Title: Effect of different mineral salt mixtures and dough extraction procedure on the physical, chemical and microbiological composition of Şalgam: a black carrot fermented beverage

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1. Introduction

Every country has a variety of traditional products that are a part of their culture and eating habits, e.g. almagro eggplant (Spain), brovada and gioddu (Italy), tarhana (Turkey), kimchi (Korea), thua nao (Thailand), tofu (China), rabadi (India), kanji (India) and tempe (Indonesia) (Erten et al., 2014; Erten, Agirman, Boyaci-Gunduz & Ben Ghorbal, 2017; Mete, Çoşansu, Demirkol & Ayhan, 2017). Şalgam is a traditional lactic acid fermented beverage that is produced at industrial scale in Turkey and is well-known throughout the country. The main ingredient of Şalgam is black carrot (*Daucus carota* var. L.) and, for this reason, Şalgam is characterized by a red colour, cloudy appearance and sour-soft taste (Agirman & Erten, 2018; Okcu, Ayhan, Altuntas, Vural & Poyrazoglu, 2016).

Şalgam is recognised as a beverage exerting positive effects on digestion process. However, this beverage also possesses functional properties, due to the presence of strong antioxidant agents (Ekinci, Baser, Özcan, Güçlü-Üstündağ, Korachi & Sofu et al., 2016). Although Şalgam is recognised to contain carbohydrates, organic acids and amino acids knowledge about the nutritional value of this product is limited. Only a few studies in the literature describe the composition of Şalgam. This product is reported to contain 0.29 g/L of total carbohydrates, 3.29-4.12 g/L of ethanol, 20.7-36.4 g/L total phenolic compounds, 0.69-0.80 g/L volatile acidity, 11.59-12.02 g/L of salt and 0.88-1.83 g/L of proteins (Ekinci, Baser, Özcan, Güçlü-Üstündağ, Korachi & Sofu et al., 2016; Tanguler, Gunes & Erten, 2014), but no information are available on vitamin and mineral composition.

In recent years, there has been an increased effort to reduce the level of sodium salt in foods. An excessive intake of sodium into the human body increases blood pressure (hypertension) as well as the risk of stomach cancer and deterioration of thirst metabolism (Agarwal, Fulgoni III, Spence & Samuel, 2015; Kloss, Meyer, Graeve & Vetter, 2015). WHO recommends a reduction to <2 g/day sodium in adults to reduce the risk of stroke and coronary heart disease. Health-related authorities suggest using potassium, magnesium and calcium minerals to replace the sodium salt. According to an industrial survey, sodium chloride has an avarage content of 14.04 g NaCl/L in Şalgam (equivalent to 5.51 g sodium/litre Şalgam). Therefore, a glass of Şalgam of 250 mL supplies approximately 70% of the recommended daily intake for sodium (WHO, 2012). Considering Şalgam consumption in Turkey, it is necessary to decrease the sodium concentration of this product.

With this in mind, the aim of the present work was to: (i) decrease the sodium level in Şalgam by partially replacing sodium chloride with potassium and calcium chloride, (ii) study the effect of different chloride salts on the physico-chemical and microbiological profile of Şalgam and (iii) deepen the knowledge on the composition of Şalgam juice.

2. Material and methods

2.1.Materials and reagents

Black carrots, bulgur flour (setik) and turnips were purchased from a market located in Adana city (Turkey). The standards used to determine the concentration of chemicals were: lactic acid (Sigma-Aldrich, St. Louis, MO, USA), acetic acid (Sigma-Aldrich, Taufkirchen, Germany), citric acid (Sigma-Aldrich, Taufkirchen, Germany), glucose (Sigma-Aldrich, Taufkirchen, Germany), fructose (Sigma-Aldrich, Taufkirchen, Germany), sucrose (Sigma-Aldrich, Taufkirchen, Germany) and ethyl alcohol (Sigma-Aldrich, Taufkirchen, Germany). Standards used for the determination of the mineral content of Şalgam were: sodium (Merck KGaA, Darmstadt, Germany), potassium

(Merck KGaA, Darmstadt, Germany), magnesium (Merck KGaA, Darmstadt, Germany), phosphorus (Merck KGaA, Darmstadt, Germany) and calcium (Merck KGaA, Darmstadt, Germany).

