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Influences of Chloride Salts on Enzymatic Activity, Lipid Oxidation and Volatile Compounds of Reduced-Sodium Salt Pastırma

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Abstract

The study investigated the effects of chloride salts (control: 100% NaCl, salt mixture I: NaCl/KCl (50/50), salt mixture II: NaCl/KCl/$CaCl_2$ (40/40/20), salt mixture III: NaCl/KCl/$CaCl_2$/$MgCl_2$ (30/40/20/10)) on enzymatic activity, lipid oxidation, and volatile compounds in reduced-sodium salt pastırma, a Turkish dry-cured meat product. Lipid oxidation and instrumental color values were not affected by different salt mixtures. Salt mixtures II and III decreased pH value ($p < 0.05$). However, the mean pH value did not fall below 5.5 in any sample. The salt mixture treatment had significant effect on water activity, cathepsin B, and cathepsin B + L. In contrast, a_w value was under 0.90 in all treatments. The highest mean values for cathepsin B and B + L were determined in the control group with 11.69 ± 2.73 and 85.82 ± 12.65 U $g^{-1} \times 10^{-3}$ dry matter, respectively. The closest correlation for lipolytic enzyme activities was determined by the mixture II and III groups, while a closer correlation was observed between salt mixtures I and III in terms of proteolytic enzyme activities. With regard to volatile compounds, there was a closer relationship between the control and salt mixture I. As a result, it can be concluded that salt mixture I in reduced-sodium salt pastırma showed closer results to the control group.

Keywords: pastırma; salt; chloride salt; cured meat product; cathepsin; volatile compound

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1. Introduction

Pastırma is a Turkish dry-cured meat product manufactured by subjecting whole pieces from water buffalo or beef carcasses to different processing steps such as curing (dry), drying, pressing, and coating with çemen (a kind of paste consisting of mashed fresh garlic, fenugreek (*Trigonella foenum graecum*) seed flour, red pepper, and water) [1]. In order to finalize production, the moisture level should be below 50%, and salt (NaCl) content should not exceed 10% in dry matter without çemen coating [2].

Salt constitutes, quantitatively, the largest proportion of additives in the pastırma curing process. It has a significant role in providing the sensory and technological properties of pastırma [3]. It has a controlling effect on the proteolysis and lipolysis phenomena in whole processed dry-cured meat products, leading to the taste and flavor formation [4,5].

Muscle enzymes have an important effect on these reactions. Among proteolytic enzymes, cathepsins have great importance in proteolysis with their activity, especially on myofibrillar proteins. Lipolytic enzymes (mainly acid lipase, phospholipase, acid esterase, and neutral esterase) affected by salt content and water activity usually show high activity at the beginning of production [6]. Lipid oxidation is another important phenomenon in flavor formation in ripened meats [3,6]. However, salt can accelerate lipid oxidation due to its prooxidative effect, and excessive lipid oxidation shows a negative effect on flavor [7]. In addition to all these, the effect of the salt content on the texture and flavor is dependent on product type [8]. The ingoing salt level used in traditional pastırma production can reach up to 10% [3,9,10]. Although ripened meats such as pastırma are highly valued by consumers for their unique taste and flavor, they are considered a major cause for concern due to their high sodium content [6]. Due to the relationship between excessive sodium intake and high blood pressure, cardiovascular diseases, and other related disorders, effective sodium replacement is needed to consistently reduce sodium intake [11–14]. For the purpose of achieving a low sodium content in meat products, various approaches were tested in different studies, and the use of different chloride salts has been outstanding within these treatments [15–19]. Similar approaches were also applied to pastırma made with salt levels higher than 5% [12,20]. Yalınkılıç et al. [12] investigated the effect of dichloride salts on the product properties in traditional pastırma production at a 10% NaCl level. Hastaoğlu and Vural [20] examined the effect of using KCl to reduce the sodium content of traditionally salted pastırma under natural and controlled drying conditions. However, in the meat industry, the production of pastırma under controlled conditions is increasing day by day and salt is usually used at 5% ratio [1,21,22]. No studies have yet been conducted on the effect of partially replacing NaCl with other chloride salts on the quality of properties in reduced-sodium salt pastırma, which is produced with a low salt level, such as 5%. Thus, the study aimed to determine the effects of partial replacement of NaCl with other chloride salts on the enzymatic activity, volatile profile, and lipid oxidation of reduced-sodium salt pastırma manufactured under controlled conditions.

2. Materials and Methods

2.1. Materials

M. longissimus thoracis et lumborum muscles of four cattle carcasses (ultimate pH < 5.8) obtained from the Meat and Dairy Board, Erzurum, Türkiye, were used for pastırma production. Each muscle was cut into two pieces, resulting in a total of sixteen pieces of meat.

