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Replacement of Sodium Chloride by Potassium Chloride in Armenian Cucumber "*Cucumis melo* Var. Flexuosu" Pickles: Sensory and Microbiological Evaluation

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ABSTRACT
Pickles are considered as high salt food mainly due to the presence of high sodium ions added for taste and preservation purposes. This high amount will increase the risk of hypertension and cardiovascular diseases. In this study, an attempt was done to replace Sodium chloride (NaCl) by salt replacer which is potassium chloride (KCl) in Armenian cucumber pickles. Nine treatments were done with different salts percentages with and without chili pepper and garlic. All treatments were incubated at room temperature for 4 weeks then pH measurement, sensory analysis, and microbial analysis were performed. The pH values decreased with incubation and the lowest pH was 3.53±0.03 for Treatment 4 (75% KCl, 25%NaCl). Treatment 4 showed the highest reduction in both total plate count and yeast count with $78.5\pm3.0\times10^3$ CFU/ml and $73.0\pm3.0\times10^2$ CFU/ml respectively. There were virtually undetectable changes in taste up to 70% KCl (Treatment 6) substitution compared to Treatment 1(100% NaCl) based on the triangle test results. Based on 5-point hedonic scale, the lowest scores for taste were for Treatment 5 (100% KCl) with 2.70±0.40 but they were higher in the samples that contain chili pepper, while the other treatments showed insignificant differences compared to Treatment 1.The different treatments did not show any significant changes with respect to texture based on 5-point hedonic scale except the samples that contain both garlic and chili pepper which showed the lowest scores for taste and texture.

***Keywords*:** Hypertension, pickles, cardiovascular diseases, sodium chloride, potassium chloride.

Introduction

Pickles are one of the oldest preserved products. They are made through the natural fermentation of vegetables and fruits. Pickling is done in presence of high concentration of salt solution in which the vegetables or fruits are dipped to ensure fermentation. Sodium chloride (NaCl) is one of the most commonly employed substance for food preservation, allowing considerable increase in shelf life (Perez-Dıaz *et al.,* 2013).

"Armenian cucumber", botanically classified as *Cucumis melo var. flexuous,* is a slender and elongated type of vegetable (Stephens, 2015). The skin has no bitterness and it is almost always used without vegetables being peeled. It is actually a melon and not a cucumber and it is harvested annually during summer (Mabberley, 1997). Pickled "Armenian cucumber" is a staple food in Middle Eastern cuisine and it is marketed as "Pickled Wild Cucumber".

Salt is one of the required components in small amounts for the human body for controlling homeostasis and nerve impulses and it is mainly consists of two elements: sodium ions (Na^+) and chloride ions (Cl^-). Na^+ is mainly responsible for the saltiness in the food. Scientists have observed that the high sodium intake leads to many health hazards by increasing the risk of heart attack and high blood pressure (Dötsch-Klerk *et al*., 2015).

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The World Health Organization (WHO) recommends that adults consume less than 2 grams of sodium daily (5g of salt) (Powles *et al*., 2013). However, average global intake exceeds this level. Concerning the Lebanese population, the average sodium intake among Lebanese adults aged 20 years and above was estimated at 2.9 g/day which exceed the WHO maximum intake level (Battcock and Azam, 1998).

All the studies have demonstrated that dietary sodium reduction towards the WHO guidelines could lead to a significant reduction in blood pressure and cardiovascular disease risk (Hughes *et al.,* 2015). The dietary sodium is associated positively with blood pressure while dietary calcium and potassium intake are associated with maintaining and decreasing blood pressure (Pierre et al., 2005). The partial substitution of sodium chloride (NaCl) by potassium chloride (KCl) or calcium chloride ($CaCl_2$) seems to provide a measure for lowering sodium content in foods. Controlled increased potassium intake is reported to protect against stroke, high blood pressure, kidney failure, and heart rhythm problems (Hall, 2016). Currently, KCl is one of the most commonly used NaCl replacers as it has a good ability to convey the perception of a saltiness taste in food products (Aburto *et al.,* 2013).