2.2. Fermentation of Şalgam using different chloride salts

Şalgams were produced at the Biotechnology Laboratory of Food Engineering Department at Cukurova University (Adana, Turkey). Şalgam production was carried out in two stages using the traditional flow diagram shown in Fig. 1 (Erten & Tanguler, 2016). The first stage, called “sourdough fermentation” with a duration of 4 d, enriches the mass with lactic acid bacteria (LAB) and yeasts. After that, sourdough was extracted three times with sufficient water. The second stage is known as “carrot fermentation” where the extracts from the sourdough mass are mixed with black carrots, different chloride salts (NaCl, KCl and $CaCl_2$), sliced turnips and adequate drinking water. A fermentation vessel of 30-L volume capacity was used to carry out the experimental productions. The fermentation was carried out at room temperature for 8 d and monitored by titratable acidity level; titratable acidity was determined daily and the fermentations were considered concluded when no increase in the amount of total acidity was registered for two consecutive days. Five different trials were set up for Şalgam production, including control production with NaCl alone and the following experimental trials: (i) NaCl-KCl (50% + 50%); (ii) NaCl-$CaCl_2$ (50% + 50%); (iii) KCl-$CaCl_2$ (50% + 50%); (iv) NaCl-KCl-$CaCl_2$ (33.3% + 33.3% + 33.3%).

2.3. General chemical analysis

The measurements of pH and total acidity of raw materials were determined by a Seven Compact S220 (Mettler Toledo, Leicester, United Kingdom) model pH meter. Dry matter and ash analysis of black carrot and bulgur flour was carried out using the method recommended by the TSE (2016). Total dry matter and ash content were determined by a Venticell 222 (MMM Medcenter, München, Germany) model ventilated oven and Protherm PLF 110/6 (Alser Technic, Ankara, Turkey) model ash furnace, respectively. The phenol-sulfuric acid method was used for the detection of the total sugar content of the raw materials according to Amrane and Prigent (1996). Mohr method was used to determine the salt level of Şalgam produced using different chloride salts (Nielsen, 2017).

2.4. Quantitation of phenolic compounds

The determination of total phenolic compounds was performed by Folin–Ciocalteu method. The absorbance of centrifuged and clarified samples was measured at 765 nm on a UV/VIS spectrophotometer (Perkin Elmer Lambda 25, Waltham, MA, USA). The amount of total phenolic compounds was determined using the standard graph prepared with gallic acid and the results were given as milligrams gallic acid equivalents per litre of Şalgam (mg GAE/L) (Ainsworth & Gillespie, 2007).

The sodium bisulphite method was used to determine the total monomeric anthocyanin (TMA) level of black carrots and Şalgam samples. Results were obtained by applying the difference in optical density between the Şalgam sample added with bisulphite and the sample lacking bisulphite to the standard curve. Reading was performed at 520 nm and 700 nm on a zeroed UV/VIS spectrophotometer (Perkin Elmer Lambda 25, Waltham, MA, USA) against purified water. The results for TMA were expressed as mg cyanidin-3-glucoside equivalents per litre of Şalgam (Giusti & Wrolstad, 2005).

2.5. Colour measurement analysis (L, a*, b*, C*, H*)*

Colour values were determined according to CIELAB colour system by Colour Quest XE (HunterLab, Virginia, USA) model device. A standard box (CQ X3708) including light trap, black card device, white tile and diagnostic tile was used for standardization. The samples were analysed by transmittance (TTRAN mode) using an optically-clear glass cell with a fixed path length of 10 mm. The illuminant and observer area were selected as D65/10°. Approximately, 30 mL of liquid Şalgam samples were placed into a special optical cell with flat parallel surfaces and colour values were measured directly after standardization.

L*, a* and b* values were measured. The L* value indicates darkness to brightness in vertical axes, the a* value indicates green to red and the b* value indicates the blue to yellow colour. Additionally, hue (h°) angle and chroma (C^*_{ab}) values were calculated according to Hunter and Harold (1987): h° = arctan (b*/a*); C^*_{ab} = sqrt (a^{*2}+b^{*2}).

2.6. Determination of organic acids, sugars and ethanol using HPLC

The amount of organic acids (lactic, acetic and citric) was determined using a LC-20AT model high performance liquid chromatography (HPLC) (Shimadzu, Tokyo, Japan) system. The system comprised a quaternary pump, a column temperature control oven (CTO-10AS), an auto sampler unit (SIL-20A), a degasser module (DGU-20A5) and a photodiode array detector (SPD-M20A). Organic acid measurements were performed at a wavelength of 210 nm and a Waters-X-Terra-MS, C18 (5 µm, 4.6 x 250 mm, Ireland) column was used.