2.2. The Production of Pastırma

First, four different salt mixtures were prepared: the control (100% NaCl), mixture I (NaCl/KCl (50/50)), mixture II (NaCl/KCl/$CaCl_2$ (40/40/20)), and mixture III (NaCl/KCl/$CaCl_2$/$MgCl_2$ (30/40/20/10)). The curing mixtures were then prepared for each treatment: 50 g salt or salt mixture, 2 g sucrose, 0.15 g KNO_3, and 0.10 g $NaNO_2$ per kg of meat pieces. Before the curing stage, excess connective tissue on the surface of the pieces was removed, and incisions were made on the pieces at a 45° angle. The meat pieces were rubbed and covered with the curing mixture at 6 ± 1 °C for 48 h. After they were hung at 15 ± 1 °C and 80 ± 2% relative humidity (RH) for 6 days (first drying) in a controllable climatic ripening chamber (Reich, Thermoprozestechnik GmbH, Schechingen, Germany), the pieces were pressed using a press machine (Yıldızlar Machine, İstanbul, Türkiye) at 7 ± 1 °C, following which they were dried again at 20 ± 1 °C and 70 ± 2% RH for 5 days (second drying). Meat pieces were pressed again at 25 ± 1 °C for 7 h and then they were dried again at 20 ± 1 °C and 70 ± 2% RH for 4 days. The dried meat pieces were then covered with çemen paste (500 g fenugreek (*Trigonella foenum graecum*) seed flour, 300 g

dried red pepper, 450 g smashed fresh garlic, and 1500 mL water) and kept in the çemen at 7 °C for 1 day before being dried again.

2.3. Determination of pH, a_w, Thiobarbituric Acid Reactive Substance Content (TBARS) and Color

For analyzing pH, a 10 g sample was mixed with 100 mL of distilled water for 1 min by Ultra-Turrax (Werk T 25, IKA, Staufen im Breisgau, Germany), and measurement was performed by a pH meter (ATI Orion 420, San Francisco, CA, USA). Before using the pH meter, it was calibrated with buffer solutions (pH 4.0 and 7.0). Water activity (a_w) was measured by a water activity device (TH-500, Novasina, Lachen, Switzerland) at 25 °C. The instrument was calibrated with 6 different salt solutions before use.

For TBARS analysis, 12 mL of trichloroacetic acid solution (0.1% EDTA, 0.1% propyl gallate and 7.5%TCA) was added to a 2 g sample and homogenized. Then, it was filtered through a filter paper (Whatman 1); thiobarbituric acid solution (0.02 M) was added to 3 mL of the filtrate. The mixture was left in a boiling water bath for 40 min and cooled. After centrifugation (5 min at 2000× *g*), the absorbance was measured at 530 nm. The TBARS value was given as μmol MDA kg^{-1} [23]. Color measurement (*L**, *a**, and *b**) was performed using a chroma meter (Konica Minolta, Osaka, Japan) in the inner surface of 2 mm thick pastırma slices.

2.4. Enzyme Activities

Cathepsin H, B, and B + L activities were fluorometrically (Perkin Elmer, Norwalk, CT, USA) measured [24]. The amount of enzyme that hydrolyzes 1 μmol of substrate per min at 37 °C was shown as one unit of enzyme activity (U).

The activities of phospholipase, acid lipase, and neutral lipase were measured fluorometrically according to the method of Motilva et al. [25] with slight modifications in the analysis of neutral lipase. An autohydrolysis was detected in 4-methylumbelliferyl oleate at pH 7.5 in the neutral lipase assay. To begin the neutral lipase assay, four different wells containing the reaction buffer and substrate without enzyme extract were used as blanks. Following the neutral lipase analysis with crude enzyme extract, the mean enzyme activity was detected after the subtraction of the results of blank testing. The enzyme activity unit (U) was defined as the amount of enzyme hydrolyzing 1 μmol substrate per hour at 37 °C.

2.5. Determination of Volatile Compounds

The method given by Kaban [3] was used for the determination of volatile compounds. For extraction of the compounds, vials containing 5 g homogenized samples were incubated at 30 °C for 1 h in a thermal block (Supelco, Bellefonte, PA, USA), and the fiber (carboxen/polydimethylsiloxane, 75 μm, Supelco, Bellefonte, PA, USA) was inserted into the headspace for 2 h. After adsorption, the fiber was injected into gas chromatography (GC, Agilent Technologies 6890 N, Santa Clara, CA, USA)/mass spectrometry (MS, 5973, Agilent Technologies, Santa Clara, CA, USA). DB-624 (J&W Scientific, 60 m × 0.25 mm i.d. × 1.4 μm film) was used as a column, and helium was used as the mobile phase at a flow rate of 1 mL min^{-1}. The temperature program was started when the fiber was inserted, and ran as follows: at 40 °C for 5 min and then the oven temperature was increased from 40 °C to 110 °C at 3 °C/min, then to 150 °C at 4 °C/min, and finally to 210 °C at 10 °C/min, where it was held for another 12 min. Quantification was performed on the basis of a total or single ion chromatogram on an arbitrary scale (eV). The GC/MS interface was maintained at a temperature of 280 °C. Mass spectra were obtained using electron impact at 70 eV, and data were acquired across the 30–400 amu range. Volatile compounds were identified by matching the mass spectrometry library (NIST, WILEY and FLAVOR), standard mix (Supelco 44585-U, Bellefonte, PA, USA for calculating of Kovats indices), and retention indices of compounds in the literature. Results were given as AU × 10^6.

