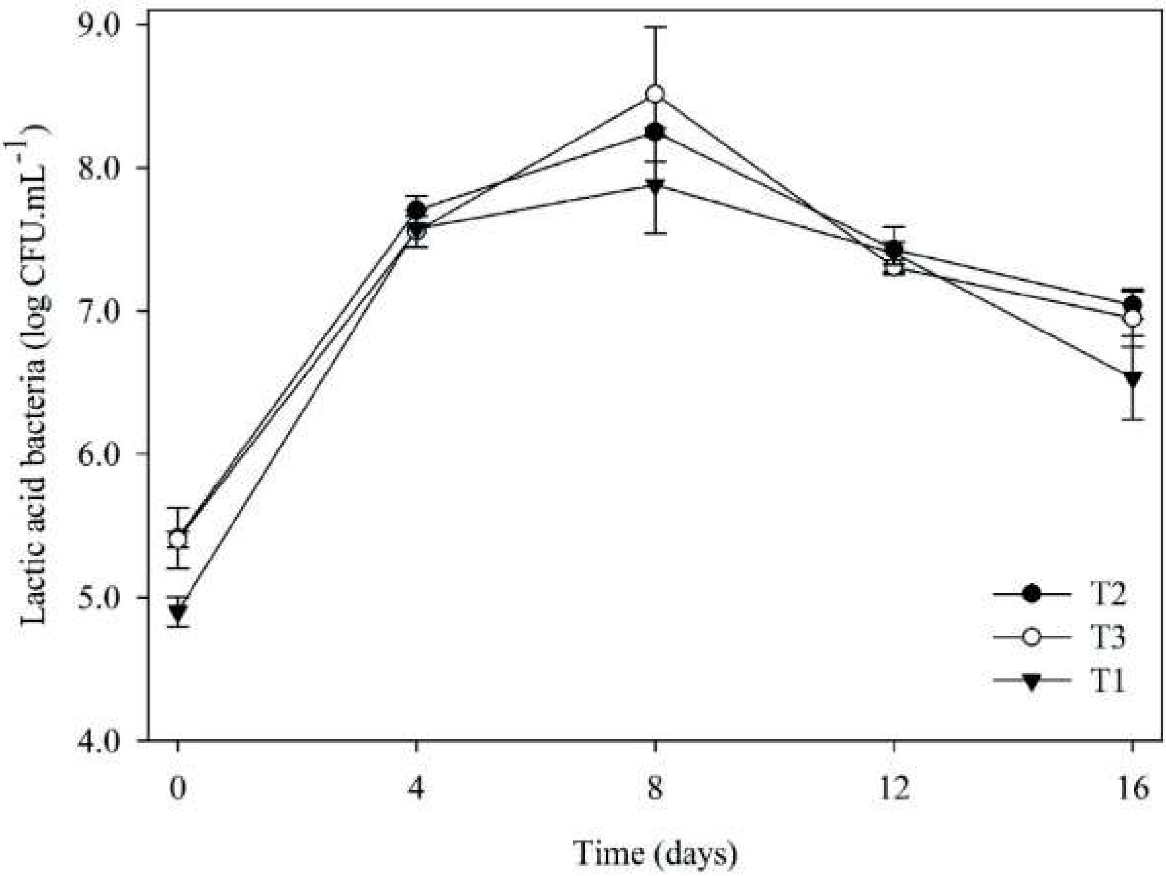
(Baylis et al., 2011).

In the evaluated conditions, the presence of coliforms at 45°C was not detected (Table 1) during the fermentation process, indicating the adoption of good practices in the raw materials cultivation and sauerkraut processing. The presence of these microorganisms in foods is indicative of inadequate hygiene or sanitation practices. In a similar study by Peñas et al. (2010a) the presence of coliforms at 35°C and 45°C was not detected in sauerkraut samples subjected to fermentation for 7 days.

LACTIC BACTERIA COUNT

The lactic acid bacteria count for the three treatments increased by about 3 log cycles until the eighth day of fermentation (Figure 2). At the end of fermentation, the bacteria count for the T_1, T_2 and T_3 treatments were 6.53, 6.95 and 7.04 log cfu mL^{-1}, respectively, with no significant difference detected among the treatments. These results show that partial substitution of NaCl by KCl in the evaluated conditions, did not affect the lactic bacteria development during the process. In the interval between the eighth day and the end of the fermentation there was a reduction of 1.5 log cycles in bacteria count in the three treatments (Figure 2), which can be explained by the inhibition caused by the acidity increase and subsequent pH reduction of the medium.

FIGURE 2. LACTIC BACTERIA COUNT DURING CABBAGE FERMENTATION.



The accentuated development of lactic acid bacteria at the beginning of the fermentation is favored by the anaerobic environment formed during the heterofermentative stage, where bacteria, less tolerant to acid lactic acid, dominate the environment. This O_2 depletion promotes the growth of homofermentative species such as *L. plantarum*, which has high resistance to acidity and is a major lactic acid producer. However, the gradual increase in lactic acid concentration throughout the fermentation, further reduces the pH of the medium and contributes to the reduction of the lactic acid bacteria count (Tamang, 2010, Xiong et al., 2012, Xiong et al., 2014). In a similar study, Xiong et al. (2012) evaluated a sauerkraut fermentation process for a 7-day period and observed that the initial lactic acid bacteria count of was approximately 2.4 log cfu mL^{-1}, followed by a significant increase in the first two days, reaching a maximum count of approximately 8.0 log cfu mL^{-1}. From the third day on there was a significant reduction in counts, resulting in a value of approximately 5.6 log cfu mL^{-1} at the end of fermentation.

