The effects of different spices on chemical, biochemical, textural and sensory properties of White cheeses during ripening

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Abstract

The aim of this study was to test the effects of various spices on the physicochemical and sensory properties of white cheese. Six different white cheeses were produced by incorporating the spices of black cumin, mint, thyme, red pepper and isot pepper at a ratio of 3 % (w/v) of the cheese milk. All cheeses were vacuum-packed and ripened at 4±1°C. On the 2nd, 15th, 30th, 60th and 90th days of ripening, dry matter, fat, pH, titratable acidity, salt, total proteins, ripening index, non-protein nitrogen rate, amino nitrogen rate, electrophoretic casein fractions, textural and sensory properties were determined. Isot pepper and black cumin increased the proteolysis rates of white cheeses with higher degradation rates on β-casein fraction, indicating that these cheese samples had more pronounced ripening. On the other hand, thyme and isot pepper were more effective on αs1-casein fraction. All tested spices, except isot pepper, contributed to the sensory properties of white cheeses, by increasing the consumer acceptance. This study reveals spice addition had positive effects on chemical, biochemical and sensory characteristics of white cheese, and thus, white cheese with spice addition is an alternative novel product with a good potential.

Keywords: spice, white cheese, ripening, texture

Introduction

Spices are defined as edible substances obtained from dried, whole and/or crumbled seeds, fruits, flowers, bark, root, stem, tubers, leaves, stalks and onions of various plants. These edible compounds are generally used to give colour, taste, smell, and flavour to food products (TGK 2013). More recently, using spices and herbs to control microbial growth in processed dairy products has been a common practice. Cumin, thyme, mint, and fennel have been the centre of focus as natural antimicrobials. Their antimicrobial properties are mainly attributed to their chemical contents namely thymol, carvacrol, linalool, menthol, menthone, transanetole, estragol, p-cymene and the cumic aldehyde etc. (Singh et al., 2015; Akramiet al., 2015; Akarca et al., 2016).

In addition, spices generally make the presentation of food more appealing (Üner et al., 2000). In recent years, an increasing demand for natural and...
whole foods containing no additives has contributed the use of spices and extracts in food industry for conservation purposes (Coşkun 2010). Several research groups used spices in white cheese production as antimicrobial agents to increase its shelf life and to make the cheese more attractive. In addition, spice addition resulted in a reduction on the amount of salt used during cheese production, and thus, structural defects caused by salt were prevented (Ayar and Akyüz, 2003).

White cheese is the most commonly produced and consumed cheese type in Turkey. It is a pickled variety similar to Feta and Teleme cheeses produced in Greece and in the Middle East countries (Cinbas and Kilic, 2006). A mixture of Lactococcus (Lc.) lactis ssp. lactis and Lc. lactis ssp. cremoris bacteria at different ratios are commonly used as the starter culture in the production of high quality-white cheeses having desirable taste and flavour (Dagdemir et al., 2003). Recently, there has been a growing interest in finding natural additives that can increase the shelf stability of dairy products as well as contribute to their taste and flavour properties. In this research, we aimed to investigate how different spices including black cumin, mint, thyme, red pepper and isot pepper affect the chemical, physicochemical, biochemical and sensory properties of white cheese.

Materials and methods

Materials

Cow milk was obtained from a local dairy plant in Ordu, Turkey. Choozit MA 16 (Danisco/DuPont) containing a mixture of Lc. lactis ssp. lactis and Lc. lactis ssp. cremoris was used as starter culture. Commercial rennet (1/16000) was obtained from Mayasan Company®, Istanbul. All spices were products of Bağdat Baharat Company (Kahramanmaraş, Turkey) and purchased from local markets. In addition, food grade CaCl₂ (Tekkim Chemicals, Bursa, Turkey) was used to maintain the calcium balance in the milk with a concentration of 0.02 % (v/v).

White cheese manufacture

Raw milk was pasteurized at 85 °C for 15 seconds and cooled to 32 °C. Starter culture was added at the level of 1 % and held for 60 min. Then milk was coagulated with rennet for 75 min. After coagulation, curd was cut into 8-10 mm cubes with a wire knife and pressed for 120 min. Whole curd was divided into six portions one being the control group (CC) without any spice. Selected spices were added (3 % (w/v), based on milk volume) to the rest of the five groups as follows; black cumin (B1), mint (B2), thyme (B3), red pepper (B4) and isot pepper (B5). Curd was weighted and spices were thoroughly mixed into the curds. Curd was pressed overnight and then cut into blocks with dimensions of 6×6×5 cm (Figure 1). The curds were salted with dry salt (the amount of dry salt used was 3 % of cheese weight) and held 24 h within salt. The cheese samples were vacuum-packed and ripened at 4±1 °C for 90 days. Two batches of cheese samples were prepared for each cheese type.