2.6. Statistical Analysis

In the study, the four experiments were carried out according to a randomized complete block design, namely salt mixture treatment (the control, salt mixture I, II, and III) with four replicates for each treatment. The results of analyses were assessed by ANOVA using a general linear model. Salt mixture treatment was the main effect for all independent parameters, and the replicates were used as random effect. Duncan's multiple range test was also performed at the $p < 0.05$ level for the comparison of differences between means by SPSS version 24 statistical software (Chicago, IL, USA). In addition, clustering analysis was performed using the Chiplot program [26] to determine the relationship between chloride mixtures and volatile compounds, and between chloride mixtures and enzyme activities.

3. Results and Discussion

The effects of salt mixture on water activity (a_w), pH, and TBARS of pastırma is given in Table 1. There was a significant effect of the salt mixture treatment on the a_w value ($p < 0.05$). The a_w values for mixtures I, II, and III were higher compared to the control, but the difference between mixture I and the control was not statistically significant. This effect exhibited by different chloride salts may be due to different degrees of hydration of the salts, as stated by Liu et al. [27]. On the other hand, the fact that $CaCl_2$ and $MgCl_2$ have more difficulty penetrating the inner parts of the muscle compared to NaCl may also have enabled the current water activity results to be achieved [17]. In pastırma, a_w has a significant hurdle effect. In the present study, although the salt mixture significantly affected the a_w value, the values were below 0.90 in all treatments. However, it is recommended that water activity does not fall below 0.85 for the sensory properties to maintain optimal [3]. As a result, the salt mixtures used may be considered a good alternative for further reducing sodium content in reduced-sodium salt pastırma without risking product safety.

Table 1. The effects of chloride salts on the water activity, pH, TBARS (μmol MDA/kg), instrumental color values of pastırma (mean ± standard deviation).

Treatment	a_w	pH	TBARS	*L**	*a**	*b**
Control	0.866 ± 0.015 b	5.88 ± 0.02 b	29.58 ± 8.31 a	36.76 ± 1.53 a	36.00 ± 0.81 a	20.00 ± 1.90 a
Salt Mixture I	0.877 ± 0.003 ab	5.93 ± 0.03 a	26.13 ± 3.83 a	38.21 ± 2.22 a	36.27 ± 2.18 a	21.87 ± 2.59 a
Salt Mixture II	0.882 ± 0.017 a	5.54 ± 0.04 c	29.75 ± 4.36 a	36.99 ± 1.74 a	36.71 ± 0.73 a	21.30 ± 1.72 a
Salt Mixture III	0.891 ± 0.010 a	5.57 ± 0.02 c	20.79 ± 3.89 a	40.12 ± 2.58 a	36.25 ± 2.64 a	23.20 ± 0.89 a
Significance	*	**	ns	ns	ns	ns

a–c: Any two means in the same column having the same letters in the same section are not significantly different at $p > 0.05$, *: $p < 0.05$, **: $p < 0.01$. ns: not significant; Control: NaCl (100); Salt Mixture I: NaCl/KCl (50/50); Salt Mixture II: NaCl/KCl/$CaCl_2$ (40/40/20); Salt Mixture III: NaCl/KCl/$CaCl_2$/$MgCl_2$ (30/40/20/10).

The pH value of pastırma was significantly affected by the salt mixture treatment ($p < 0.01$). As shown in Table 1, 50% KCl substitution (salt mixture I: NaCl/KCl (50/50)) in the curing mixture caused an increase in the pH value. This finding was also observed in other studies on dry-cured lacon [28], Jinhuan ham [29], pastırma [20], and dry-cured foal Cecina [18]. In contrary, the use of KCl (NaCl/KCl (50/50)) did not cause a significant change in the pH of the traditional pastırma produced with 10% NaCl compared to the control [12]. On the other hand, as shown in Table 1, the mixtures containing $CaCl_2$ and $MgCl_2$ (salt mixtures II and III) caused a decrease in pH value. Divalent salts are known to decrease the pH value of dry-cured meat products, and our results are in agreement with the findings in the dry-cured foal Cecina [18] and traditionally produced pastırma [12]. Also, Vidal et al. [30] reported that the jerked-beef group containing $CaCl_2$ showed a lower pH value than the control and NaCl + KCl (50/50) groups. Similar results were observed in salted meat products by Vidal et al. [31]. Although changes in the pH value of pastırma

were observed in our study, the pH did not decrease below 5.5 in any group. In fact, Kaban [3] reported that the pH value of pastırma should not decrease below 5.5 in terms of sensory properties (>5.5).

