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A comparative analysis of microbial, chemical composition, and quality parameters in Kürtün-Araköy and Gümüşhane breads

Kürtün-Araköy ve Gümüşhane ekmeklerinde mikrobiyal, kimyasal bileşim ve kalite parametrelerinin karşılaştırmalı analizi

Büşra ÇİMEN[1], Fırat YILMAZ*[1]

[1]*Gumushane University, Faculty of Engineering and Natural Sciences, Department of Food Engineering, 29100 Gumushane, Türkiye*

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Abstract

The objective of this study was to ascertain the distinguishing characteristics of Kürtün-Araköy bread and Gümüşhane bread, two local breads produced in Gümüşhane province in the Eastern Black Sea region. Furthermore, the bread dough samples employed in bread production were subjected to microbiological analyses. It was determined that the number of microorganisms in the sourdough samples used in the production of Gümüşhane bread was high ($P<0.001$). In this context, breads sold in the Gümüşhane province were collected and subjected to a series of analyses. These included chemical, microbiological, colour and HMF analyses of the bread samples. As a result of microbiological analyses performed on bread samples; It was observed that there were statistical differences in terms of lactic acid bacteria on MRS agar and yeast-mold counts. However, based on the chemical analyses performed on the bread samples, it was determined that there were statistically significant differences between the bread samples. The resulting data were then subjected to statistical analyses, with the objective of determining the differences between the bread samples. This was achieved through the use of PCA analysis, which also enabled the determination of the significance levels of these differences. In addition, the relationship between the colour values of the bread samples and the amount of HMF was also investigated in this study. The findings indicated that as the L^* and b^* colour values decreased, the HMF content of the bread samples increased. In this study, it was determined that Kürtün-Araköy and Gümüşhane breads have distinctive characteristics.

Keywords: Bread, Chemical analysis, HMF, Microbiological analysis, Principal companent analysis (PCA)

Öz

Bu çalışmanın amacı, Doğu Karadeniz Bölgesi'nde Gümüşhane ilinde üretilen iki yerel ekmek olan Kürtün Araköy ekmeği ve Gümüşhane ekmeğinin ayırt edici özelliklerini tespit etmektir. Ayrıca, ekmek üretiminde kullanılan ekmek hamuru örnekleri mikrobiyolojik analizlere tabi tutulmuştur. Gümüşhane ekmeğinin üretiminde kullanılan hamur mayası örneklerindeki mikroorganizma sayısının düzeyde yüksek olduğu belirlenmiştir ($P<0.001$). Bu kapsamda Gümüşhane ilinde satılan ekmekler toplanmış ve bir dizi analize tabi tutulmuştur. Bunlar arasında ekmek örneklerinin kimyasal, mikrobiyolojik, renk ve HMF analizleri yer almaktadır. Ekmek örneklerinde yapılan mikrobiyolojik analizler sonucunda; MRS agarda gelişen laktik asit bakteri ve maya-küf sayıları bakıından istatistiksel farklılıkların olduğu gözlenmiştir. Bununla birlikte; ekmek örnekleirnde yapılan kimyasal analizler doğrultusunda, ekmek örnekleri arasında istatistiksel önemli farklılıkların olduğu belirlenmiştir. Elde edilen veriler daha sonra ekmek örnekleri arasındaki farklılıkları belirlemek amacıyla istatistiksel analizlere tabi tutulmuştur. Bu da PCA analizi kullanılarak gerçekleştirilmiş ve bu farklılıkların anlamlılık düzeylerinin belirlenmesini sağlamıştır. Ayrıca, bu çalışmada ekmek örneklerinin renk değerleri ile HMF miktarı arasındaki ilişki de araştırılmıştır. Ekmek örneklerinin L ve b* renk değerleri azaldıkça, ekmek örneklerinin HMF miktarının arttığı tespit edilmiştir. Bu çalışmada Kürtün-Araköy ve Gümüşhane ekmeklerinin ayırt edici özelliklere sahip olduğu belirlenmiştir.*

Anahtar kelimeler: *Ekmek, Kimyasal analiz, HMF, Mikrobiyolojik analiz, Başlıca bileşen analizi (PCA)*

*Fırat YILMAZ; asst.prof.firatyilmaz@gmail.com

1. Introduction

Bread is a dietary staple in Turkey, with a significant proportion of the population consuming it on a daily basis (Taşçı et al., 2017). It constitutes an important source of energy and a fundamental element of the national diet. Türkiye is renowned for its extensive assortment of breads, many of which are crafted using traditional methods that are unique to specific regions (Köten & Ünsal, 2007). These local breads possess distinctive characteristics, including the utilisation of locally sourced ingredients, bespoke baking techniques, artisanal expertise, and region-specific equipment. They are officially registered as a geographical indication. Two examples of geographically labelled breads from the Gümüşhane region are the Gümüşhane Bread and the Kürtün-Araköy bread. Gümüşhane Bread is characterised by a round shape and a long shelf life, which can be attributed to the use of sourdough and baking in stone ovens with forest products as fuel. Kürtün-Araköy bread is free from additives and exhibits a distinctive texture resulting from the fermentation process, which creates air pockets. It is traditionally baked in stone ovens and has a longer shelf life than other breads (Şen & Ekinci, 2020).