LC-20AD model HPLC system (Shimadzu, Tokyo, Japan) combined with a dual-pump, a column oven (CTO-10AS), a degasser module (DGU-20A5) and refractive index detector (RID-10A) was used for the determination of glucose, fructose, sucrose and ethyl alcohol in samples. An Aminex-HPX-87H (300 x 7.8 mm, Bio-Rad, Richmond, CA, USA) column maintained in column oven at 50 °C and mobile phase (H_2SO_4 solution) with a concentration of 0.01 N was used for sugar and ethyl alcohol analyses.

The mobile phase was used at a flow rate of 0.5 mL/min. Şalgam samples were centrifuged at 8,000 × g for 15 min at 4°C and then two-stage filtration (a 0.45 µm and then 0.22 µm filter, Millipore, Germany) was applied through PTFE filter (Tanguler & Erten, 2012a). The external standard method was used for the quantitative determination of organic acids, ethyl alcohol and sugars. Peak identifications were carried out according to standard spectrums and elution time for organic acids, sugars and ethyl alcohol. HPLC analyses were carried out in duplicate.

2.7. Isolation and identification of lactic acid bacteria

Culturable LAB were collected from dough fermentation, extract of dough and Şalgam during fermentation at day 0, 2, 4, 6 and 8). Enumeration, isolation and purification of LAB were carried out at 30 °C for 48 h on MRS and M17 (Merck, Darmstadt, Germany) for rod and coccus shaped species, respectively. ~~Counts of LAB were presented in previously published manuscript (Agirman & Erten, 2018).~~ Presumptive LAB were checked by means of shape, color, elevation, surface and edge. After collecting at least three colonies per each of the morphology observed, Gram and catalase reactions were tested. Gram positive, catalase negative cultures were purified by sequential culturing and then stored in glycerol (40 % v/v) in – 80 °C. The entire process of LAB collection, purification and preliminary characterization was performed as described by Alfonzo et al. (2017).

Cells were harvested after 24 h incubation in M17 and MRS broth medium at 30 °C and washed two times with sterile ultra pure water. Genomic DNAs were extracted by Insta Gene Matrix Kit (Bio-Rad, Hercules, USA) according to manufacturer's instructions. DNAs were quantified by Qubit 3.0 Fluorometer (Thermo Fisher, Waltham, MA, USA). Random amplification of polymorfic DNA (RAPD-PCR) method was used to differentiate the isolates at strain level. To this purpose, M13 (5’-GAGGGTGGCGGTTCT-3’), AB106 (5’-TGCTCTGCCC-3’) and AB111 (5’-GTAGACCCGT-3’) primers were employed (Gaglio et al., 2017). Amplification reactions were carried out by AG 22331 model thermocycler (Eppendorf, Hamburg, Germany).

PCR products were seperated by electrophoresis on 1.5 % agarose gel and visualized by Infinity VX2 model UV transulliminator (Vilber Lourmat, Collégien, France) after staining with Syber® Safe DNA Gel Stain (S33102) (Thermo Fisher, Waltham, MA, USA). 100 bp (Thermo Fisher, Waltham, MA, USA) DNA ladder was used as molecular size marker. RAPD-PCR profiles were analysed with the pattern analysis software MEGA-X. Unweighted pair group method with arithmetic average (UPGMA) clustering algorithm was used to obtain dendograms. Bootstrap method was used as test of phylogeny.

Three isolates sharing the same RAPD-PCR profile were subjected to 16S rRNA gene sequencing using the primers fD1 (5’ AGAGTTTGATCCTGGCT 3’) and rD1 (5’ AAGGAGGTGATCCAGCC 3’) primers as described by Weisburg, Barns, Pelletier and Lane (1991). Sequencing data were edited in BioEdit 7.2 sequence alignment editor software and compared using the sequence database on Nucleotide Basic Local Alignment Search Tool with BLAST search in Genbank/EMBL/DDBJ database.