Lipid oxidation is an important phenomenon in whole processed meat products such as bacon, dry-cured ham, lacon, and pastırma [32,33]. In these products, the TBARS value increases depending on the ripening time. Lipid oxidation is directly influenced by many factors such as nitrite, pH, heavy metals, and storage conditions, and NaCl is also considered to be an important factor in this oxidation [7,33]. High salt levels generally promote lipid oxidation, but the degree of substitution, especially by sodium substituents, can modulate this effect [34,35]. In this study, however, the substitution of NaCl did not have any effect on TBARS (Table 1). This result demonstrates that the amount of salt used and the ratio of monovalent and divalent salts in the salt mixtures are appropriate for the product. In other studies that were conducted on pastırma made using the traditional method, it was also indicated that the substitution of NaCl does not induce the oxidation of the lipid [12,20]. In another study by Cittadini et al. [18], it was found that $CaCl_2$ in the salt mixture increased lipid oxidation in foal Cecina. However, in the same study, lower value for TBARS was observed in the group with the NaCl/KCl (50/50) mixture.

The instrumental color parameters (L^*: lightness (brightness), ranges from 0 (black) to 100 (white); a^*: redness–greenness axis, with positive value: red, negative value: green; b^*: yellowness–blueness axis, with positive value: yellow, negative value: blue) were determined in the sliced sample, and no statistical differences were observed between salt mixture treatments for all color parameters (Table 1). Alino et al. [36] and Hastaoğlu and Vural [20] also reported that chloride salts had no significant effect on instrumental color values in dry-cured loin and pastırma, respectively. In contrast, Yalınkılıç et al. [12] stated that the salt mixtures with $CaCl_2$ or $CaCl_2$ + $MgCl_2$ decreased the a^* value of traditional pastırma produced with an ingoing salt level of 10%. In addition, it has also been reported by Vidal et al. [30] that the intensity of red color in dried beef made by wet and dry curing decreases in the presence of KCl or $CaCl_2$. These differences in color values are thought to depend on the amount of salt used as well as the proportions of chloride salts in production.

The enzyme activities of pastırma groups were showed in Table 2. No statistical difference was found between the control and other groups for each lipolytic enzyme activity ($p > 0.05$). In a study using chloride salts such as $MgCl_2$, KCl, and $CaCl_2$ in traditional pastırma production (initial salt level 10%), it was found that the neutral lipase values were similar to our findings, but the acid lipase and phospholipase activity were significantly affected by chloride salt replacement [12]. In a study by Ripolles et al. [15] in which KCl, $CaCl_2$, and $MgCl_2$ salts were used in dry-cured ham, the use of different chloride salts was found not to have a significant effect on the acid lipase value. Similarly, in a study carried out on dry-cured loin salted with KCl, no significant difference was detected in neutral lipase and acid lipase activities, except for phospholipase which was affected by KCl [29].

The use of different chloride salts in the curing mixture was found to affect the cathepsin B and B + L activities of the samples significantly ($p < 0.01$). Cathepsin H activity was under the detectable level in each treatment. The results on cathepsin H activity were observed to be consistent with results of previous studies as determined in dry-cured ham [16] and traditional pastırma [12]. However, it is emphasized that cathepsin H was the most sensitive to water activity [37]. Cathepsins B and L, due to notable stability during processing, are regarded as the primary endopeptidases accountable for muscle proteolysis and the development of flavor in dry-cured ham [38]. The highest cathepsin B and cathepsin B + L values of the samples were detected in the samples with only 100% NaCl ($p < 0.05$), and it was determined that the modification of the curing mixture with

alternative chloride salts caused a decrease in the proteolytic enzyme activity of the samples. Similar results were also reported by Yalınkılıç et al. [12] in traditionally produced pastırma, where the cathepsin B activity decreased due to the use of different chloride salts except for the group containing KCl and $CaCl_2$, and the cathepsin B + L values were lower in each group compared to the control. It was determined that the use of KCl in the production of dry-cured loin caused an increase in cathepsin B and cathepsin B + L activities [39]. However, Liu et al. [27] found that the use of KCl in dry-cured beef caused a decrease in cathepsin B and cathepsin B + L activities. On the other hand, Armenteros et al. [16] found that there were no differences between the dry cured ham samples produced with KCl, $MgCl_2$, and $CaCl_2$ groups in terms of cathepsin B and cathepsin B + L values.

Table 2. The effects of chloride salts on the proteolytic and lipolytic enzyme activities of pastırma (mean ± standard deviation).