The Turkish Patent Institute has granted geographical indication status to several breads originating from the Eastern Black Sea region, thereby underscoring their significance at the local level (Şen & Ekinci, 2020). The process of sourdoughing, which has been employed for centuries, is of paramount importance in the production of bread with the desired qualities, including volume, texture, flavour, nutritional value and shelf life (Poutanen et al., 2009). In traditional sourdough, a combination of wild yeasts, lactic acid bacteria, citric acid bacteria, acetic acid bacteria, and cultured yeasts work in concert to ferment the dough (Behera & Ray, 2015). The natural sourdough flora is primarily composed of heterofermentative and homofermentative lactic acid bacteria and yeasts belonging to genera such as *Lactobacillus*, *Saccharomyces*, and *Candida*. The interaction between these microorganisms is responsible for the distinctive characteristics of sourdough bread, including its flavour, acidity, and elasticity. Furthermore, the sourdough process is a crucial factor in developing the distinctive taste of sourdough bread through the release of flavours during fermentation (Şahin & Meral, 2012).

Lactic acid bacteria exert a considerable influence on the expansion, resilience, acidity, and flavour of bread. Lactobacillus, a specific type of lactic acid bacteria, is of particular importance in the bread industry due to its distinctive metabolic properties. Sourdough bread, which contains beneficial microorganisms, may be considered a probiotic product. The most commonly occurring bacteria in sourdough are *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Leuconostoc* mesenteroides, and *Pediococcus spp.* Conversely, *Lactobacillus plantarum*, *Lactobacillus sanfranciscensis*, and *Lactobacillus fermentum* are employed in the production of traditional breads. Additionally, yeast species such as *Saccharomyces cerevisiae* and *Saccharomyces exigus* are present in sourdough (Gerçekaslan et al., 2012; Çifci, 2017). The presence of lactic acid bacteria is of paramount for the controlled and safe fermentation process in bread production, as they exert a profound influence on the quality of the final product in a number of ways, including lactic acid formation, proteolytic activity, phage resistance, and bacteriocin production (Göçmen, 2001).

The accurate detection, isolation and identification of microflora are essential for the study of sourdough in order to identify the most suitable microflora for bread production. The presence of rope disease and mould can result in microbiological spoilage of bread (Bakırcı & Köse, 2017). This may be caused by the use of unsuitable raw materials, inadequate hygiene standards, or contamination throughout the production process. To circumvent this issue, chemical preservatives and lactic starter cultures are frequently employed (Menteş et al., 2004).

Nevertheless, the baking of bread at elevated temperatures can result in the formation of hydroxymethylfurfural (HMF), a chemical compound that has the potential to impact the quality, safety, and human health of the food product (Türker & Elgün, 1998). The formation of HMF is a consequence of the exposure of carbohydrates to elevated temperatures or prolonged storage periods. This can occur as a result of processes such as the Maillard reaction and caramelisation. It is particularly prevalent in heat-treated foods that contain fructose and glucose. Therefore, it is essential to regulate HMF formation in order to produce bread of the highest quality for consumer health (Civelek et al., 2024).

HMF is acknowledged as a highly toxic substance with the capacity to induce mutations, carcinogenesis and cytotoxicity. The accumulation of HMF is an indicator of deterioration in food quality, which can be attributed to improper storage or processing. This has resulted in the monitoring of HMF levels in the food industry and

the implementation of rigorous regulatory measures. The Consumer Food Codex imposes restrictions on the levels of these compounds permitted in foodstuffs, given that they are formed through heat treatment (Gülcan, 2017).

The aim of this study was to investigate the quality parameters of Kürtün-Araköy and Gümüşhane breads produced in Gümüşhane province and to determine the differences between the breads based on the data obtained.

2. Material and method

2.1. Material

In this context, a total of 30 bread samples (15 Gümüşhane bread (GB) and 15 Kürtün-Araköy bread (KAB) and 8 dough samples (4 Gümüşhane bread dough (GB-D) and 4 Kürtün-Araköy bread dough (KAB-D)) obtained from the market in Gümüşhane province were subjected to examination for their microbiological and chemical properties.

2.2. Method

2.2.1. Preparation of samples

Ten grams of each collected bread and dough sample were transferred to sterile special bags under aseptic conditions and homogenised in a stomacher device for five minutes by adding 90 ml of 0.85% physiological saline. This resulted in a dilution of 10^{-1}, and the bread and dough samples were diluted up to 10^{-5} for microbiological analysis (Certel et al., 2009).

2.2.2. Microbiological analyses

The total mesophilic aerobic bacteria (TAMB) counts were determined by inoculating diluted samples onto Plate Count Agar (PCA) and enumerating the resulting colonies after incubation (Halkman, 2005, 2007). The enumeration of lactic acid bacteria (LAB) on MRS agar was conducted using the smear method under anaerobic conditions, and the resulting colonies were counted. The same methodology was employed for the enumeration of LAB on M17 agar (Hendek Ertop, 2017; Cebeci & Doğan, 2021). For the enumeration of yeast and moulds, diluted samples were inoculated on Potato Dextrose Agar (PDA) incubated for 5 days (Halkman, 2005; Erginkaya et al., 2016). For the enumeration of coliform bacteria, samples were transferred to Violet Red Bile Agar (VRBA) and incubated at 37°C for 24 hours. At the end of incubation, colonies with a diameter of 0.5 mm or larger and showing a dark red colour were identified as coliforms and counted (Morul & İşleyici, 2012).