Wolkers-Rooijackers Thomas and Nout (2013) evaluated the sauerkraut production with different concentrations of NaCl, KCl, $CaCl_2$ and $MgCl_2$, and verified that lactic acid bacteria counts were similar in all treatments, reaching values of 8-9 log cfu g^{-1}, without interference of the sodium substitution in the lactic bacteria count, results similar to those found in this study.

Sensory analysis

The difference-from-control test allowed to verify if the samples with partial replacement of NaCl differed significantly from the control sample (sauerkraut with 2.5% NaCl) regarding color, flavor and texture parameters.

The results evaluated by analysis of variance and the Dunnett mean test presented no significant difference between treatments, showing that the partial replacement of NaCl by KCl did not affect the evaluated parameters.

Based on the comparison of the average scores of the treatments in the sensory scale, all treatments, regarding the color attribute, presented values close to 2 (two) ("mild/moderate difference" on the scale); for the flavor attribute, T_1 and T_2 treatments showed values close to 2 (two) and the T_3 treatment, a value close to three (3) ("moderate difference" on the scale). For the texture attribute, all treatments showed values close to two (2) (Table 2).

TABLE 2. TREATMENT MEANS OBTAINED IN THE SAUERKRAUT SENSORY ANALYSIS.

Treatments	Parameters		
	Color	Flavor	Texture
T1	1.56 ± 1.34^a	2.34 ± 1.67^a	1.70 ± 1.61^a
T2	1.82 ± 1.41^a	2.48 ± 1.53^a	1.98 ± 1.50^a
T3	1.86 ± 1.49^a	2.76 ± 1.44^a	2.04 ± 1.60^a

* Means with the same superscript letters in the same column do not differ from control (P>0.05) by the Dunnet's test.

It is therefore concluded that the sauerkraut with partial replacement of 20% and 40% of NaCl by KCl did not differ from the standard formulation, presenting no discernible sensory changes in the product.

Marsilio et al. (2002), when analyzing green olives, observed that green olives with replacement of 50% NaCl by KCl obtained a good sensory evaluation acceptance. That is a result similar to that shown by Panagou et al. (2011), who detected no sensory differences between black olives fermented with 100% NaCl and olives with 50% NaCl and 50% KCl, indicating that these salts in equal proportions were suitable for the production of olives with lower sodium content and acceptable sensory characteristics.

Currently, the possibility of reducing the NaCl concentration in fermented vegetables is becoming a reality. However, this reformulation must always be assessed in each individual product (Bautista-Gallego et al., 2013).

CONCLUSION

The results demonstrate that partial substitution of NaCl by KCl did not influence the development of lactic acid bacteria involved in sauerkraut fermentation and provided the same inhibitory effect on coliforms. In addition, there was no significant difference in the physicochemical and sensory parameters evaluated. Thus, potassium chloride was shown to be a promising substitute for sodium chloride in the sauerkraut manufacturing process, since besides ensuring the technological properties of the product, which is a desirable feature for the food industry, it can also provide benefits to consumer health by reducing the sodium concentration in the product.

RESUMO

INFLUÊNCIA DA SUBSTITUIÇÃO PARCIAL DE CLORETO DE SÓDIO POR CLORETO DE POTÁSSIO NA FERMENTAÇÃO DE REPOLHO

Este estudo teve como objetivo avaliar a influência da substituição de NaCl por KCl nas características fisicoquímicas, microbiológicas e sensoriais do chucrute. A substituição parcial de NaCl por KCl foi eficaz na redução do pH ao longo da fermentação, onde todas as amostras apresentaram pH final abaixo de 4,5. A porcentagem de ácido láctico do chucrute aumentou gradualmente até a estabilização, sem diferença significativa entre os tratamentos a partir do quarto dia de fermentação. Observou-se que nas condições avaliadas, NaCl e KCl foram eficazes na redução de coliformes a 35° C, onde a partir do oitavo dia de fermentação, foi detectada uma contagem de < 0.3 MPN.mL^{-1}. A contagem de bactérias láticas aumentou em 3 ciclos de log até o oitavo dia e, em seguida, foi reduzida em até 2 ciclos até o final do processo, onde não foi observado diferença significativa. Na avaliação sensorial, não houve diferença significativa para parâmetros de cor, sabor e textura de chucrute. De acordo com esses resultados podemos concluir que a substituição parcial de NaCl por KCl na produção de chucrute não alterou as características físico-químicas e microbiológicas, além de não ter influenciado negativamente nas características sensoriais do produto.

PALAVRAS-CHAVE: CHUCRUTE, PICLES, FERMENTAÇÃO LÁTICA, REDUÇÃO DE SÓDIO

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