2.8. Mineral analysis by ICP-OES

2.8.1. Instrumentation

The determination of sodium (Na), potassium (K), calcium (Ca), magnesium (Mg) and phosphorus (P) was carried out using ICP-OES for Şalgam samples. ICP-OES measurements were achieved using a model Optima 2100 DW ICP-OES (Perkin Elmer, Wellesley, USA) inductively coupled plasma optical emission spectrometer. Employed automatic dual viewing (DV) ICP-OES was equipped with a charged coupled device detector (CCD) and cross-flow gem tip nebulizer. Instrument conditions for mineral determination by ICP-OES are presented in Table S1.

2.8.2. Samples and standard preparation

At the end of fermentation, 10 samples were taken from each trials and analysed to determine the mineral concentrations. The samples were diluted 1:2 with 0.1 M nitric acid solution which brought the samples and standards to the same concentration. The samples were then centrifuged for 15 min at 4,000 × rpm by using Universal 320/320R centrifuge (Hettich, Tuttlingen, Germany). Before analysis, all vials, cups and glasswares were cleaned by soaking in HNO_3 (10% v/v) solution, and then rinsed with tap water followed by deionized water and then with an acid solution. Finally, all apparatuses were rinsed again 4-5 times with deionized water. Teflon digestion vessels were rinsed with acetone and washed with deionized water. Furthermore, vessels were covered with 0.1 M HNO_3 for 30 min followed by rinsing with deionized water and left to dry.

Working standards were prepared daily in polypropylene vials by suitable dilutions of a 1,000 mg/L stock solution. Single element standard solutions of Na, K, Mg, Ca and P were used for ICP-OES measurement. Ultra-pure water was used for the preparation of all solutions (Paneque, Morales, Burgos, Ponce & Callejón, 2017). Samples and standard solutions for the the calibration curve were diluted using 5% v/v HNO_3. Water acidified with nitric acid at a ratio of 5% v/v HNO_3 was used as the calibration blank.

2.8.3. Digestion procedure

Microwave acid-digestion was applied in order to minimize the troubles (i.e. sample contamination) related with sample pre-treatment and transfer the whole of the analytes into solution. The acid digestion of the samples was performed in three stages using a Speedwave MWS-2 model microwave oven (Berghof, Eningen, Germany). To this purpose, 2 mL of each sample were transferred into a polytetrafluoroethylene (PTFE, Teflon) vessel. Subsequently, 2 mL of 65% HNO_3 and 1 mL of 35% H_2O_2 were added to the Şalgam samples which were digested into a microwave under the conditions given in Table S2. The digested solutions were transferred to 25 mL volumetric flasks and ultra-pure water was used to adjust the final volume. The blank was digested using the same procedure.

2.9. Statistical analysis

A one-way analysis of variance (ANOVA) test was used to compare the results of minerals, organic acids, sugars, ethyl alcohol, colour, total phenol, TMA, pH, salt, total acidity, ash and total dry matter analysis between the different salt treatments. Duncan multiple comparisons was applied as a post-hoc test to determine significant differences using the software SPSS.20. A p value of <0.05 was considered significant.

3. Results and discussion

3.1. Composition of raw materials

Black carrots and setik were characterized by a pH of 6.39 and 5.95, respectively. As expected, total acidity was very low levels in black carrots (0.94 g/kg) and setik (3.45 g/kg) (Table 1). The levels of dry matter, ash and total sugars of setik were higher than those registered in black carrots. The sugar level of raw materials has to be significant in order to supply nutrients to the microorganisms during fermentation. From this perspective both raw materials used in Şalgam production showed that the total amount of sugars of the mixture was adequate to support the growth of LAB and yeasts. The TMA level of black carrots was detected as 606.9 mg/kg in terms of cyanidin-3-glucoside. The amount of anthocyanin determined in this study was in the range of total anthocyanin content reported in literature for black carrot (10 - 980 mg/kg) (Kammerer, Carle & Schieber, 2004).

Lactic acid was not found in black carrot, while acetic acid and citric acid were detectd at 0.57 and 2.70 g/kg, respectively. So far, no information on the content of organic acids in black carrots have been published. Regarding sugars, glucose, fructose and sucrose levels of the black carrots were recorded at a total amount of 41.79 g/kg. Indeed, the amount of sugars found in black carrots varies considerably depending on the cultivar and the cultivation region. To support this opinion Kammerer et al. (2004) stated that the total saccharides level of black carrot roots can be high or low, which is in accordance with documented data varying from 59 to 520 g/kg dry matter. Erten and Tanguler (2016) indicated that the main soluble fermentable sugars of black carrots cultivated in Turkey were sucrose (49.95-331 g/kg), glucose (6.66-56.4 g/kg) and fructose (6.65-43.6 g/kg). Finally, no ethyl alcohol was detected in the samples of black carrots.