2.2.3. Chemical and HMF analyses

Dry matter analysis was carried out by weighing 3 g of the homogenised sample and drying at 105°C until a constant weight was obtained (Elgün & Ertugay, 2002). In order to ascertain the ash content, a quantity of 2 g of the sample was subjected to incineration at a temperature of approximately 550°C until a white residue was produced (Demirci, 2019). For the analysis of protein content, the Kjeldahl method was used, in which 1 g of sample was digested with concentrated sulfuric acid and a digestion tablet. The ammonia obtained from the distillation was kept in boric acid and the resulting distillate was titrated with 0.1N HCl (Gamlı, 2022). The pH of the bread samples was determined by immersing a pH electrode in a homogenised sample, in accordance with the AOAC 943.02 method (Akgün, 2007; Gamlı, 2022). In addition, acidity was determined by homogenising 10 g of sample with water and titrating with 0.1 N NaOH solution (Gamlı, 2022).

In this study, the HMF content of bread samples was determined by HPLC chromatography. The method applied by Zappalà et al. (2005) was used to determine the HMF content of the samples. To 10 g of bread sample, 5 mL of deionised water was added followed by 1 mL of Carrez I and Carrez II solutions. After a one minute mixing time, the mixture was centrifuged and the clear supernatant was then transferred to a separate tube. The volume was then adjusted to 10 mL with deionised water. The supernatant was passed through a 0.45 micrometre diameter injector filter into vials. Chromatography was performed using a C18 column and a

DAD detector at 280 nm wavelength. Acetonitrile:water (5:1) was used as mobile phase in the HPLC system and the column oven temperature was set to 32°C. A standard curve was prepared using the HMF standard and the amount of HMF in the sample was calculated according to this standard curve.

2.2.4. Colour measurement

A colour measurement instrument (Konica, Minolta, Tokyo, Japan) was used for colour analysis. Three parallel measurements were made separately on the outer and inner parts of the bread samples and these parallels were averaged. The instrument measured *L**, *a** and *b** values on the crust and inner parts of the samples. The *L** value is known as darkness-lightness, the *a** value as green-red intensity and the *b** value as yellow-blue intensity (Elgün et al., 2002).

2.2.5. Statically analyses

The statistical analysis of the data obtained in the study was conducted using the IBM SPSS 22 software program. A one-way ANOVA test was conducted on all data sets to ascertain the differences between the sample groups. PCA and correlation analysis were performed using the XLSTAT program.

3. Result and discussion

3.1. Microbiological analysis

The results of the microbiological analysis in dough samples of KAB and GB were presented in Table 1. The results of the analyses demonstrated statistically significant differences between the microbiological values of the bread doughs. The microbiological results of the Gümüşhane bread dough samples were found to be higher than those of the Kürtün-Araköy bread dough samples. Furthermore, the utilisation of solely traditional sourdough in KAB-D and the combination of sourdough with fresh baker's yeast in GB-D may have proved an efficacious approach with regard to LAB and yeast counts. The number of lactic acid bacteria (LAB) and yeast varied depending on the fermentation process. In a further study by Çetin et al. (2021) investigated the impact of yam powder supplementation on sourdough fermentation. Their findings indicated that the number of lactic acid bacteria (LAB) increased with the addition of yam powder, although higher rates of supplementation resulted in a reduction in the LAB count. Moreover, Alver Oral (2016) conducted an analysis of breads produced by the addition of kefir to sourdough and wet yeast combinations. The microbiological analysis revealed notable discrepancies between the dough samples, which could be attributed to alterations in the raw materials and fermentation processes. In a previous study (Akgün, 2007), the effects of sourdough powder on bread production were investigated by fermenting dough with combinations of fresh yeast and a lactic starter.

Table 1. Microbiological analysis results of dough samples

Sample	TAMB		LAB on MRS		LAB on M17		Yeast-Mould	
	KAB-D	GB-D	KAB-D	GB-D	KAB-D	GB-D	KAB-D	GB-D
1	7.17±0.05	7.98±0.25	6.96±0.07	8.48±0.13	7.07±0.09	8.42±0.01	6.80±0.13	7.81±0.12
2	6.70±0.20	8.16±0.08	6.91±0.11	8.26±0.25	7.01±0.07	8.39±0.02	6.33±0.37	8.03±0.03
3	7.11±0.16	8.07±0.01	6.94±0.12	8.08±0.10	6.97±0.02	8.16±0.07	6.86±0.14	7.89±0.01
4	7.10±0.02	7.65±0.16	7.10±0.05	7.95±0.08	7.19±0.03	7.87±0.08	6.63±0.09	7.41±0.36
Means	**7.02±0.20^{A}**	**7.96±0.23^{B}**	**6.98±0.11^{A}**	**8.19±0.25^{B}**	**7.06±0.10^{A}**	**8.21±0.23^{B}**	**6.65±0.28^{A}**	**7.79±0.29^{B}**
Anova *P*-Sample	***0.000***		***0.000***		***0.000***		***0.000***	

KAB-D: Kürtün-Araköy Bread Dough, GB-D: Gümüşhane Bread Dough, TAMB: Total Aerobic Mesophilic Bacteria, LAB: Lactic Acid Bacteria

Microbiological analysis results of KAB and GB samples were given in **Table 2.** The TAMB count of KAB samples exhibited a range of 1.99 to 4.01 log cfu/g, with an average of 2.59 log cfu/g. In comparison, the TAMB count of GB samples ranged from 1.99 to 3.21 log cfu/g, with an average of 2.68 log cfu/g. The maximum total aerobic mesophilic bacteria count in bread was found to be 7 log cfu/g, which was not exceeded in KAB and GB samples. In a separate study (Postoğlu, 2018), the TAMB count in sourdough bread samples was 1.99 log cfu/g, which was lower than the average values observed in KAB and GB samples. Furthermore, the TAMB count in normal and wholemeal breads was observed to be affected by different storage conditions (Certel et al., 2009), the impact of diverse storage conditions on the quality of both standard and wholemeal breads produced with soluble dry yeast was investigated.