As a result, potassium chloride has gained regulatory acceptance for use in food products in the United States and European Union and numerous other international scientific bodies and regulatory authorities (US Department of Agriculture, 2010). Replacement of NaCl by KCl would result in better compliance to the WHO potassium intake guideline (4.7 g of potassium per day). Therefore, based on the intake data, KCl is recommended as a valuable, safe replacer for sodium chloride in foods products (Buren *et al.,* 2016).

Therefore, the present work has been undertaken with the following objectives: To investigate the possibility of replacing sodium chloride by potassium chloride and produce low sodium "Armenian cucumber" pickles, to study the effect of NaCl substitution on both microbial and sensorial properties of pickles, to optimize salt mixture components for low sodium "Armenian cucumber" pickles without majorly affecting its microbiological and sensory qualities, and to assess primarily the effects of addition of chili pepper and garlic on masking the bitter taste of pure KCl.

Materials and Methods

1. Raw materials:

1. The Armenian cucumbers were purchased around June in 2017 and 2018 (peak season) from local market specifically for pickles.
2. Vinegar with 5% acetic acid was purchased from local supermarket.
3. Salts: Food grade sodium chloride was used for pickling and potassium chloride was from Nu-Salt® used without further purification.
4. Chili pepper and garlic were purchased from local market.

2. Preparation of pickles:

Different 8% brine solutions were prepared at a ratio of 1:2 fruits: brine (W/V) by boiling the drinking soft water followed by the addition of salt with different proportions as follows:

1. Treatment 1 (T1) : 8% sodium chloride
2. Treatment 2 (T2) : 6% sodium chloride + 2% potassium chloride
3. Treatment 3 (T3): 4% sodium chloride + 4% potassium chloride
4. Treatment 4 (T4): 2% sodium chloride + 6% potassium chloride
5. Treatment 5 (T5): 8% potassium chloride

Then vinegar was added to all the brine solutions. After cooling the brine solutions to about 40°C, the prepared solutions were distributed equally among different jars. Four jars were made for each treatment to increase precision. The vegetables were separated according to size (CODEX STAN 115-1981). A is for small size averaging 8-10 cm in length and B is for medium size averaging 10-16 cm in length. In addition to five jars prepared with diced vegetables (D) of 1cm width instead of whole vegetables (Stephens, 2015). Another 5 jars were made with both chili pepper and garlic (G+Pr) and additional 5 jars with just chili pepper (Pr) to study the effect of this addition on taste.

A space of 1 cm is left in jars as a headspace. Also all the vegetables were immersed in the brine and no floating on the surface was noticed. Jars were closed immediately and inverted upside down for 5 minutes.

A full set of new experiments were prepared only for sensorial analysis purpose in order to find the preferable combination where there is the highest amount of KCl with good sensorial evaluation. So, another 4 treatments each with 4 replications were done with the same procedures as above but with different proportions of salt as follows:

1. Treatment 6 (T6): 2.4%NaCl,5.6%KCl
2. Treatment 7 (T7): 2.8%NaCl,5.2%KCl
3. Treatment 8 (T8): 3.2%NaCl,4.8%KCl
4. Treatment 9 (T9): 3.6%NaCl,4.4%KCl

The prepared pickles were stored in sterile glass jars. During the entire storage period at room temperature it was ensured that the pickles were stored in aerated, dry, dark, and hygienic conditions.

pH measurement:

The pH was measured using standard pH meter (MARTINI instruments Mi 151) based on chapter 13 "pH and titratable acidity" in Food analysis book, 4th edition (2010) (Sadler and Murphy 2010).

Microbial count:

The colony forming units for bacteria, yeast, and molds were calculated using Bacteriological Analytical Manual 6th edition (FDA, BAM 1992).