[Table 1 near here]

3*.2. Evaluation of fermentation and results of basic analysis*

Data on acidification (Table 2) showed that carrot fermentation carried out in this study terminated more rapidly than other studies, since the process was considered completed after 8 d. Previously, Tanguler et al. (2014) reported a fermentation duration of 11 days for the second step of Şalgam production. However, Erten et al. (2017) indicated that the total fermentation period can vary from 13 to 25 d depending on the temperature and other factors. Therefore, it is clear that the dough extraction process used in this study shortened the second fermentation step, which is an important result for the application at industrial scale level.

The acidity level of Şalgam is one of the most important sensory properties for consumers. Another important feature of this product is represented by its salt content, which directly affects the preference for Şalgam. The pH level of Şalgam produced using different chloride salts ranged between 3.26 to 3.48, the total acidity, as lactic acid, between 7.40 to 8.71 g/L and salt, in terms of sodium chloride, between 1.50 and 1.61%. Values obtained in this study were compatible with the Turkish Şalgam Standard which was recently revised in 2016 (Table 2).

3.3. Total phenolic compounds and the total monomeric anthocyanin level of Şalgams

Phenolics belong to the anthocyanin group and there is a correlation between phenolics and anthocyanin content (Paneque et al., 2017). The total phenolic content of Şalgams ranged from 627.0 to 748.5 (mg GAE/L) in this study ($p>0.05$). The lowest total phenolic content was determined in Şalgam produced using the salt combination $KCl+CaCl_2$ while the highest level was obtained in presence of $NaCl+CaCl_2$ (Table 2). Total phenolic content detected in our study is higher than the results of Ekinci et al. (2016) who reported a total phenolic content of 517.21 mg GAE/L for Şalgam.

Black carrots, due to their colour pigments, represent one of the most significant natural food colourants (Erten & Tanguler, 2016). In this context, the purplish red colour of the Şalgam beverage comes from anthocyanins, which are the most abundant group of pigments found in black carrots. The TMA content of Şalgam samples ranged from 198.4 mg/L to 238.4 mg/L as cyanidin-3-glucoside equivalent ($p>0.05$). It can be inferred that the TMA content of black carrots is transferred to Şalgam at an average of

36%, which is a quite low ratio ($p<0.05$). The total anthocyanin level found in this study is higher than the results of Turker, Aksay, Istanbullu and Artuvan (2007) and Tanguler (2010) who found the anthocyanin level to be between 27.9 mg/L to 205.4 mg/L for Şalgam produced at commercial and laboratory scale. These differences are imputable to the different ratios between black carrots and other ingredients used for Şalgam production as well as the amount of anthocyanins of the roots.

3.4. Colour scores of Şalgam

The effect of using different chloride salts on the colour character was assessed during the production of Şalgam. A low value (0-50) on the L* scale indicates a dark colour, while the a* scale indicates red when it has a positive number. L* values ranged from 18.75 to 20.94 and a* values were between 50.38 to 53.15 in the Şalgam produced in this study. Thus, the results of L* and a* values showed that whole Şalgam produced under the conditions of this study has its own characteristic colour, as the typical colour of Şalgam seems to be dark red. The b* values, another dimension for colour representation, changed limitedly from 32.06 to 35.84. The positive values of b* in all Şalgam samples indicated that there is also yellowness in the red colour of Şalgam samples. The h° value is attributed to colour perception by means of which an object is judged to be red, yellow, green, blue or purple, while the C^*_{ab} parameter indicates the saturation (Yuksel & Koca, 2008). C^*_{ab} and h° values in the present study ranged from 59.75 to 64.20 and 32.42 to 33.98, respectively. Tanguler et al. (2014) ascertained the h° values to be between 14.73 and 23.79 and C^*_{ab} values between 34.84 and 37.70, which are lower in comparison to the present study.