Table 2. Microbiological analysis results of bread samples

Sample	TAMB		LAB on MRS agar		LAB on M17 agar		Yeast-Mould		Coliform	
	KAB	GB	KAB	GB	KAB	GB	KAB	GB	KAB	GB
1	2.82±0.02	2.97±0.33	3.00±0.00	< 2	2.30±0.00	< 2	< 2	< 2	< 1	< 1
2	2.80±0.10	2.97±0.33	2.78±0.24	< 2	2.48±0.00	< 2	< 2	< 2	< 1	< 1
3	3.98±0.05	2.49±0.29	4.12±0.10	< 2	3.71±0.07	2.00±0.00	< 2	< 2	< 1	< 1
4	2.80±0.05	2.66±0.33	< 2	< 2	<2	< 2	< 2	< 2	< 1	< 1
5	< 2	2.82±0.09	< 2	< 2	2.00±0.00	2.00±0.00	< 2	< 2	< 1	< 1
6	2.90±0.05	3.21±0.24	3.73±0.27	< 2	2.00±0.00	2.84±0.06	< 2	< 2	< 1	< 1
7	2.09±0.10	2.69±0.31	< 2	< 2	<2	2.48±0.00	2.33±0.33	< 2	< 1	< 1
8	2.00±0.00	2.43±0.44	< 2	< 2	<2	< 2	2.32±0.33	< 2	< 1	< 1
9	4.01±0.01	2.59±0.30	3.15±0.01	< 2	3.88±0.02	2.00±0.00	2.86±0.13	< 2	< 1	< 1
10	2.32±0.16	2.79±0.12	< 2	< 2	<2	2.00±0.00	2.32±0.33	< 2	< 1	< 1
11	2.40±0.20	< 2	< 2	< 2	<2	< 2	2.32±0.33	2.00±0.00	< 1	< 1
12	< 2	2.75±0.15	< 2	< 2	<2	2.60±0.00	2.66±0.33	< 2	< 1	< 1
13	< 2	2.96±0.06	< 2	< 2	<2	2.83±0.12	2.32±0.33	< 2	< 1	< 1
14	2.20±0.10	2.09±0.10	< 2	< 2	<2	< 2	2.66±0.33	< 2	< 1	< 1
15	2.63±0.03	2.84±0.18	< 2	< 2	<2	2.20±0.10	2.32±0.33	2.30±0.00	< 1	< 1
Mean	**2.59±0.10^{A}**	**2.68±0.07^{A}**	**2.45±0.11^{B}**	**< 2^{A}**	**2.29±0.09^{A}**	**2.19±0.05^{A}**	**2.27±0.07^{B}**	**2.01±0.01^{A}**	**< 1**	**< 1**
Anova	***0.459***		***0.000***		***0.377***		***0.000***		-	
P-Sample										

KAB: Kürtün Araköy Bread, GB: Gümüşhane Bread, TAMB: Total Aerobic Mesophilic Bacteria, LAB: Lactic Acid Bacteria, $^{A-C}$: Capital letter indicates the difference between the samples

The findings revealed that the TAMB number was 1.15 log cfu/g in the case of standard bread and 1.92 log cfu/g in wholemeal bread. The lactic acid bacteria (LAB) count in KAB samples ranged from 1.99 to 4.12 log cfu/g, with an average of 2.45 log cfu/g. In contrast, all GB samples exhibited a LAB count of 1.99 log cfu/g. The LAB count on M17 agar in KAB samples ranged from 1.99 to 3.88 log cfu/g, with an average of 2.29 log cfu/g, while GB samples exhibited a range of 1.99 to 2.84 log cfu/g, with an average of 2.19 log cfu/g.

The lowest count for KAB samples was 1.99 log cfu/g, the highest was 2.86 log cfu/g, and the average was 2.27 log cfu/g. Similarly, the lowest count for GB samples was 1.99 log cfu/g, the highest was 2.30 log cfu/g, and the average was 2.01 log cfu/g. The maximum acceptable limit for mould-yeast in bread is 3 log cfu/g (Anonymous, 2009). Although the highest count for KAB samples was close to this limit, it remained within the permissible range. A comparison of these results with those of previous studies (Fırat, 2016; Patır & Güran, 2018) revealed no significant differences in mould-yeast counts. The overall mould-yeast counts in the bread samples analysed exhibited a relatively consistent pattern, falling within the acceptable limits. The maximum permissible level of coliform group bacteria in bread, as established by Anonymous (2009), is 3 log cfu/g. The analysis of bread samples revealed the absence of coliform bacteria <1 log cfu/g in all samples, indicating compliance with the Turkish Food Codex communiqué on microbiological criteria. Furthermore, the number of coliform bacteria in this study was found to be lower than that observed in previous studies (Karaoğlu, 2002; Patır & Güran, 2018).

3.2. Chemical analyses

The results of chemical analyses of bread samples were given in Table 3. The dry matter content of the KAB samples ranged from 53.03% to 61.28%, while in the GB samples, it ranged from 57.63% to 63.91%.

It was thus established that the dry matter values of GB samples exceeded those of KAB samples, with the mean dry matter values between the two sample groups exhibiting a statistically significant discrepancy. However, prior studies (Gülcan, 2017; Patır & Güran, 2018; Candal et al., 2020) had documented higher dry matter values in both the KAB and GB samples.