Sensory analysis:

The sensory analysis was done using the Sensory Evaluation Techniques book (2007), 4th edition, using 12 trained panelists from School of Science, Lebanese International University. The different pickles samples were tested for texture and taste using 5-points hedonic scale. Triangle test method was used determine whether a sensory difference exists between two samples (Morten *et al*., 2007).

Cluster Analysis

This analysis was done to figure out grouping relationship for the results of pH, texture, and taste based on average linkage (Squared Euclidean distance) using the IBM SPSS statistics 23.

Statistical analysis:

Data for different parameters were analyzed using mean separation method (Duncan) using IBM SPSS statistics 23 (Morten *et al*., 2007).

Results and Discussion

1. Microbial Study

Yeast, gram positive, gram negative enterobacteria, LAB and others colonize the fresh vegetables and are present in the processing water and the environment (Perez-Dıaz et al., 2013). The results for the total plate count and yeast are found in table 1. Molds were not seen on the surface of the liquor and no scum formation was found during storage period. The colony forming units (CFUs) for all the treatments were within the critical limits for safe products (5×10^5) according to the International Commission on Microbiological Specifications for Foods (ICMSF 1986)**.** Concerning the total plate count, T3 (50%NaCl, 50%KCl) and T4 (25%NaCl, 75%KCl) showed the lowest Colony forming unit (CFU) with $79.5\pm3.0\times10^3$ CFU/ml and $78.5\pm3.0\times10^3$ CFU/ml respectively. On the other hand, there were insignificant differences among the CFUs of T1 (100% NaCl), T2 (75%NaCl, 25%KCl), and T5 (100%KCl). Concerning the yeast count, T3 and T4 showed less count comparing to other treatments with $73.30\pm3.0\times10^2$ CFU/ml and $73.00\pm3.0\times10^2$ CFU/ml respectively which follows the same pattern as the total plate count. Also, there were insignificant differences among T1, T2, and T5. The addition of garlic and chili pepper did not undermine the safety of the product. Also, the different sizes (A, B, and diced) gave similar results. During the fermentation process, lactic acid bacteria gain predominance by producing lactic acid which leads to reduced pH (Perez-Dıaz et al., 2013). While the other normal

microflora tend to decrease as fermentation is proceeding. In addition to various species of fermentative yeasts are also typically present during fermentation .The fermented vegetables may contain up to 10^5 of total aerobic count and up to 10^4 of yeast depending on the species of the vegetables (Perez-Dıaz et al., 2013). The results above matched with the results of Mateus *et al.* (2016). They concluded that a decline of enterobacteria population was found in samples that contain mixture of salts .So the presence of different chlorides in the samples will lead to a lower amount of microorganisms when compared to the samples that contain one type of salt (Youssef *et al*., 2017; Mani and Wilson 2017) .The combination of different salts will lead to a good microbiological quality that meets the standards of food safety.

Table 1: Average Values for Colony Forming Unit CFU/ml (mean ± SE)

Treatment	Total Plate Count	Yeast count	Molds
T1	$81.8\pm3.0\times10^{3}$ a*	$90.0\pm3.0\times10^{2}$ a	ND
T2	$81.7\pm3.0\times10^{3}$ a	$87.0\pm2.0\times10^{2}$ ab	ND
T3	$79.5\pm3.0\times10^{3}$ b	$73.3\pm3.0\times10^{2}$ c	ND
T4	$78.5\pm2.8\times10^{3}$ b	$73.0\pm2.0\times10^{2}$ c	ND
T5	$81.6\pm3.0\times10$ a	$90.0\pm3.0\times10^{2}$ a	ND

* Means with the same letter are not significantly different at $p<0.05$

pH measurement

Two measurements were done at 4th week and 8th week. Pickles are considered as high acidic food meaning that the pH shall be 4.6 or lower (Perez-Dıaz et al., 2013). Table 2 shows the pH values for all the treatment at 4th week and 8th week and all the treatments have pH lower than 4.6. T3 and T4 showed the lower pH after 4 weeks with 3.55±0.03 and 3.53±0.03 respectively. On the other hand, the pH values of T1, T2, and T5 were insignificantly different. So there is an increase in acidity (lower pH) in samples that contain high concentration of potassium. These results are similar to the results that were shown by Bansal and Rani (2014) that concluded that lower pH values were found in the samples that contain high amounts of potassium after 60 days of storage. After 8 weeks, the pH values were lower than the values of the 4th week. The same pattern of pH decrease was noticed, which means that T3 and T4 had lower values with 3.45±0.03 and 3.46±0.04 respectively.