Figure 1. Cheeses with added spices: black cumin, mint, thyme, isot pepper, red pepper and plain

Chemical analysis

Dry matter content was determined in a laboratory oven at 105 °C until a constant weight was obtained. Total nitrogen concentration of samples was determined by micro-Kjeldahl procedure described by Case et al. (1985) with a micro-Kjeldahl analyzer UDK-149 (VELP Scientifica, Usmate, Italy) and calculated by the following formula; Total N % = normality of acid used in the titration x volume of acid used in the titration (mL) x 0.014 x 100 /
sample weight (g). Total protein was calculated by multiplying % total nitrogen with the factor of 6.38. Fat content was measured by the Van-Gulik method (IDF, 1986). Salt content was determined according to the Mohr method (Nielsen, 2003), pH was determined with a digital pH meter (Start- er 3100, USA) described by Case et al. (1985). All analyses were performed in duplicate.

Proteolysis analysis

Water-soluble nitrogen (WSN), trichloroacetic acid-soluble nitrogen (TCA-SN), and phosphotungstic acid-soluble nitrogen (PTA-SN) were determined according to the method described by Kamaly et al. (1989) and Butikofer et al. (1993).

Urea-PAGE electrophoresis

The electrophoretic analysis of protein patterns was conducted as previously described by Cream- er (1991) with some modifications made by Çelik and Tarakçı (2017).

Colour analyses

Colour measurements were performed using a colorimeter (Minolta Chroma Meter, CR-400, Osaka, Japan). The L*, a* and b* colour measurements were determined according to the CIE Lab colour space system. Three readings were taken for each sample and arithmetic means were calculated.

Texture profile analyses

For the texture analysis, the temperature of the cheeses was adjusted to 20±2 °C. The cheeses were cut into cubes with dimensions of 20x20x20 mm with a cutting wire. Texture profile analyses (hardness, springiness, gumminess, cohesiveness, adhesiveness, resilience and chewiness) of cheese samples were performed using TA-XT2 (Stable Mi- cro Systems Ltd., Surrey, UK). Analysis conditions: P/36 aluminium cylinder probe (36 mm diameter, AACC) and cell strength 25 kg weight, test speed 0.4 mm/s, initial test speed 1.0 mm/sec, print 40 %, hold time 5 sec. (Everard et al., 2006).

Sensory analysis

Sensory assessments of White cheeses were carried out by a six-member panel consisting of the university staff based on their experience in sensory evaluation of White cheeses. Prior to assessment, each cheese was cut into 10 g cubes, equilibrated to room temperature (20 °C) by laying for 4 h at room temperature and served to the panelists randomly. The overall sensory quality was assessed using a hedonic scaling method (1-10 points), where 1 is unacceptable and 10 is very good for appearance and colour, body and texture, flavour and overall acceptability. Panelists were also instructed to report any defects in colour and appearance (wet, dry, cracks), texture (hard, soft, pasty, crumbly, grainy), odour, flavour (rancid, salty, bitter, sour) and overall acceptability as well. Water was provided for mouth rinsing between evaluations of the sample.

Statistical analysis

All the analyses were performed in duplicate. All statistical calculations were performed using Statistical Software (SPSS 2000), and the obtained values are presented as mean ± standard deviation. Variance analysis (ANOVA) was performed for the determination of statistical differences between mean values for cheese types and ripening periods; then, differences found statistically significant were compared using Duncan’s multiple range test. The significance levels of P<0.05 was used for statistical differences.

Results and discussion

Chemical changes in white cheeses

The dry matter content in cheese varies depending on the characteristics of the process parameters, the type of cheese, and the degree of cheese ripening. Throughout 90 days of ripening, dry matter, fat and salt contents increased significantly (P<0.05) for all types of cheeses, possibly because of salt diffusion into the cheese (Tarakçi and Tunçtürk, 2008) and concomitant syneresis. As shown in Table 1, there is a significant difference in dry matter ratio between cheese varieties
(P<0.05). Interaction between cheese type and ripening period is also important at P<0.05 level. Whey drainage was increased with added spices and lead to higher dry matter content in these cheeses compared to the control cheese.

The ratio of fat among the cheeses was found to be the lowest with 19.8 % in the control samples and highest with 23.2 % in the pepper-enriched cheeses. There was no steady increase or decrease in fat rate during ripening with an overall mean of 21.93±1.43 %. Significant differences were found among the cheese varieties in terms of protein ratios. The highest value in terms of salt amount was recorded on the 60th day of ripening and the lowest on the 15th day, and this difference was statistically significant.