The ash content of the KAB samples ranged from 1.65% to 2.14%, while in the GB samples, it ranged from 1.27% to 2.31%. KAB samples exhibited higher average ash content compared to GB samples ($P<0.001$). As a result of the studies (Certel et al., 2009; Erdem & Gökmen, 2022) carried out on various bread samples; it was determined that the determined ash amounts exhibited similar results with both KAB and GB samples. The protein content of the KAB samples exhibited a range of 9.82% to 12.46%, while the GB samples demonstrated a range of 8.64% to 11.49%. Although the protein amounts of KAB (10.95%) and GB (9.85%) samples were at similar levels, it was determined that the protein values of both sample groups were statistically different. The average protein amounts of KAB and GB samples were lower than the protein amounts in the study conducted by Üçüncüoğlu (2021) and higher than the protein amounts in the study conducted by Karaağaoğlu et al. (2008).

The pH measurements of the KAB samples ranged from 4.73 to 5.38, with an average value of 5.03. The pH measurements of GB ranged from 5.89 to 6.36, with an average value of 6.04. There is a statistical difference in pH values between bread sample groups. In previous studies (Moroni et al., 2011; Erdem & Gökmen, 2022), bread samples exhibited higher pH levels compared to KAB and GB samples.

The acidity levels in KAB ranged from 5.72% to 9.01%, with an average of 7.81%, while GB exhibited acidity levels ranging from 2.74% to 4.96%, with an average of 3.88%. The difference between the mean titratable acidity values of bread samples is statistically significant. In the study conducted by Hendek Ertop (2017); bread samples produced using sourdough showed higher titratable acidity levels compared to control bread (without sourdough) samples. In addition, in the study conducted by Gül et al. (2021); It was reported that the titratable acidity levels of Isparta bread samples produced using sourdough varied between 3.90% and 8.80%. While KAB bread samples contained an average of 20.36 mg/kg HMF, GB samples were found to contain an average of 5.91 mg/kg HMF. In consideration of these findings, it was determined that the HMF content of KAB samples was statistically significantly higher than that of GB samples. The study revealed that as the ratio of whey to other ingredients in breads produced with whey addition increased, the amount of HMF in the bread also increased. The bread sample with 100% whey added exhibited HMF levels approaching those of the KAB (Candal et al., 2020). Furthermore, different bread varieties exhibited higher levels of HMF compared to the GB samples (Gülcan, 2017; Candal et al., 2020).

3.3. Colour measurement

The colour values of bread samples were given in Table 4. According to these results; it was determined that the values except the a value were statistically different in the color measurements made on the crust part of the bread samples. As a result; GB samples exhibited higher L^* and b^* values on average compared to KAB samples. In addition, when the color values of the inner parts of the bread samples were examined, it was seen that there were statistical differences between all color values. Accordingly; KAB samples exhibited higher L^*, a^* and b^* values on average compared GB samples. In a study conducted by Uslu et al. (2021), the effects of sourdough obtained from apples and figs on whole wheat bread were investigated. The findings revealed that as the sourdough rate increased, the L^* and b^* values decreased and the a^* value increased in the breads. In the other study, the L^* value of the outer of the bread was determined to be 54.02, the a^* value was 11.51, and the b^* value was 29.47. The L^* value of the inner of the bread was found to be 68.59, the a^* value was 0.11, and the b^* value was 13.72 (Aplevicz et al., 2014).

3.4. Correlation between colour and HMF values of bread samples

The correlation between the colour values (L^*, a^*, b^*) and HMF amounts of bread samples is presented in Table 5 and Table 6, along with the associated significance levels (P-values). Additionally, the correlation matrix for the related analysis is provided in Figure 1. These results indicate a highly negative correlation between the L^* and b^* values of the outer part of the bread samples and the HMF content of the bread samples. Furthermore, the correlation between the a^* value of the outer part of the bread samples and the HMF content of the bread samples is minimal and statistically insignificant.