Treatment	Cathepsin B	Cathepsin B + L	Cathepsin H	Acid Lipase	Neutral Lipase	Phospholipase
	U g^{-1} DM × 10^{-3}			U g^{-1} DM		
Control	11.69 ± 2.73 a	85.82 ± 12.65 a	nd	1.12 ± 0.11 a	0.60 ± 0.34 a	0.64 ± 0.08 a
Salt Mixture I	7.22 ± 1.93 b	47.75 ± 14.44 c	nd	1.09 ± 0.06 a	0.45 ± 0.32 a	0.68 ± 0.02 a
Salt Mixture II	7.29 ± 1.75 b	65.49 ± 16.54 b	nd	1.05 ± 0.15 a	0.39 ± 0.20 a	0.58 ± 0.13 a
Salt Mixture III	4.97 ± 1.74 c	47.87 ± 10.16 c	nd	1.07 ± 0.18 a	0.39 ± 0.16 a	0.61 ± 0.16 a
Significance	**	**	ns	ns	ns	ns

a–c: Any two means in the same column that has the same letters in the same section are not significantly different. ns: not significant; **: $p < 0.01$; nd: not detected; Control: NaCl (100); Salt Mixture I: NaCl/KCl (50/50); Salt Mixture II: NaCl/KCl/$CaCl_2$ (40/40/20); Salt Mixture III: NaCl/KCl/$CaCl_2$/$MgCl_2$ (30/40/20/10).

The results of the lipolytic (a) and proteolytic (b) enzyme activities of the pastırma groups were evaluated using heatmap analysis (Figure 1). Two main clusters were formed for both lipolytic and proteolytic enzymes, and the control group and the groups containing other chloride salts were separated from each other. The closest correlation for lipolytic enzyme activity was determined by the mixture II and III groups. On the other hand, a closer correlation was observed between salt mixtures I and III in terms of proteolytic enzymes. According to these results, the use of different salt mixtures causes changes in enzyme activities.

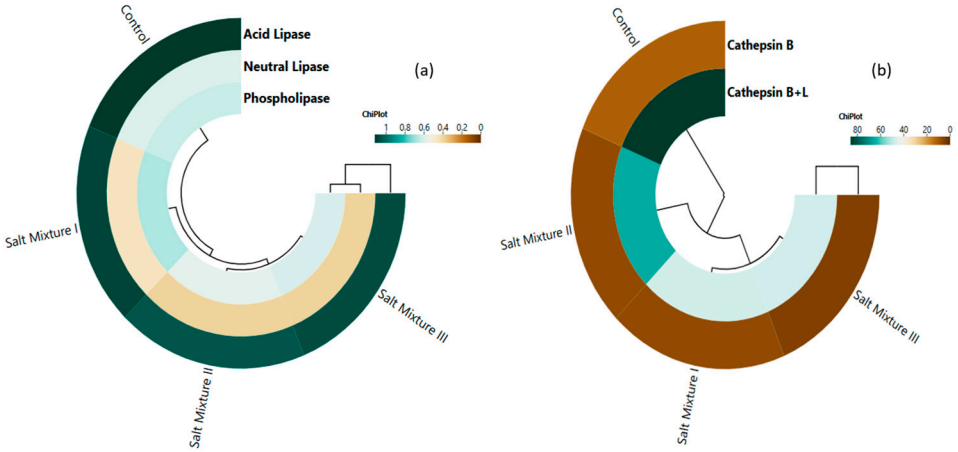

Figure 1. Cluster analysis of the relationship between salt mixtures and lipolytic (**a**) and between salt mixtures and proteolytic enzyme activities (**b**).

Fifty volatile compounds were detected in the pastırma samples (Table 3). The use of different chloride salts was found to affect eight volatile compounds at a significant ($p < 0.05$) level and one at a very significant ($p < 0.01$) level. While the volatile compound profile consists of nine different chemical groups, the most abundant chemical groups

were aldehydes and sulfur compounds, respectively. In a study where KCl, $CaCl_2$, and $MgCl_2$ were used in traditional pastırma production, aldehyde and sulfur compounds were determined as the volatile groups that had the highest areas [12]. In contrast to these findings, ketone and acid groups had the highest abundance in a study on dry-cured meats salted with KCl and $CaCl_2$ [40]. The differences between our research findings and those found by Nachtigall et al. [40] are probably due to the raw material, process conditions, and çemen coating. As a matter of fact, it was determined by Kaban [3] that çemen coating, which is a mixture of different spices, garlic, and *Trigonella foenum graecum* flour, affects the chromatographic area of many volatile compound groups, especially sulfur compounds.

Table 3. The effects of chloride salts on the volatile profile of pastırma (mean ± standard deviation) (Au × 10^6).