**TABLE 1.** Changes in dry matter, fat, protein and pH values of cheese samples during ripening*

| Cheese Types | Ripening time (days) | 2 | 15 | 30 | 60 | 90 | X |
|--------------|----------------------|---|----|----|----|----|---|
| Dry matter (%) | CC   | 47.64±0.51 | 44.19±0.19 | 45.99±0.20 | 44.48±0.33 | 43.55±0.25 | 44.77±1.50a |
| B1 | 48.61±0.23 | 46.10±0.18 | 46.61±0.11 | 47.73±0.25 | 46.36±0.17 | 47.37±0.83a |
| B2 | 48.53±0.49 | 51.13±2.71 | 47.98±0.21 | 49.40±0.06 | 46.80±0.09 | 48.77±1.91a |
| B3 | 48.95±1.49 | 49.02±0.30 | 45.99±0.84 | 48.44±0.35 | 49.07±0.10 | 48.47±1.54a |
| B4 | 46.91±0.41 | 46.09±0.10 | 45.58±0.16 | 48.03±0.21 | 46.39±0.05 | 46.63±0.85a |
| B5 | 47.38±0.78 | 47.59±1.39 | 44.65±0.10 | 47.08±0.46 | 46.09±0.19 | 46.56±1.32a |
| X | 48.00±1.06a | 47.76±2.60a | 45.80±1.35a | 47.52±1.56a | 46.38±1.62a |
| Fat (%) | CC | 18.75±0.25 | 20.00±0.50 | 19.25±0.25 | 20.75±0.25 | 20.25±0.25 | 20.25±0.25 |
| B1 | 21.50±1.50 | 21.50±0.50 | 21.50±0.50 | 22.00±0.50 | 22.50±0.75 | 21.75±0.90a |
| B2 | 21.50±0.50 | 25.50±0.50 | 23.50±0.50 | 25.25±0.25 | 22.25±0.25 | 23.20±1.42a |
| B3 | 21.75±0.25 | 23.25±0.25 | 25.50±0.50 | 25.00±0.50 | 22.25±0.25 | 22.45±0.61a |
| B4 | 22.00±0.01 | 21.25±0.25 | 22.50±0.50 | 23.50±0.01 | 22.25±1.25 | 22.50±0.95a |
| B5 | 22.50±0.50 | 21.75±0.25 | 21.00±1.00 | 23.25±0.25 | 21.75±0.25 | 22.05±0.93a |
| X | 21.33±1.39a | 22.21±1.80ab | 21.79±1.48ab | 22.38±1.06b | 21.92±1.02ab |
| Protein (%) | CC | 17.98±0.34 | 18.43±0.56 | 16.76±0.11 | 16.93±0.90 | 16.43±0.01 | 17.31±0.91a |
| B1 | 21.90±1.34 | 18.20±0.11 | 19.90±0.34 | 18.84±0.11 | 18.58±0.83 | 19.48±1.52a |
| B2 | 22.57±0.22 | 19.88±1.57 | 19.22±0.56 | 19.96±0.34 | 18.83±0.25 | 20.09±1.52a |
| B3 | 21.45±1.34 | 20.11±0.45 | 20.57±0.11 | 19.85±0.45 | 20.48±0.41 | 20.49±0.88a |
| B4 | 19.77±0.56 | 18.20±0.56 | 18.39±0.56 | 19.40±0.22 | 18.08±0.01 | 18.76±0.82a |
| B5 | 20.56±0.01 | 18.43±0.78 | 19.00±0.56 | 18.39±0.34 | 17.92±0.17 | 18.86±1.03a |
| X | 20.71±1.73a | 18.87±1.14a | 18.96±1.28a | 18.89±1.13a | 18.39±1.27a |
| pH | CC | 4.60±0.02 | 4.83±0.01 | 4.65±0.01 | 4.68±0.01 | 4.67±0.03 | 4.68±0.08a |
| B1 | 4.81±0.02 | 4.81±0.01 | 4.77±0.02 | 4.80±0.01 | 4.70±0.01 | 4.77±0.04a |
| B2 | 4.65±0.01 | 4.90±0.01 | 4.69±0.01 | 4.68±0.02 | 4.54±0.01 | 4.69±0.12a |
| B3 | 4.81±0.01 | 4.85±0.01 | 4.74±0.01 | 4.72±0.02 | 4.66±0.01 | 4.75±0.06a |
| B4 | 4.76±0.01 | 4.86±0.01 | 4.67±0.01 | 4.76±0.01 | 4.55±0.01 | 4.72±0.11a |
| B5 | 4.85±0.01 | 4.89±0.01 | 4.67±0.01 | 4.71±0.04 | 4.64±0.06 | 4.74±0.10a |
| X | 4.74±0.09a | 4.85±0.03a | 4.69±0.04a | 4.72±0.05a | 4.62±0.06a |
| Salt (%) | CC | 2.52±0.06 | 2.40±0.06 | 2.90±0.09 | 3.13±0.03 | 2.95±0.09 | 2.78±0.28a |
| B1 | 2.52±0.06 | 2.05±0.06 | 2.78±0.03 | 2.69±0.01 | 2.87±0.06 | 2.58±0.29a |
| B2 | 2.40±0.06 | 2.98±0.06 | 2.93±0.12 | 2.52±0.06 | 2.57±0.01 | 2.68±0.24a |
| B3 | 2.34±0.01 | 2.63±0.06 | 2.57±0.01 | 2.60±0.03 | 2.54±0.03 | 2.54±0.11a |
| B4 | 2.40±0.06 | 2.34±0.01 | 2.72±0.03 | 3.04±0.01 | 2.98±0.06 | 2.70±0.29a |
| B5 | 2.93±0.12 | 2.52±0.06 | 2.60±0.03 | 3.10±0.06 | 2.81±0.01 | 2.79±0.22a |
| X | 2.52±0.21a | 2.49±0.29a | 2.75±0.15a | 2.85±0.25a | 2.79±0.18a |

*Values are means of two replicated trials ± standard deviation; different superscript lowercase letters indicate significant difference between cheese types and ripening time (P<0.05)
cally significant. The highest salt content (2.79 %) was found in isot pepper cheese samples, and the lowest (2.54 %) was found in thyme-added samples and the difference was statistically significant (Table 1). The dry matter and salt contents showed fluctuations during the ripening period, probably as an integrated consequence of dry salting and vacuum packaging. The dry matters increased due to intense salt penetration and whey drainage in the first fifteen days. However, release of whey was not possible due to the vacuum packaging and there were probably releases and intakes of whey that lead to fluctuations in dry matter contents. This condition also affected the protein contents similarly.