The highest values of L*, a* and b* were observed in Şalgam produced combining NaCl and KCl in equal proportions, although the lowest values for all properties were found in the Şalgam trial produced without sodium salt (KCl+$CaCl_2$). Şalgam produced with NaCl+KCl salts exhibited a better quality than the control trial in terms of colour due to the intense redness and saturated colour properties. On the other hand, the only experiment in which the sodium salt was not used (KCl+$CaCl_2$) had the lowest grade of redness, yellowness, darkness and hence, saturation. Consequently, the decrease in the colour standard of Şalgam with no NaCl salt added appears to be obvious. Another important finding is that the use of KCl salt resulted in an increased colour quality. In summary, NaCl and KCl contributed to the colour of the Şalgam beverage. This effect was previously revealed by Bautista-Gallego, Arroyo-López, López- López and Garrido-Fernández (2011) on fermented olives using equal proportions of KCl and NaCl or $CaCl_2$.

3.5. Organic acids, sugars and ethanol composition of Şalgam

Homofermentative and heterofermentative LAB form lactic acid as the primary end product of glucose fermentation via the Embden Meyerhof Parnas and pentose phosphate pathways (Erten & Tanguler, 2016). In addition, some other metabolic products, such as acetic acid, citric acid and ethanol, can also be produced during fermentation. In the present study, the amount of lactic acid in Şalgam samples was found to be at least 5.90 times higher than the quantity of acetic acid and citric acid (Table 2). The lactic acid content ranged from 7.43 g/L to 8.26 g/L and the highest concentration was observed in Şalgam produced using a combination of the three different salts. However, the lowest lactic acid concentration was found in the control sample. The results of HPLC analyses indicated that the amounts of lactic acid were compatible with the minumum value as 4.5 g/L lactic acid which was specified in the Şalgam standard. On the other hand, the level of two minor organic acids, acetic and citric acid, ranged from 0.43 g/L to 0.90 g/L and 0.71 g/L to 1.26 g/L, respectively

($p<0.05$). The content of the three organic acids was comparable with those reported by the study of Ekinci et al. (2016) who found the level of lactic acid, acetic acid and citric acid at 8.90 g/L, 1.29 g/L and 1.25 g/L, respectively. However, the amounts of lactic acid and citric acid in this study were significantly higher than the results of Tanguler and Erten (2012b) reporting a lactic acid concentration between 2.66 - 4.74 g/L. Table 2 shows that the highest acetic and citric acid values were obtained in the experiment using the three salts together, while the lowest citric acid content was found in Şalgam produced using only the NaCl salt. Furthermore, the lowest acetic acid content was found in the Şalgam produced with NaCl+$CaCl_2$ salts, followed by the control trial. As a result of the organic acid values, the use of potassium and calcium salts in Şalgam production has a greater effect on acid formation, homo- and heterofermentation than sodium salt alone. The obtained data were in agreement with Panagou, Hondrodimou, Mallouchos and Nychas (2011) who reported that the use of KCI and $CaCl_2$ determined a higher lactic acid formation than NaCl during olive fermentation.

[Table 2 near here]

The residual sugar content of Şalgam indicated that the fermentation successfully completed. Additionally, the majority of consumers prefer Şalgam with a high acid content and low sugar in terms of sensory properties; hence, sugar free or minimal sugar content is desirable in the end product. Sucrose was detected as the main sugar in Şalgam fermented using different chloride salts with its concentration being at least 29.65% higher than glucose and fructose (Table 2). The sucrose content of Şalgam ranged from 160.71 mg/L to 295.57 mg/L, while glucose from 69.11 mg/L to 123.96 mg/L and fructose from 67.07 mg/L to 101.34 mg/L ($p>0.05$). It is remarkable that the lowest value for these three sugars was determined in Şalgam produced using the three salts together. The content of sucrose and glucose in the tested Şalgam was higher than what reported in literature (sucrose 75 mg/L and glucose 41 mg/L), while fructose was lower (104 mg/L) (Ekinci et al., 2016). In another comparative study, five different commercially produced Şalgam samples were examined and the sugar content was generally found to be higher than that found in the present study. Glucose ranged from 117 mg/L to 1,902 mg/L and fructose from 60 mg/L to 1,310 mg/L in the study of Tanguler and Erten (2012b).

Besides lactic acid, ethanol can be produced in small amounts during Şalgam fermentation. In this study, the level of ethanol produced barely ranged from 3.98 g/L to 4.42 g/L ($p<0.05$). These results are highly comparable with the ethanol concentrations recorder in other studies (Tanguler & Erten, 2012b; Tanguler et al., 2014).