Table 3. Chemical analysis results of bread samples

Sample	Dry matter (%)		Ash (%)		Protein (%)		pH		Titrable acidity (%)		HMF (mg/kg)	
	KAB	GB	KAB	GB	KAB	GB	KAB	GB	KAB	GB	KAB	GB
1	61.28±0.41	63.91±0.80	2.14±0.05	1.64±0.01	11.30±0.29	10.03±0.25	5.38±0.03	5.98±0.00	8.32±0.12	4.61±0.25	22.52±0.41	6.34±0.25
2	57.80±0.18	57.92±0.14	1.99±0.01	1.27±0.02	9.82±0.21	9.31±0.53	5.10±0.01	6.04±0.00	8.74±0.18	3.95±0.28	22.99±0.16	5.84±0.02
3	58.84±0.35	63.38±0.68	1.99±0.03	1.49±0.02	10.97±0.32	8.68±0.09	5.07±0.00	6.01±0.00	7.63±0.09	3.61±0.12	21.03±0.13	6.31±0.08
4	57.98±0.06	60.92±0.40	1.95±0.04	1.82±0.01	10.55±0.25	10.06±0.57	4.92±0.00	6.00±0.00	8.42±0.22	4.58±0.06	16.81±1.00	6.24±0.23
5	58.55±0.28	58.47±0.26	1.83±0.01	1.53±0.01	11.72±0.40	8.71±0.22	5.05±0.00	6.07±0.00	7.35±0.18	3.50±0.09	22.22±0.14	5.29±0.06
6	57.75±0.62	61.59±0.63	1.80±0.01	1.55±0.01	10.68±0.24	8.84±0.26	5.16±0.01	6.09±0.01	7.66±0.15	3.15±0.19	15.55±0.14	6.51±0.03
7	58.78±0.24	61.35±0.45	2.11±0.02	1.52±0.02	10.14±0.17	9.26±0.29	5.00±0.00	6.11±0.00	7.31±0.03	3.43±0.26	19.23±0.27	7.38±0.02
8	57.81±0.58	63.78±0.40	1.96±0.01	2.31±0.01	10.71±0.21	11.31±0.32	5.14±0.00	5.94±0.01	7.80±0.06	4.96±0.44	19.80±0.33	5.48±0.02
9	54.52±0.21	62.99±0.45	1.65±0.01	2.26±0.02	10.59±0.18	11.32±0.33	5.00±0.00	5.93±0.01	7.90±0.06	4.61±0.15	20.33±0.48	5.97±0.11
10	56.14±0.30	63.22±0.39	1.91±0.02	1.86±0.01	11.06±0.20	10.27±0.17	4.73±0.00	6.36±0.04	8.08±0.24	2.74±0.30	20.66±0.05	6.05±0.36
11	54.47±0.17	59.17±3.80	1.90±0.01	1.84±0.01	10.94±0.59	9.86±0.53	5.16±0.01	6.17±0.01	5.72±0.06	2.74±0.09	19.00±0.16	5.85±0.46
12	53.03±0.06	58.15±0.49	1.70±0.01	1.58±0.05	12.46±0.70	9.86±1.20	4.79±0.01	5.96±0.01	7.07±0.10	3.71±0.23	22.94±0.03	4.79±0.31
13	56.60±0.43	57.63±0.24	2.06±0.02	1.51±0.01	11.49±0.18	8.64±0.14	5.09±0.01	5.89±0.00	7.42±0.25	3.81±0.24	22.74±0.31	4.35±0.03
14	53.32±0.10	62.57±0.26	1.85±0.01	1.91±0.06	11.16±0.13	11.49±1.21	4.92±0.00	6.01±0.01	8.67±0.24	4.44±0.39	17.20±0.86	6.27±0.02
15	56.52±0.28	63.11±0.08	1.98±0.01	1.92±0.03	10.62±0.24	10.00±0.24	4.99±0.01	6.02±0.01	9.01±0.43	4.30±0.45	22.38±0.25	5.98±0.21
Mean	**56.89±0.34^{A}**	**61.21±0.41^{B}**	**1.92±0.02^{B}**	**1.73±0.04^{A}**	**10.95±0.12^{B}**	**9.84±0.18^{A}**	**5.03±0.02^{A}**	**6.04±0.02^{B}**	**7.81±0.13^{B}**	**3.88±0.12^{A}**	**20.36±0.40^{B}**	**5.91±0.11^{A}**
Anova P-Sample	***0.000***		***0.000***		***0.000***		***0.000***		***0.000***		***0.000***	

KAB: Kürtün Araköy Bread, GB: Gümüşhane Bread, HMF: Hydroxymethylfurfural, $^{A-C}$: Capital letter indicates the difference between the samples.

However, a negative correlation was observed between the *L** value of the inner part of the bread and the HMF content of the bread samples ($P < 0.0001$). Conversely, a positive correlation was identified between the a and values of the bread core and the HMF content of bread samples ($P < 0.0001$).

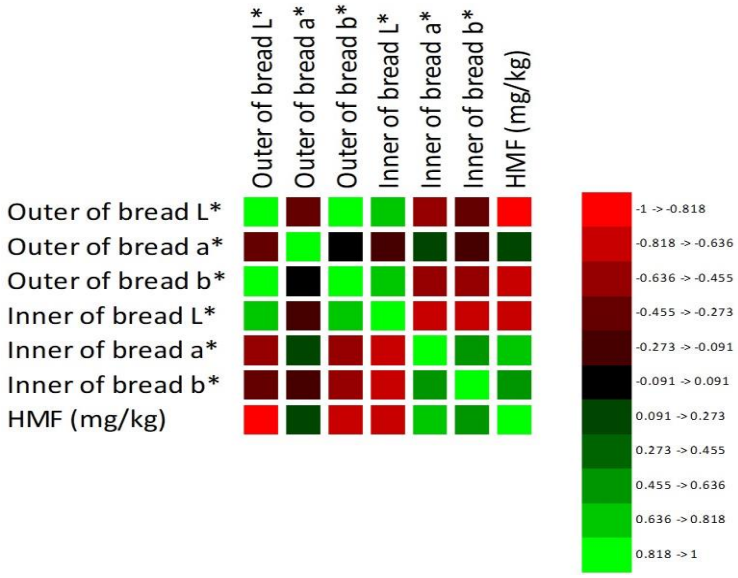

Figure 1. Correlation matrix between colour values and HMF content of bread samples

3.5. PCA analysis

Principal Component Analysis (PCA) was employed to conduct a comprehensive investigation into the variations in the physicochemical properties of the bread samples. The results of the principal component analysis (PCA) are presented in Figure 2. The data analysis of the KAB and GB samples revealed that the F1 and F2 axes collectively accounted for 77.14% of the total data. The F1 axis was responsible for 61.49% of the observed variance, while the F2 axis accounted for an additional 15.65%. The variable loadings analysis demonstrated that ash, protein, and titrable acidity exerted a negative influence on the F1 axis, whereas dry matter and pH exhibited a positive impact. Furthermore, the *L**, *a**, and *b** parameters also had a significant effect. The GB samples were found to cluster on the right side of the plot and were strongly associated with dry matter, pH, and colour values. In contrast, the KAB samples clustered on the left side with high ash and protein content. The F1 axis clearly differentiated between these two product groups, while the F2 axis highlighted the diversity within the sample distances. Therefore, it can be concluded that there are significant component differences between the KAB and GB samples, with colour values playing a crucial role in their separation on the F1 axis.