Table 2: Average values for pH of different treatments after 4 weeks and 8 weeks (mean ± SE)

Treatment	pH at 4th week	pH at 8th week
T1	$3.60\pm0.03^{a*}$	3.50 ± 0.04^{a}
T2	3.60 ± 0.02^{a}	3.51 ± 0.05^{a}
T3	3.55 ± 0.03^{b}	3.46 ± 0.03^{b}
T4	3.53 ± 0.03^{b}	3.45 ± 0.04^{b}
T5	3.59 ± 0.03^{a}	3.49 ± 0.04^{a}

* Means with the same letter are not significantly different at $p<0.05$

In addition, there were insignificant differences among T1 and T2, and T5. In each treatment, the pH values were insignificantly different in samples with size A, size B, and diced vegetables meaning that the size did not affect the pH of the final product. Also, the addition of garlic and chili pepper did not change the pH results. As a conclusion, the pH will decrease with storage due to the production of lactic acid produced by the fermentation of the carbohydrates and other nutrients found by lactic acid bacteria (Perez-Dıaz et al., 2013). This low pH increases the shelf life of pickles by the inhibition of the growth of the pathogens that will cause spoilage.

Sensory Evaluation

Concerning triangle test, the numbers of correct answers were counted to determine to which point the substitution was undetectable. At T4 (75% KCl, 25% NaCl) there was significant difference in comparison with T1 (100% NaCl). The panelists did not detect any significant difference up to 70% KCl (T6) in comparing with 100% NaCl pickles. So the pickles that contain 70% KCl and 30% NaCl will have essentially the same taste as 100% NaCl pickles which means there is reduction of initial

sodium amount from 16 g to 5 g with undetectable changes. The same results were concluded in 25% (T2), 50% (T3), 55% (T9), 60% (T8), and 65% (T7) substitutions which means there were insignificant differences in comparison to 100% NaCl samples.

The results of the category scaling were based on 5-point hedonic scale for taste and texture. Table 3 shows the scores for the taste and texture for all the treatments. The lowest scores concerning the taste were for T5 (100% KCl) followed by T4 (75% KCl, 25% NaCl) with 2.70±0.40 and 3.70±0.41 respectively. There were insignificant differences among T1, T2, and T3. These results are compatible with those of Bansal and Rani, (2014) in lemon pickle and with Youssef *et al.* (2017) in cucumber and carrot pickles.

In all treatments, the lowest scores were for the samples that contain both garlic and chili pepper followed by the samples with chili pepper except treatment 5 (100% KCl) since the highest scores were for the samples that contain chili pepper and this may due to the masking of the bitter taste that is caused by KCl. These results are matching with the previous studies that tackled the usage of KCl as a substitution of NaCl in different kinds of food (Youssef *et al.,* 2017; Bansal and Rani 2014).Concerning the texture, there were insignificant differences among the treatments with lowest scores in samples that contain both garlic and chili pepper. As a result, using different combinations of salts will not affect the texture of pickles since it is an essential criteria for the acceptability of pickles so the softer the pickles the less the acceptability (Youssef *et al.,* 2017).