Compounds	RI	KI	Control	SM I	SM II	SM III	S
Aldehydes							
Acetaldehyde	b	<500	7.91 ± 4.52 a	9.97 ± 11.82 a	10.41 ± 11.96 a	9.46 ± 6.79 a	ns
Pentanal	a	742	5.21 ± 1.26 a	6.66 ± 1.95 a	7.07 ± 1.65 a	5.56 ± 0.78 a	ns
2-methyl-2-butenal	c	788	3.63 ± 1.51 b	6.77 ± 2.57 a	4.97 ± 0.98 b	4.40 ± 1.12 b	*
Hexanal	a	837	79.94 ± 29.78 a	100.79 ± 36.67 a	102.16 ± 20.55 a	82.27 ± 17.47 a	ns
2-Hexenal	c	895	1.07 ± 0.81 a	0.73 ± 0.64 a	0.97 ± 0.25 a	0.99 ± 0.30 a	ns
Heptanal	a	955	3.50 ± 1.23 a	4.31 ± 0.84 a	4.03 ± 0.54 a	4.06 ± 0.54 a	ns
2-Heptenal	c	1024	1.75 ± 0.54 a	2.70 ± 0.29 a	2.20 ± 0.45 a	2.04 ± 0.12 a	ns
Benzaldehyde	a	1026	44.17 ± 8.16 a	54.94 ± 28.56 a	34.41 ± 21.23 a	26.40 ± 8.33 a	ns
Octanal	a	1053	6.62 ± 1.53 a	6.29 ± 0.97 a	6.98 ± 1.54 a	8.67 ± 3.00 a	ns
2,4-Heptadienal	c	1086	0.58 ± 0.10 a	1.34 ± 1.11 a	0.76 ± 0.11 a	0.79 ± 0.26 a	ns
2-Octenal	c	1120	5.54 ± 1.31 a	6.07 ± 1.10 a	6.19 ± 1.83 a	6.24 ± 1.63 a	ns
Nonanal	b	1144	14.88 ± 2.93 a	20.63 ± 3.78 a	19.14 ± 2.49 a	26.57 ± 8.79 a	ns
2-Nonenal	c	1228	2.91 ± 0.63 a	4.30 ± 1.40 a	2.87 ± 0.69 a	3.85 ± 0.58 a	ns
2,4-Nonadienal	c	1263	2.80 ± 3.76 a	3.49 ± 4.19 a	3.45 ± 3.31 a	2.36 ± 0.84 a	ns
2,4-Decadienal	c	1422	0.36 ± 0.24 a	0.87 ± 0.24 a	0.64 ± 0.52 a	0.89 ± 0.75 a	ns
Ketones							
2,3-Butanedione	a	630	3.44 ± 0.76 a	2.80 ± 2.87 a	4.24 ± 3.35 a	5.80 ± 1.58 a	ns
3-Hydroxy-2-butanone	b	779	3.48 ± 2.63 a	4.56 ± 2.29 a	5.99 ± 2.51 a	6.87 ± 2.18 a	ns
2-Heptanone	c	946	1.36 ± 1.09 a	1.24 ± 0.24 a	0.96 ± 0.13 a	1.96 ± 0.48 a	ns
2,5-Octanedienone	c	1031	17.30 ± 19.07 a	17.32 ± 12.03 a	13.39 ± 6.24 a	8.32 ± 5.68 a	ns
6-Methyl- 5-hepten-2-one	c	1049	2.04 ± 1.19 a	1.66 ± 0.52 a	1.29 ± 0.30 a	1.65 ± 0.32 a	ns
2-Octanone	c	1050	0.49 ± 0.57 ab	0.86 ± 0.71 a	0.35 ± 0.42 ab	0.00 ± 0.00 b	*
3-Octen-2-one	c	1114	2.59 ± 0.90 a	4.04 ± 1.04 a	2.74 ± 0.61 a	3.17 ± 0.46 a	ns
3,5-Octadien-2-one	c	1158	1.44 ± 0.72 a	2.87 ± 1.40 a	1.79 ± 0.44 a	2.63 ± 2.14	ns
Alcohols							
Ethanol	a	539	11.99 ± 8.09 a	10.16 ± 5.03 a	13.29 ± 8.27 a	13.11 ± 6.89 a	ns
1-Hexanol	a	930	1.69 ± 0.79 a	3.18 ± 1.16 a	1.97 ± 0.73 a	1.92 ± 1.42 a	ns
Sulfur compounds							
Allyl methyl sulfide	b	730	2.71 ± 0.50 a	4.32 ± 0.67 a	2.90 ± 1.94 a	3.46 ± 0.64 a	ns
3,3′-thiobis-1-propene	b	889	14.48 ± 7.31 a	24.77 ± 6.49 a	16.05 ± 4.89 a	20.83 ± 2.79 a	ns
Methyl 2-propenyl disulfide	c	957	0.82 ± 1.64 a	0.67 ± 1.34 a	1.26 ± 1.53 a	1.21 ± 1.42 a	ns
Diallyl disulphide	a	1138	70.51 ± 21.59 a	103.37 ± 26.85 a	73.35 ± 15.34 a	79.00 ± 9.04 a	ns
Methyl-2-propenyl trisulfide	c	1210	1.50 ± 0.59 a	2.70 ± 1.38 a	2.97 ± 1.08 a	2.81 ± 0.45 a	ns
Esters							
Ethyl acetate	a	639	3.34 ± 2.57 a	2.50 ± 3.08 a	2.69 ± 3.13 a	4.12 ± 1.02 a	ns
2,4-Hexadienoic acid, methyl ester	c	1075	0.37 ± 0.47 b	0.93 ± 0.51 b	1.04 ± 0.36 ab	1.63 ± 0.60 a	*
Butanoic acid, hexyl ester	b	1216	2.10 ± 1.50 a	1.55 ± 0.68 a	1.19 ± 0.31 a	1.74 ± 1.49 a	ns
Furans							
2-Butyl furan	c	924	0.61 ± 0.46 ab	0.19 ± 0.37 b	0.96 ± 0.22 a	1.13 ± 0.16 a	**
2-Pentyl furan	b	1021	3.13 ± 1.19 b	3.77 ± 1.05 ab	4.78 ± 1.07 a	4.48 ± 0.15 a	*
Terpenes							
D-Limonene	a	1054	2.39 ± 0.67 a	3.75 ± 1.27 b	3.36 ± 0.72 b	3.57 ± 0.66 b	*
Carvone	c	1332	4.75 ± 2.49 a	4.43 ± 1.40 a	4.81 ± 1.26 a	4.54 ± 0.55 a	ns
Copaene	c	1434	1.06 ± 0.82 a	2.13 ± 1.13 a	1.91 ± 0.73 a	1.78 ± 0.59 a	ns
Caryophyllene	c	1490	0.93 ± 0.86 a	2.13 ± 1.13 a	1.91 ± 0.73 a	1.78 ± 0.59 a	ns
Aromatic hydrocarbons							
1-Methyl-3-(1-methylethyl)-benzene	c	1060	3.31 ± 1.44 b	5.33 ± 1.88 a	4.76 ± 1.09 a	5.31 ± 0.74 a	*
1,2-Dichlorobenzene	c	1068	2.31 ± 0.67 a	3.18 ± 2.21 a	3.53 ± 0.62 a	3.99 ± 1.14 a	ns
3-vinyl-1,2-dithiacyclohex-4-ene	c	1244	2.25 ± 1.52 a	2.07 ± 0.88 a	1.51 ± 0.45 a	2.71 ± 1.08 a	ns