The highest pH value (4.77±0.04) was determined in B1 samples and the lowest (4.68±0.08) in control cheeses (K). The pH values of minty cheese samples (B2) were statistically similar to the control cheese samples. The highest mean pH values of cheese samples during ripening were 4.85±0.03 after 15 days, and the lowest values were 4.62±0.06 after 90 days. The overall average pH value was calculated as 4.73±0.09. These values were lower than the pH values reported by Peralta (1986) in pineapple quesq de pma cheeses, and the white soft cheeses in the studies by Dimitreli et al. (2017). On the other hand, it was higher than the pH values determined by Topçu and Saldamlı (2006) and Soltani et al. (2015) in Turkish and Iranian white cheeses, respectively.

Changes in nitrogen fractions of cheese

Proteolysis is the most complex and the primary biochemical event that occurs during ripening in most cheese varieties, including white cheese. An increase in the concentrations of proteolysis products such as peptides and amino acids is an indicator of ripening in cheese. Variations in the nitrogen fractions of the cheeses after 2, 30, 60, and 90 days of ripening are given in Table 2. There is a continuous increase of nitrogen fractions during ripening. Statistically significant differences were found in terms of the degree of ripening among the cheese varieties. The increase in ripening rate was initially achieved with the aid of microbial enzymes in addition to the enzymatic activity (Kaminarides et al., 1990). The increase in parameters of proteolysis during cheese ripening is closely related to the increase in bacterial activity and enzyme activity over time. Several authors have found that advancing the ripening time increases the ripening values and leads to an increase in protein degradation in white cheeses (Dagdemir et al., 2003; Hayaloglu et al., 2004; Goncu and Alpkent, 2005). The non-protein nitrogen (NPN) is comprised of amino acids and medium and short chain peptides. The dry matter and protein concentrations together with the pH values are effective on the NPN ratios of cheese samples (Fritsch et al., 1992). The highest NPN ratio was recorded in the B3 cheese samples, followed by B2 cheese samples and then B4 and B1 cheese samples, which were not statistically different among each other. The lowest NPN ratios were found in B5 and control cheese samples and there was no significant difference between them (P<0.05). These values were higher than those obtained by Topçu and Saldamlı (2006) in white cheeses and by Agboola and Radovano-vic-Tesic (2002) in Australian herb cheeses, while the values obtained by Hayaloglu and Karabulut (2013) and Tunçtürk et al. (2014) were found to be lower than herby cheese. Dimitreli et al. (2017) found similar NPN values in herby cheeses.

Protease peptone nitrogen (PPN) is comprised of peptides and amino acids with less than 600 daltons in molecular weight and those are soluble in 5 % phosphotungstic acid (PTA) (Hannon et al., 2003). It is believed that the level of free amino acids and peptides soluble in 5 % PTA was a reliable indicator of the level of cheese flavor development (McSweeney and Sousa, 2000) and this nitrogen fraction is closely related to peptidolytic activity of starter and non- starter microflora (Scolari et al.,1993). Table 2 shows that there was a continuous increase in the PPN ratio during ripening as well as in the nitrogen ratio, the ripening index and the NPN ratio.
TABLE 2. Changes in the ripening index, TCA-SN/TN and PTA-SN/TN during ripening of cheese samples*