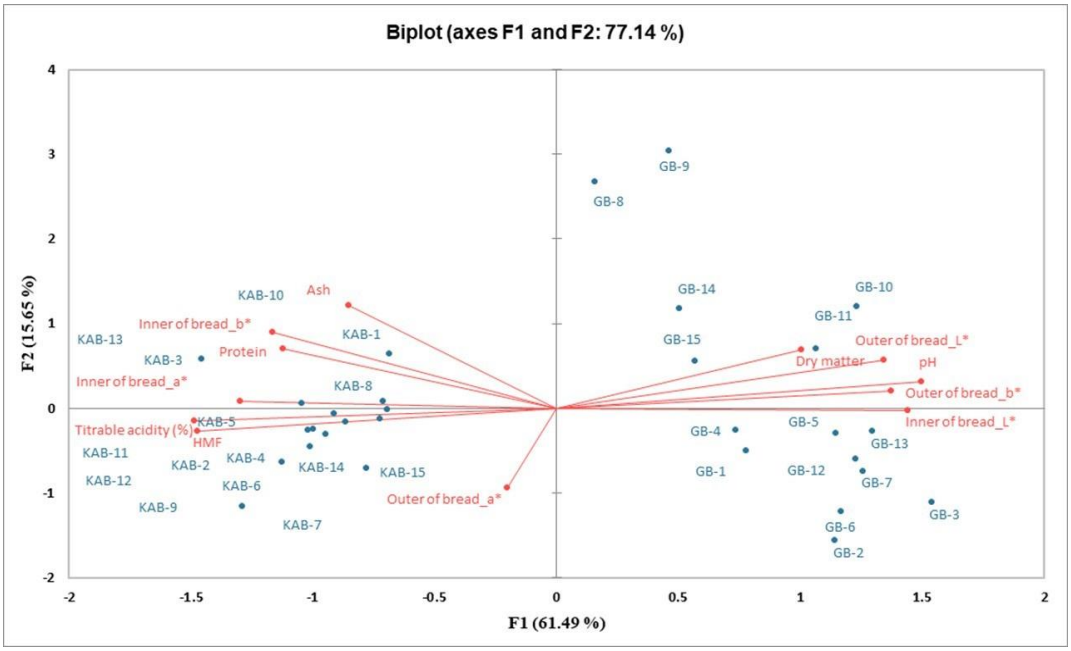

Figure 2. PCA analysis of bread samples

Table 4. Measurement of colour values in bread samples

Sample	Outer of bread						Inner of bread					
	*L**		*a**		*b**		*L**		*a**		*b**	
	KAB	GB	KAB	GB	KAB	GB	KAB	GB	KAB	GB	KAB	GB
1	45.26±0.52	50.97±2.32	10.88±0.40	13.12±1.24	22.48±0.32	30.66±1.36	66.73±1.07	68.20±1.53	0.39 ±0.13	0.19±0.091	19.06±0.42	16.74±0.43
2	40.53±0.16	52.58±1.41	10.97±0.41	11.71±0.05	20.11±0.27	31.16±0.96	62.74±1.31	70.84±1.41	0.72±0.09	-0.30±0.03	19.20±0.26	15.64±0.19
3	43.21±0.89	52.84±0.45	10.83±0.19	11.16±0.38	17.20±0.90	30.38±0.51	63.43±1.16	74.99±0.93	0.91±0.14	-0.49±0.05	19.33±0.23	13.93±0.33
4	40.02±0.89	50.63±1.35	11.06±0.15	11.22±0.21	18.16±0.96	27.41±1.67	64.88±0.23	70.58±2.18	0.72±0.17	0.13±0.08	19.80±0.66	15.43±0.48
5	46.85±0.12	57.84±2.39	10.92±0.24	9.53±1.14	24.51±0.21	30.33±0.53	62.20±0.18	68.79±0.22	-0.03±0.05	-0.46±0.28	18.76±0.19	17.60±0.60
6	42.24±0.34	52.29±1.50	12.73±0.26	12.34±0.15	21.82±0.21	30.50±1.03	61.95±1.31	70.38±1.12	0.47±0.09	0.28±0.02	19.44±0.86	14.44±0.55
7	41.50±0.83	55.06±0.70	11.23±0.76	11.41±0.14	20.87±0.78	32.82±0.78	62.62±0.25	68.98±1.27	-0.24±0.04	-0.52±0.01	19.04±0.29	15.61±0.15
8	45.93±0.78	57.84±0.59	10.86±0.27	9.73±0.26	22.33±0.63	31.35±0.18	64.13±1.62	65.73±0.39	0.65±0.18	0.76±0.13	19.46±0.21	21.67±1.15
9	38.44±0.35	62.41±1.48	10.97±0.25	6.42±0.67	15.13±0.40	28.45±0.10	60.77±0.74	67.99±0.84	1.02±0.055	0.27±0.12	19.29±0.25	19.99±0.34
10	44.63±1.45	54.43±3.61	11.10±0.71	8.43±0.53	23.49±0.40	28.80±1.99	61.82±0.74	73.14±1.17	0.46±0.14	-0.83±0.08	19.53±0.28	17.84±0.26
11	41.90±0.94	56.45±0.46	10.38±0.22	8.94±0.41	21.37±1.41	29.71±0.62	61.97±1.98	70.42±1.04	0.33±0.17	-0.72±0.21	19.99±0.27	17.92±0.10
12	43.95±1.04	54.56±0.93	11.67±0.12	9.90±0.40	24.32±0.85	28.93±0.47	62.86±0.93	72.50±0.87	0.53±0.04	-1.01±0.19	19.50±0.16	15.08±0.32
13	34.76±1.82	57.60±0.63	7.94±0.59	7.53±0.52	14.18±1.57	29.87±0.87	61.31±0.48	70.02±0.99	0.81±0.14	-0.94±0.06	20.15±0.42	16.22±0.18
14	48.05±0.94	53.09±0.74	10.67±0.32	11.35±0.42	25.15±0.87	30.94±0.31	62.78±1.06	68.28±1.01	0.71±0.17	-0.24±0.03	19.34±0.27	20.09±0.14
15	47.74±1.82	52.33±0.67	10.64±0.92	12.20±0.67	26.15±0.09	31.61±0.68	61.99±1.83	62.92±2.31	0.75±0.09	-0.31±0.06	18.96±0.35	19.22±0.29
Means	43.00±0.57^{A}	54.73±0.57^{B}	10.86±0.17^{A}	10.33±0.30^{A}	21.15±0.55^{A}	30.20±0.29^{B}	62.81±0.33^{A}	69.59±0.51^{B}	0.55±0.06^{B}	-0.28±0.08^{A}	19.39±0.10^{B}	17.16±0.35^{A}
Anova P-sample	*0.000*		*0.137*		*0.000*		*0.000*		*0.000*		*0.000*	