Table 3: Average values for 5-point hedonic scale concerning taste and texture (mean ± SE)

Treatment	Taste	Texture
T1	4.00±0.33^{a*}	4.00±0.35^{a}
T2	4.00±0.30^{a}	4.00±0.25^{a}
T3	3.80±0.30^{ab}	3.95±0.31^{a}
T4	3.70±0.41^{ab}	3.95±0.35^{a}
T5	2.70±0.40^{d}	3.95±0.33^{a}

* Means with the same letter are not significantly different at $p<0.05$

Cluster Analysis

The results of cluster analysis for all treatments standardized by z-score are shown in figure 1. This dendrogram based on average linkage (Squared Euclidean distance) contains the gradual clustering of all treatments based on sensory analysis and pH measurement.

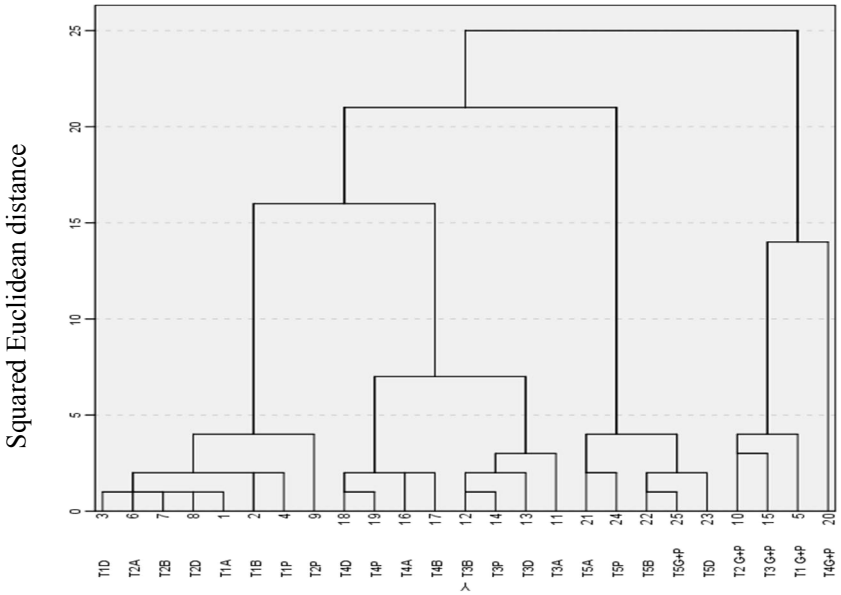

Fig. 1: Dendrogram based on average linkage (Squared Euclidean distance) for the different treatments based on pH, texture, and taste results

Five main clusters were determined on the basis of the cluster analysis using the SPSS software. T1 and T2 were grouping into one cluster. T3 and T4 formed two clusters that are near to each other. With the exception of the samples of T1, T2, T3, and T4 that contain garlic and chili pepper which formed a separated cluster with significant distance to the remaining clusters. T5 apparently formed a cluster which is further to T1 and T2 but near to T3 and T4.

This grouping confirms that T1 and T2 are somehow similar to each other concerning the pH, taste, and texture. Also, the same conclusion is for T3 and T4. T5 forms separated cluster which is nearer to T3 and T4. With the exception of the samples with garlic and chili pepper that were different comparing to the other treatments and this may due to the changes especially in taste that was detected after the addition of these two ingredients in the same sample.

Conclusion and Recommendation

Pickling is considered as one of the preservation methods for various foods such as vegetables and fruits. The main drawback is the high amount of sodium ions that will lead to many adverse effects on human health and cause different diseases such as hypertension. Based on this study, the effects of potassium chloride substitution in Armenian cucumber pickles that it is widely used in the Middle East are promising. Sodium chloride was substituted with potassium chloride up to 70% and initial sodium amount was reduced from 16 g to 5 g without any significant detectable changes in taste and texture and without affecting the safety of pickles. The addition of both garlic and chili pepper in the same samples didn't affect the safety of the product but it reduced the acceptability of these samples while the addition of chili pepper increased the scores for the 100% potassium chloride samples. Further investigation is needed to calculate shelf life of sodium substituted product, to improve the sensory properties of sodium substituted food by masking the bitterness of KCl using flavor enhancer such as glutamic acid E620, and to study in depth the effects of KCl on lactic acid bacteria's activities.

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