| Cheese Types | Ripening time (days) | 2    | 15   | 30   | 60   | 90   | X    |
|--------------|----------------------|------|------|------|------|------|------|
| Ripening index (WSN/TN) (%) |                      |      |      |      |      |      |      |
| CC           |                      | 8.82±0.33 | 9.89±0.11 | 11.91±0.02 | 17.84±0.82 | 24.65±0.79 | 14.62±5.93 |
| B1           |                      | 7.46±0.50 | 10.04±0.34 | 12.68±0.12 | 16.57±0.13 | 21.83±0.74 | 13.72±5.07 |
| B2           |                      | 8.00±0.67 | 10.23±0.56 | 12.96±0.30 | 16.51±0.16 | 24.96±0.59 | 14.53±5.96 |
| B3           |                      | 8.45±0.48 | 9.29±0.66 | 13.32±0.39 | 18.78±0.23 | 25.62±0.83 | 15.09±5.96 |
| B4           |                      | 8.05±0.36 | 9.88±0.33 | 13.58±0.31 | 16.99±0.20 | 24.56±0.48 | 14.61±5.86 |
| B5           |                      | 7.65±0.05 | 10.31±0.75 | 12.29±0.11 | 14.86±0.27 | 22.00±0.32 | 13.42±4.92 |
| X            |                      | 8.07±0.46 | 9.94±0.61 | 12.79±0.62 | 16.92±1.27 | 23.94±1.61 |      |
| TCA-SN/TN (%) |                      |      |      |      |      |      |      |
| CC           |                      | 3.40±0.41 | 4.01±0.23 | 4.94±0.02 | 5.40±0.12 | 7.88±0.16 | 5.13±1.56 |
| B1           |                      | 3.15±0.15 | 3.94±0.08 | 4.87±0.01 | 4.97±0.05 | 9.12±0.27 | 5.21±2.07 |
| B2           |                      | 3.26±0.01 | 4.33±0.24 | 5.57±0.28 | 6.13±0.15 | 10.73±0.10 | 6.00±2.57 |
| B3           |                      | 3.64±0.32 | 4.07±0.29 | 6.75±0.03 | 7.12±0.11 | 11.05±0.35 | 6.53±2.67 |
| B4           |                      | 3.85±0.06 | 4.15±0.20 | 6.05±0.43 | 5.51±0.06 | 7.35±0.14 | 5.38±1.31 |
| B5           |                      | 2.69±0.32 | 3.24±0.08 | 4.39±0.20 | 5.17±0.04 | 8.24±0.17 | 4.75±1.96 |
| X            |                      | 3.35±0.45 | 3.95±0.40 | 5.45±0.82 | 5.72±0.73 | 9.06±1.42 |      |
| PTA-SN/TN (%) |                      |      |      |      |      |      |      |
| CC           |                      | 0.68±0.01 | 0.95±0.27 | 1.07±0.12 | 1.66±0.12 | 1.89±0.11 | 1.25±0.48 |
| B1           |                      | 0.82±0.05 | 0.93±0.06 | 1.02±0.01 | 1.31±0.04 | 1.68±0.07 | 1.15±0.31 |
| B2           |                      | 0.61±0.26 | 1.19±0.17 | 1.62±0.15 | 2.30±0.06 | 2.66±0.04 | 1.68±0.75 |
| B3           |                      | 1.29±0.06 | 1.36±0.01 | 2.04±0.03 | 2.34±0.05 | 2.61±0.05 | 1.93±0.55 |
| B4           |                      | 0.52±0.34 | 0.60±0.04 | 1.41±0.02 | 1.74±0.04 | 2.05±0.05 | 1.27±0.63 |
| B5           |                      | 0.48±0.10 | 0.66±0.07 | 1.02±0.05 | 1.42±0.05 | 1.69±0.06 | 1.05±0.46 |
| X            |                      | 0.74±0.33 | 0.95±0.30 | 1.36±0.38 | 1.79±0.40 | 2.10±0.41 |      |

*Values are means of two replicated trials ± standard deviation; different superscript lowercase letters indicate significant difference between cheese types and ripening time (P≤0.05)

Assessment of proteolysis

Figure 2 shows the urea-PAGE electrophoretogram for the casein fractions of α-s1-, α-s2-, β-, αs1-I peptide and other breakdown products of White cheeses with different types of spices during ripening. During the ripening period, the amounts of α- and β-caseins of all cheese samples were decreased. However, the amounts of αs1-I peptide, γ-casein and other metabolites were increased.

Proteolysis of αs1-casein follows a three-step pattern (Fox 1989). Initially, the αs1-casein is cleaved from the Phe23-Phe24 linkages into αs1-casein (f1-23) and αs1-casein (f24-199). Secondly, αs1-casein (f24-199) is hydrolysed to medium and small sized peptides by the coagulant enzyme, the indigenous enzymes and the bacterial enzymes and finally, bacterial enzymes break down small peptides into amino acids (Dave et al., 2003). αs1-casein was continuously degraded during the ripening period. The highest decrease in the amount of αs1-casein was found in the thyme and isot pepper-added cheese samples. Tarakçı et al. (2004), Hayaloğlu and Karabulut (2013), Tunçtürk et al. (2014) and Dimitreli et al. (2017) also found a decrease in the amount of αs1-casein in herby cheese during ripening. Furthermore, during the ripening period, the decrease in the amount of αs1-casein seems
to be greater than the decrease in the amount of β-casein. However, the decrease in the amount of αs1-casein in mint, red pepper and cumin-added cheese was lower than the decline in the control group cheese. Considering starters’ contribution to the proteolysis of αs1-casein it would be plausible to say that the antimicrobial activities of tested spices inhibited the microbial protease activities. However, a more comprehensive study is required to understand the whole mechanism.

The amount of αs2-casein determined in cheese samples also decreased during ripening. It has been reported that αs2-casein hydrolysis in cheese was also related to the activity of plasmin, the natural milk proteinase (Tarakçı and Tunçtürk, 2008).