3.6. Species distrubition of LAB in Şalgam

A total of 184 LAB belonging to three different genera and thirteen species or sub-species were identified from Şalgam fermentations (Table 3). Different species were found in different samples as shown by the dendogram (Fig. 2). Some LAB species detected in this study (*Leuconostoc mesenteroides* subsp. *jonggajibkimchii*, *Lactococcus lactis* subsp. *cremoris*, *Lactobacillus coryniformis* subsp. *coryniformis* and *Lactobacillus paraplantarum*) were reported to be associated to Şalgam fermentation for the first time. Two acid tolerant species, *Lactobacillus paracasei* and *Lactobacillus plantarum*, were found to be dominant among the LAB community representing 36.40 and 26.70% of total isolates, respectively (Table 4). Dominant microorganisms were followed by *Lactobacillus paracasei* subsp. *tolerans* (9.78%), *Leuconostoc mesenteroides* subsp. *jonggajibkimchii* (7.60%) and *Lactobacillus brevis* (5.97%).

[Table 3 near here]

[Figure 2 near here]

Lactobacillus paracasei, *Lactobacillus plantarum* and *Lactobacillus brevis* species were isolated from all different salt experiments while newly reported heterofermentative LAB species *Leuconostoc mesenteroides* subsp. *jonggajibkimchii* was not found only in the trial containing NaCl + KCl. *Lactobacillus casei* subsp. *casei*, *Lactobacillus coryniformis* subsp. *coryniformis* and *Lactobacillus pentosus* were found only in control Şalgam. Productions with NaCl + KCl salt combination showed the highest number of isolates followed by KCl + $CaCl_2$, NaCl +KCl + $CaCl_2$, control and NaCl +$CaCl_2$, respectively. Extract liquid obtained from dough fermentation included 19.6% of the total isolates. Dough fermentation determined a good starter for carrot fermentation, since the liquid extracts contained high numbers of LAB, responsible for the rapid termination of the second fermentation. Therefore, the application of the extraction process led to produce Şalgam in a shorter time in comparison to Şalgam produced with the direct method or dough fermented in pouch without the extraction process. Although *Leuconostoc mesenteroides* was not isolated during Şalgam fermentation, it was found both in dough and extracts. This species was detected at the beginning of the fermentation but was no more found due to its low acid tolerance (Erten & Tanguler, 2016). Recently, *Lactococcus lactis* subsp. *cremoris* strains are preferred to continual use in food fermentation (Gaglio et al., 2016) for their superior contribution to product flavor via unique metabolic mechanisms (Vieira, Cabral, da Costa Lima, Paschoalin, Leandro, & Conte-Junior, 2017). Five *Lactococcus lactis* subsp. *cremoris* strains were detected in dough, extract and Şalgam fermented with KCl+$CaCl_2$ and NaCl+KCl+$CaCl_2$. *Lactobacillus paraplantarum*, closely related to *Lactobacillus plantarum* group, was isolated from Şalgams fermented with NaCl+KCI and NaCl+$CaCl_2$ salt combinations. Eighteen *Lactobacillus paracasei* subsp. *tolerans* and two *Lactobacillus plantarum* subsp. *argentoratensis* species were identified in this study and this finding similarly have been reported by the study of Ekinci et al. (2016) with regard to Şalgam. *Lactococcus lactis*, which is effective in various vegetable fermentation, was isolated from all Şalgam experiments except control group, dough and extraction liquid.

[Table 4 near here]

The deepen microbiological investigation clearly showed that *Lactobacillus paracasei* and *Lactobacillus plantarum* are the typical species of Şalgam fermentations, because comparison with previous works confirmed this trend (Tanguler, 2010; Erten & Tanguler, 2016). *Lactobacillus coryniformis* subsp. *coryniformis* was also isolated in this study, but also at the end of fermentation of the control Şalgam trial.

3.7. Mineral content of Şalgam including different chloride salts

Minerals such as calcium, magnesium, potassium, sodium and phosphorus, which are naturally present in the structure of raw materials used in Şalgam production, contribute to the mineral content of the beverage (İncedayı, Uylaşer & Çopur, 2008).

Salts containing different minerals were used to reduce the amount of Na in Şalgam. Major (Na, K, Ca) and minor (Mg, P) mineral concentrations were determined at ppm level (Table 2).