Table 3. *Cont.*

Compounds	RI	KI	Control	SM I	SM II	SM III	S
Phenol	c	1249	1.37 ± 1.14 a	0.84 ± 1.68 a	1.28 ± 1.76 a	1.71 ± 1.49 a	ns
1-methoxy-4-(1-propenyl)- benzene	c	1251	0.82 ± 0.96 a	0.74 ± 0.86 a	0.38 ± 0.75 a	0.00 ± 0.00 a	ns
Aliphatic hydrocarbons							
1,3-Pentadiene	c	567	1.39 ± 2.52 b	2.21 ± 2.03 b	10.19 ± 2.92 a	13.85 ± 7.68 a	*
2-Hexene	c	1051	1.04 ± 0.24 a	1.94 ± 0.88 a	1.51 ± 0.60 a	1.45 ± 0.34 a	ns
3-Ethyl-2-methyl-1,3-hexadiene	c	1094	0.61 ± 0.22 b	1.39 ± 0.47 a	0.80 ± 0.22 b	0.98 ± 0.21 ab	*
Dodecane	a	1200	0.75 ± 0.07 a	1.06 ± 0.59 a	0.53 ± 0.05 a	0.61 ± 0.58 a	ns
Tridecane	a	1300	2.98 ± 2.99 a	3.13 ± 3.29 a	1.63 ± 0.26 a	2.28 ± 0.74 a	ns
Tetradecane	a	1400	0.92 ± 0.39 a	1.33 ± 0.84 a	0.89 ± 0.45 a	1.75 ± 1.38 a	ns

Any two means in the same column that has the same letters in the same section are not significantly different. ns: not significant; *: $p < 0.05$; **: $p < 0.01$; Control: NaCl (100); SM I: NaCl/KCl (50/50); SM II: NaCl/KCl/$CaCl_2$ (40/40/20); SM III: NaCl/KCl/$CaCl_2$/$MgCl_2$ (30/40/20/10). Results are expressed in arbitrary area units ($\times 10^6$); RI: reliability of identification; a: mass spectrum and retention time identical with an authentic sample; b: mass spectrum and Kovats index from the literature in accordance; c: tentative identification by mass spectrum; KI: Kovats index calculated for DB-624 capillary column (60 m × 0.25 mm × 1.4 µm) installed on a gas chromatograph equipped with a mass selective detector.