The highest decrease in the amount of β-casein was observed in the thyme-added cheese, followed by the black cumin and isot pepper pepper-added cheeses. The least decrease in the amount of β-casein occurred in mint and red pepper-added cheeses. The amount of β-casein in the control group cheese was lower than that of mint and red pepper-added cheese; thyme, cucumber and isot pepper-added cheese. Many researches have reported that β-casein decreased during cheese ripening (Kim et al., 2004).

γ-casein is a breakdown product of β-casein. Therefore, the amount of γ-casein increased in cheese samples during ripening. It has also been observed in other studies that the amount of γ-casein increased during ripening (Taivosalo et al., 2018).

The αs1-I peptide is a large molecule peptide formed by hydrolysis of 24/25 peptide bond within the αs1-casein. The formation of αs1-I peptide is largely catalysed by rennin (Grappin et al., 1985). From the second day of ripening all the cheeses were observed to have αs1-I peptides. At the beginning of the ripening period the amount of αs1-I peptide increased due to the effect of rennin and decreased in later periods. The obtained result is similar to the results obtained by Ceruti et al. (2016).

**Color changes in white cheese**

L, a, and b values are given by a three-dimensional coordinate system in which the L* value indicates brightness (100) and darkness (0), a* indicates redness/greenness (+/-) and b* indicates yellow/blue (+/-) (Jha, 2010). The L value was found to be high in the control group cheese samples compared
to the spice-added cheese samples and it was close to the full score value. The highest L value was 96.18±2.84 in the control group, as expected. The highest decrease in L value was found in the cheese samples with thyme, isot pepper and mint in the order of 79.69±4.65, 80.89±4.78, 82.10±6.43 (Table 3). It is thought that thyme and mint crumbles are light and have more scattering property in cheese, whereas isot pepper is thought to decrease L value due to its coloring property.

**TABLE 3.** Changes in colour L, a and b values of cheese samples*

| Cheese Types | Ripening time (days) | 2     | 15    | 30    | 60    | 90    | X     |
|--------------|----------------------|-------|-------|-------|-------|-------|-------|
| L            |                      | CC    | 98.80±0.84 | 98.15±0.73 | 98.29±0.45 | 92.09±0.27 | 93.58±0.26 | 96.18±2.84a |
|              |                      | B1    | 92.83±1.00  | 93.12±0.92  | 90.25±1.94  | 73.57±1.02  | 88.25±0.97  | 87.60±7.34a |
|              |                      | B2    | 83.54±0.55  | 83.72±0.69  | 83.05±0.94  | 73.45±0.26  | 74.71±0.47  | 79.69±4.65a |
|              |                      | B3    | 88.65±2.01  | 87.81±2.29  | 83.90±0.17  | 71.96±0.65  | 78.20±0.32  | 82.10±6.43a |
|              |                      | B4    | 89.31±1.11  | 90.54±0.11  | 87.96±0.82  | 82.24±1.28  | 84.02±0.53  | 86.81±5.29a |
|              |                      | B5    | 85.17±1.59  | 84.43±2.16  | 82.73±1.81  | 72.81±0.96  | 79.31±0.89  | 80.89±4.78a |
|              |                      | X     | 89.72±5.20a | 89.63±5.21a | 87.69±5.60b | 77.69±7.35d | 83.01±6.43c |
| a            |                      | CC    | 1.54±0.04   | 1.16±0.15   | 1.28±0.20   | 2.39±0.26   | 1.79±0.04   | 1.63±0.47b  |
|              |                      | B1    | 0.63±0.08   | 0.60±0.29   | 0.86±0.32   | 1.60±0.04   | 1.95±0.09   | 1.13±0.58a  |
|              |                      | B2    | 0.32±0.45   | 0.25±0.15   | 0.10±0.64   | 0.50±0.39   | 0.63±0.22   | 0.36±0.45a  |
|              |                      | B3    | 1.89±0.21   | 0.74±0.31   | 1.41±0.57   | 1.71±0.26   | 2.33±0.45   | 1.62±0.65a  |
|              |                      | B4    | 6.84±0.26   | 7.20±0.71   | 8.83±0.52   | 9.58±1.04   | 7.64±0.47   | 8.02±1.22c  |
|              |                      | B5    | 4.86±0.52   | 4.12±0.59   | 4.45±0.21   | 5.63±0.46   | 3.98±0.36   | 4.61±0.74a  |
|              |                      | X     | 2.68±2.39ab | 2.35±2.56a  | 2.82±3.04b  | 3.57±3.17c  | 3.05±2.30b  |
| b            |                      | CC    | 11.09±1.30  | 11.37±0.95  | 13.05±1.90  | 13.00±0.18  | 12.73±0.50  | 12.25±1.42a |
|              |                      | B1    | 9.81±0.80   | 9.89±1.59   | 10.38±1.62  | 11.24±1.95  | 10.93±1.53  | 10.45±1.64a |
|              |                      | B2    | 10.56±0.70  | 9.55±0.46   | 9.48±0.63   | 15.31±0.27  | 10.71±0.89  | 11.12±2.24a |
|              |                      | B3    | 7.05±0.99   | 10.03±0.32  | 10.53±1.64  | 16.49±1.70  | 11.46±0.76  | 11.11±3.29a |
|              |                      | B4    | 18.12±0.27  | 21.00±0.67  | 19.50±1.84  | 22.42±0.70  | 20.57±0.79  | 20.32±1.76c |
|              |                      | B5    | 12.20±1.97  | 12.94±1.09  | 10.51±0.53  | 15.10±4.29  | 13.34±0.55  | 12.82±2.66b |
|              |                      | X     | 11.47±3.55a | 12.46±4.10b | 12.24±3.73b | 15.59±4.06c | 13.29±3.51b |