The sodium level was 6,148.50 mg/L ($p<0.05$), potassium 806.40 mg/L ($p<0.05$), calcium 159.90 mg/L ($p<0.05$), magnesium 99.225 mg/L ($p<0.05$) and phosphorus 78.485 mg/L ($p>0.05$) in control Şalgam. The level of minerals inherently found in Şalgam could be estimated according to the results of the control Şalgam. The ratio of sodium salt found naturally in Şalgam can be determined based on the results of mineral analyses of Şalgam produced without NaCl. The sodium content was determined at

181.05 mg/L in Şalgam produced with the $KCl+CaCl_2$ salt combination. According to this result, the most common mineral in Şalgam was potassium followed by sodium and calcium, which is in the same order as the amount of minerals found in black carrots. The composition of magnesium and phosphorus were comparable between control samples and Şalgams produced with the different salt combinations. This is expected as magnesium or phosphorus salts were not added during the production of Şalgam in this study. On the other hand, significant differences were expected between the amounts of sodium, calcium and potassium minerals according to the different experiments. The highest potassium content was 4,773 mg/L in the trial using a combination of NaCl+KCl, while the highest calcium content was 2,821 mg/L and was detected for the Şalgam produced with $KCl+CaCl_2$. The control experiment was the trial which had the greatest amount of sodium mineral. It is noteworthy that the ICP-OES results showed great consistency with the amount of salts added at the beginning of the production stage. This statement is supported by the findings of Bautista-Gallego et al. (2011) who reported that Na and Ca content of olive produced via different chloride salts depended mainly on the initial salt concentration.

According to the mineral results it can be concluded that Şalgam beverage produced via different chloride salts appear to be rich in minerals, in particular potassium, calcium and sodium. These chloride salts have been reported to meet many significant functions in the body (Ursell, 2001). Therefore, it is important to highlight that Şalgams produced via application of different mineral salts supply a critical part of the daily mineral demand. For an adult, the daily mineral requirements are 3 g for potassium, 500 mg for calcium and 500 - 2,500 mg for sodium (Ursell, 2001). Thus, one glass of Şalgam per day (about 250 mL) when produced with low sodium content will meet the need of these three minerals.

To our knowledge, there is no comparative study in the literature on minerals in the Şalgam beverage. However, there are a few studies concerning the mineral composition of black carrots, with Uzel (2017) recently reporting the nutrients present in black carrots. According to this study, the sodium level in black carrots was 690 mg/kg, potassium 3,200 mg/kg, calcium 330 mg/kg, magnesium 120 mg/kg, phosphorus 350 mg/kg, iron 3 mg/kg, zinc 2.4 mg/kg and manganese 1.43 mg/kg.

Taste of food stuffs plays a crucial role in the final quality definition due to its impact on the acceptability by consumers. In this study, the reduction and replacement of sodium chloride with potassium and calcium salts greatly influenced the sensory properties of the end-product. The effect of utilization of different chloride salts on the sensory parameters of Şalgam were discussed in a previously published paper (Agirman and Erten, 2018) showing that Şalgam juice fermented with 0.85% NaCl–0.85% KCl mineral salt combination received the best sensory results among the different salt substitutions. Furthermore, the results also showed that calcium chloride determined a bitter taste of şalgam, resulting in an unacceptable product. Hence, $CaCl_2$ was not found to be suitable for the production of şalgam (Agirman and Erten, 2018).

4. Conclusions

In summary, this study revealed the effect of different chloride salts (KCl, $CaCl_2$) on Şalgam fermentation and product composition. All Şalgams were characterized by a chemical composition in accordance with the specifications given by the Turkish Şalgam Standard. The extraction process applied during production accelerated the second fermentation and shortened the production process. The use of KCl with NaCl in Şalgam production led to an improvement in product colour. It is clear from the results that the use of potassium and calcium salts had a positive effect on the formation of lactic acid during Şalgam fermentation. From the microbiological point of view, *Lactobacillus paracasei* and *Lactobacillus plantarum* dominated the fermentation process. According to the results of the mineral composition, if no external salt is added, the most abundant mineral found in Şalgam is potassium. From the health point of view, the results of this study indicated that potassium salt should replace sodium salt in Şalgam production.

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Fig. 1. Şalgam production using the traditional method

Fig. 2. Cluster analysis of RAPD-PCR patterns obtained with three primers for LAB strains isolated from Şalgam fermented with different mineral salts.
The evolutionary history was inferred using the UPGMA method. The optimal tree with the sum of branch length = 0.38856776 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the

number of base substitutions per site. This analysis involved 13 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1564 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.