The use of KCl was detected to be effective on 2-methyl-2-butenal and 2-octanone ($p < 0.05$). These compounds were found at the highest level in samples with the NaCl/KCl mixture (Table 3). On the contrary, Yalınkılıç et al. [12] detected higher 2-methyl-2-butenal in traditional pastırma salted with KCl/$CaCl_2$/$MgCl_2$. Although this compound could not be identified in other dry-cured meat products produced with different chloride salts [28,40,41], among the aldehydes, the hexanal was the most abundant compound in all groups (Table 3). Hexanal, nonanal, and pentanal, whose main precursors are assumed to be oleic and linoleic fatty acids, are the most abundant aldehydes in whole processed cured meat products. Of these, hexanal is considered to be an important indicator of lipid oxidation and although it contributes to the overall aroma of these products, its excessive presence can cause off-flavors [42]. In another study in which 2-octanone was detected, this compound was detected at the highest level in dry-cured hams with NaCl/KCl [41]. While the alcohol and sulfur compounds were not affected ($p > 0.05$), the use of chloride salts significantly affected the 2-pentyl furan and 2-butyl furan compounds. It is possible to state that the use of $CaCl_2$ and $MgCl_2$ increases the level of volatile compounds in the furan group (Table 3). The data obtained for the 2-pentyl furan were in accordance with the literature [12,28,40]. On the other hand, the addition of alternative monovalent and divalent salts to the curing mixture increased the level of 2,4-hexadienoic acid methyl ester, 1-methyl-3-(1-methylethyl)-benzene, and D-limonene compounds ($p < 0.05$). It was determined that the use of KCl in dry-cured Jinhua ham increased the abundance of the D-limonene compound in the final product [29]. In contrast, an increase in D-limonene level was reported by Yalınkılıç et al. [12] in traditional pastırma salted with $CaCl_2$ and $MgCl_2$. While the level of 3-ethyl-2-methyl-1,3-hexadiene increased ($p < 0.05$) with the use of KCl, divalent salts also significantly increased ($p < 0.05$) the 1,3-pentadiene ratio in this group. In contrast, no significant effect of the salt treatment was found on the 3-ethyl-2-methyl-1,3-hexadiene compound detected in traditionally produced pastırma salted with different chloride salts [12]. In studies on dry-cured meat products salted with other salts [12,28,29,40], it can easily be seen that the effect of monovalent and divalent salts on the volatile compound profile is quite different, and the number of compounds and the compound groups have different distributions. These differences are probably derived from differences in raw material, process conditions, production time, product type, etc.

Concerning the volatile compounds, two main clusters were observed: the first one contained the control and SMI, and the second one contained SMII and SMIII. These results

show that SMI group has the closest volatile compounds values to the control in pastırma samples produced with different salt mixtures (Figure 2).

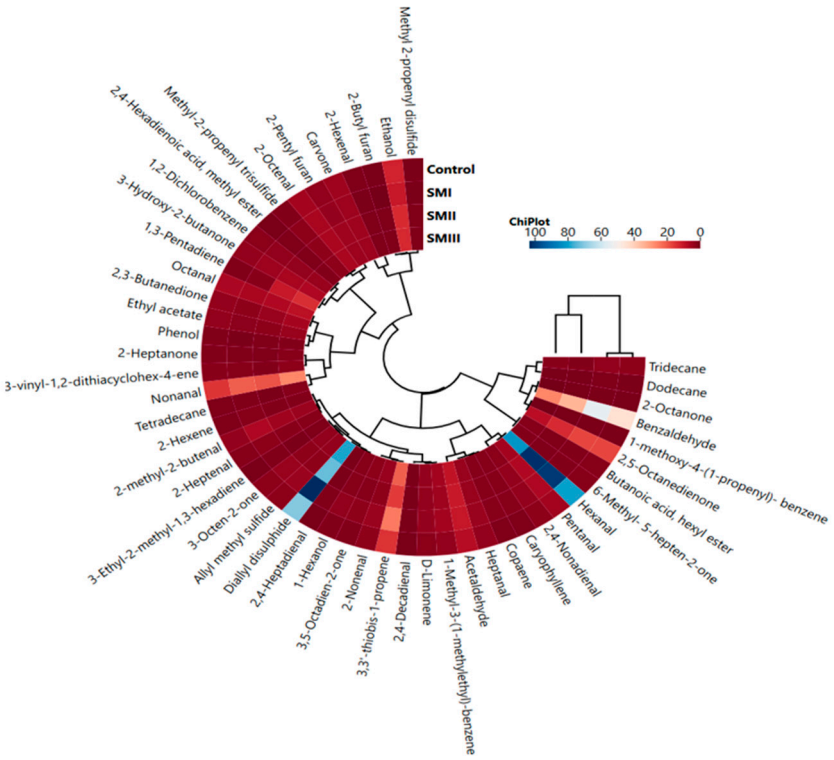

Figure 2. Cluster analysis of the relationship between salt mixtures and volatile compounds.

4. Conclusions

The use of different salt mixtures in production of reduced-sodium salt pastırma caused a change in pH and aw values of the final product. However, in all treatments, the aw value was below 0.90 and the pH value varied between 5.5 and 6.0. In addition, pastırma produced with different chloride mixtures showed lower activity of cathepsin B and cathepsin B + L than the pastırma produced with only NaCl. The use of different chloride salts had no significant effect on lipid oxidation, instrumental color values (L^*, a^*, and b^*), acidic lipase, neutral lipase, and phospholipase. In addition, correlation analysis showed that different chloride salt mixtures caused differences in the enzyme activity of reduced-sodium salt pastırma. Among the volatile compounds, only a few compounds were affected by the mixtures, and a closer correlation was observed between NaCl% 100 and mixture I. However, studies are needed to determine the sensory properties of chloride salts in reduced-sodium salt pastırma. Furthermore, studies on the use of other alternative salts, such as $MgSO_4$, are also important.

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