*Values are means of two replicated trials ± standard deviation; different superscript lowercase letters indicate significant difference between cheese types and ripening time (P<0.05)
As mentioned above, the highest $L$ value was found in the control group cheeses, followed by cumin-added cheese and red pepper flaked-blended cheese, which are not statistically different. The lowest $L$ value was detected in cheese samples with thyme and isot pepper addition.

The value indicates redness and greenness and it varies between 0.36±0.45 and 3.57±3.17 for the cheese samples. The highest $a$ value during ripening was determined as 3.57 at 60 days, and the lowest as 2.35 at 15 days. The highest $a$ value was determined as 8.02 in B4 while the lowest one was 0.36 in B2 samples. High values were detected in most of the red pepper flakes-added cheese samples, followed by isot pepper-added samples. The lowest $a$ value, which is indicator of green colour, was found in minty cheese and it is significantly different from other cheeses. The high $b$ value is the indicator of yellow colour. B4 cheese had the maximum $b$ value and it was statistically different from other samples. The lowest $b$ values were recorded for samples B1, B3, B2 and CC that were not statistically different from each other.

**Texture profile analysis (TPA) in white cheeses**

There are seven texture parameters in the TPA, which are derived from the power-time curve. These are resilience, hardness, springiness, gumminess, cohesiveness, adhesiveness and chewiness. Hardness is the maximum force applied to the first compression of the cheese (Kim et al., 2004). Hardness is related to the proportion of salt within the cheese and water. If the salt content in the water is increased, the hardness increases (Kaya, 2002). The highest hardness value was 5.61±1.17 kg and the lowest was 3.10±0.69 kg. As can be understood from the analysis of the dry matter, it is considered that the control group cheeses have lower hardness value than the spice-added cheese (Table 4). There was a prominent increase in hardness values on the 15th day of ripening for all cheeses, especially for sample B2. This could be attributed to the increase in salt and dry matter concentrations in B2 cheese (Table 1).

The cohesiveness values increased on the 15th day of ripening and then started decreasing. The highest values belong to isot pepper cheese (B5), followed by other cheese samples with no statistically significant difference (Table 4). The adhesiveness values of cheese samples were found to be -14.75±11.33 g.s.n and -47.01±28.30 g.s.n (Table 4). External tackiness values increased until the 30th day of ripening and then decreased slightly. The highest external tackiness value was -23.62±13.04 g.s.n for thyme added cheese and the lowest value was -42.79±20.03 g.s.n.

Resilience is defined as the degree of restoration ability of foods during chewing (Truong et al., 2002). The values varied between 0.68±0.135 mm and 0.76±0.09 mm. In the study conducted by Karaman et al. (2012), it was determined that the elasticity-spreadability values of full-fat cheese produced from non-homogeneous creams were 0.56-0.73 mm. The values we obtained in our research are close to the values of the investigator. The gumminess values ranged from 0.52±0.14 kg to 2.23±0.45 kg with an overall average of 1.28±0.61 kg. Mehenktaş and Metin (2007) determined the average value of gumminess as 1.29 kg in full fat white cheese samples which is similar to the results of this study. The gumminess value was highest in the thyme-added cheese but it is not statistically different from other spice-added cheese. However, the control cheese had a significantly different gumminess value compared to the spice added cheeses.

Chewiness is defined as the chewing force required making a food ready for swallowing (Raphaelides et al., 1995). The values varied between 0.24±0.10 kg.mm and 1.71±0.28 kg.mm. The mean chewiness values during the ripening period increased from 2nd day (0.35 kg.mm) to 15th day (1.31 kg mm), then decreased at 90th day. According to the results of multiple comparison tests of chewiness values of cheese varieties, the highest value (1.09 kg mm) was observed in the cheese with thyme and the lowest value (0.62 kg mm) was in the control group cheese. The springiness values varied between 0.07±0.01 and 0.28±0.18. In terms of ripening period, it is hard to say that there is a real alteration in the springiness values. The highest springiness was determined in the isot pepper cheese (0.14) while the lowest (0.10) was the control, black cumin and mint-added cheeses.
TABLE 4. Changes in texture profile values of cheese samples