KAB: Kürtün Araköy Bread, GB: Gümüşhane Bread, *L**: 0-100 (Darkness- Lightness), *a**: (+/-, red/green), *b**: (+/-, yellow, blue), $^{A-C}$: Capital letter indicates the difference between the samples.

Table 5. Correlation levels (*R*) between colour values (*L**, *a**, *b**) and HMF amounts of bread samples

Variables	Outer of bread *L**	Outer of bread *a**	Outer of bread *b**	Inner of bread *L**	Inner of bread *a**	Inner of bread *b**	HMF (mg/kg)
Outer of bread *L**	1	-0.333	0.888	0.651	-0.600	-0.366	-0.821
Outer of bread *a**	-0.333	1	0.034	-0.106	0.202	-0.149	0.136
Outer of bread *b**	0.888	0.034	1	0.653	-0.629	-0.467	-0.812
Inner of bread *L**	0.651	-0.106	0.653	1	-0.651	-0.664	-0.744
Inner of bread *a**	-0.600	0.202	-0.629	-0.651	1	0.594	0.668
Inner of bread *b**	-0.366	-0.149	-0.467	-0.664	0.594	1	0.519
HMF (mg/kg)	-0.821	0.136	-0.812	-0.744	0.668	0.519	1

Values in bold are different from 0 with a significance level alpha=0.05. ***L**:*** 0-100 (Darkness- Lightness), ***a**:*** (+/-, red/green), ***b**:*** (+/-, yellow, blue)

Table 6. Significance levels (*P*) of correlation levels between colour values and HMF amounts of bread samples

Variables	Outer of bread *L**	Outer of bread *a**	Outer of bread *b**	Inner of bread *L**	Inner of bread *a**	Inner of bread *b**	HMF (mg/kg)
Outer of bread *L**	0	0.001	<0.0001	<0.0001	<0.0001	0.000	<0.0001
Outer of bread *a**	0.001	0	0.750	0.319	0.056	0.161	0.200
Outer of bread *b**	<0.0001	0.750	0	<0.0001	<0.0001	<0.0001	<0.0001
Inner of bread *L**	<0.0001	0.319	<0.0001	0	<0.0001	<0.0001	<0.0001
Inner of bread *a**	<0.0001	0.056	<0.0001	<0.0001	0	<0.0001	<0.0001
Inner of bread *b**	0.000	0.161	<0.0001	<0.0001	<0.0001	0	<0.0001
HMF (mg/kg)	<0.0001	0.200	<0.0001	<0.0001	<0.0001	<0.0001	0

Values in bold are different from 0 with a significance level alpha=0.05. ***L****: 0-100 (Darkness- Lightness), ***a****: (+/-, red/green), ***b****: (+/-, yellow, blue)

4. Conclusion

A comprehensive evaluation of the microbiological and chemical characteristics of Gümüşhane and Kürtün-Araköy bread dough samples revealed statistically significant differences. The analysis of bread samples from Kurdün-Araköy revealed a substantially elevated presence of lactic acid bacteria (LAB) on MRS agar and yeast-mold populations. However, no statistical differences were detected in LAB on M17 agar and TAMB counts. Chemical analyses distinguished the Kürtün-Araköy breads with significantly lower dry matter content and pH values. In contrast, higher levels of ash, protein, titratable acidity and hydroxymethylfurfural (HMF) concentrations were observed in the Kürtün-Araköy bread samples compared to the Gümüşhane bread samples. Furthermore, a significant variation was identified in the colourimetric profiles of the two distinct groups of bread, indicative of their varied compositional characteristics. The results obtained demonstrate the microbiological and chemical uniqueness of Kürtün-Araköy and Gümüşhane breads, and also provide critical baseline data for the conservation, evaluation and future scientific research of traditional bread varieties unique to the region.

CRediT authorship contribution statement

Büşra ÇİMEN: Formal Analysis; Investigation; Methodology; Writing - review & editing. Fırat YILMAZ: Conceptualization; Data curation; Investigation; Resources; Writing - original draft; Writing - review & editing. Formal analysis.

Declaration of competing interest

The authors declared that there is no conflict of interest.

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