| Cheese Types | Ripening time (days) | 2      | 15     | 30     | 60     | 90     | X      |
|--------------|----------------------|--------|--------|--------|--------|--------|--------|
|              |                      | CC     | B1     | B2     | B3     | B4     | B5     |
| Hardness     |                      | 1.86±0.35 | 2.46±0.52 | 3.81±1.01 | 2.32±0.72 | 2.17±0.34 | 2.10±0.62 |
|              |                      | 3.50±0.33 | 4.94±0.13 | 5.04±0.34 | 6.06±0.55 | 4.34±0.26 | 3.85±0.12 |
|              |                      | 3.52±0.42 | 5.65±0.29 | 5.99±0.93 | 5.45±0.37 | 4.85±0.42 | 3.70±0.10 |
|              |                      | 3.78±0.26 | 3.94±0.47 | 5.81±0.24 | 6.77±0.52 | 4.88±0.17 | 5.26±0.98 |
|              |                      | 3.10±0.69 | 4.41±1.20 | 5.61±1.17 | 4.95±1.57 | 4.05±0.99 | 3.96±1.10 |
| Cohesiveness |                      | 2.45±0.08a | 4.72±1.53a | 4.62±0.90a | 4.86±1.14a | 5.07±1.16a |
| Adhesiveness |                      | 0.03±0.00 | 0.02±0.00 | 0.01±0.00 | 0.02±0.00 | 0.02±0.00 | 0.01±0.00 |
| Resilience   |                      | 0.16±0.10 | 0.19±0.07 | 0.19±0.06 | 0.19±0.06 | 0.15±0.03 | 0.17±0.03 |
| Gumminess    |                      | 0.82±0.03 | 0.79±0.04 | 0.76±0.06 | 0.73±0.11 | 0.72±0.11 | 0.71±0.01 |
| Chewiness    |                      | 0.89±0.10 | 0.83±0.17 | 0.72±0.11 | 0.73±0.11 | 0.73±0.06 | 0.70±0.05 |
|              |                      | 0.11±0.12 | 0.11±0.12 | 0.10±0.04 | 0.11±0.04 | 0.10±0.01 | 0.09±0.01 |

*Values are means of two replicated trials ± standard deviation; different superscript lowercase letters indicate significant difference between cheese types and ripening time (P<0.05)
**Sensory properties of white cheeses**

All sensory scores are presented in Table 5. The control group (8.71) and black cumin added cheese samples (8.14) were the most preferred in terms of colour and appearance among fresh cheeses; while the lowest scores were determined in isost pepper (5.14) and red pepper-added cheese samples (5.71). Colour and appearance scores generally increased in cheese samples during the ripening period. The mean values also showed that control and black-cumin added cheeses were the most preferred ones regarding colour and appearance. The odour values varied between 6.14 and 9.44. Regarding the mean odour values, only isost pepper-added cheeses had significantly different odour value however, it was similar to those of red pepper and control. The structure and texture scores of the cheese samples changed between 6.71±1.16 and 9.13±0.78. Texture scores tended to increase as ripening proceeded; however, there is no significant difference found between mean textural scores of the cheese samples.

*Values are means of two replicated trials ± standard deviation; different superscript lowercase letters indicate significant difference between cheese types and ripening time (P≤0.05)*

**Table 5. Changes in sensory scores in White cheese during ripening period**
Cilj ovog rada bio je ispitati utjecaj različitih začina na fizikalno-kemijska i senzorska svojstva bijelih sireva u salamuri. Šest različitih vrsta sira u salamuri proizvedeno je dodatkom 3 % (w/v) začina poput crnog kima, mente, timijana, crvenog papra i crnog papra u mlijeko za podsiravanje. Svi sirevi su pakirani u vakuum i dozrijevali su pri 4±1°C. Uzorcima su određivani ukupna suha tvar, udio masti, pH, titracijska kiselost, ukupni protein, indeks zrenja, udjel neproteinskih dušičnih tvari, udjel dušika u amino obliku, frakcije kazeina pomoću elektroforeze, senzorska i teksturalna svojstva. Navedeni analize provođene su nakon 2, 15, 30, 60 i 90 dana skladištenja. Crni papar i crni kim utjecali su na povećanje stupnja proteolize bijelih sireva, i to prije svega na povećanje stupnja degradacije frakcije β-kazeina što je ukazivalo na intenzivnije zrenje kod ovih sireva. S druge strane, timijan i crni papar više su poticali hidrolizu αs1-kazeina. Osim crnog papra, svi ostali začini pozitivno su utjecali na senzorska svojstva bijelih sireva te povećavali njegovu prihvatljivost od strane potrošača. Rezultati ove studije potvrđuju kako dodatak začina pozitivno utječe na kemijsku, biokemijsku i senzorska svojstva bijelog sira, a tako obogaćeni sir predstavlja alternativni novi proizvod visokog potencijala.

**Ključne riječi:** začin, bijeli sir, zrenje, tekstura

**Conclusions**

This study showed that white cheese with spice addition is an alternative novel product with a good potential. Spice addition had positive effects on chemical, biochemical and sensory characteristics of White cheese. Cheeses with black cumin and isot pepper possessed higher ripening rates than others. Thereby, thyme, black cumin and isot pepper were found to be more effective on the β-casein fraction hydrolysis while thyme and isot pepper were better degraders for αs1-casein fraction. Except isot pepper added cheese, all tested spices contributed to the sensory properties of white cheeses. These new varieties of white cheese will present new choices for consumers looking for new tastes.

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