



Hydrogen incorporation into butter improves its microbial and chemical stability, biogenic amine safety, quality attributes, and shelf-life

Duried Alwazeer^{a,b,c,*}, Menekşe Bulut^{b,c,d}, Mehmet Murat Ceylan^e, Yasemin Çelebi^f, Enes Kavrut^{b,c,d}, Yunus Çetintaş^g, Ali Tekin^{h,i}, Ali Adnan Hayaloğluⁱ

^a Department of Nutrition and Dietetics, Faculty of Health Sciences, Iğdır University, 76000 Iğdır, Turkey

^b Research Center for Redox Applications in Foods (RCRAF), Iğdır University, 76000 Iğdır, Turkey

^c Innovative Food Technologies Development, Application, and Research Center, Iğdır University, 76000, Iğdır, Turkey

^d Department of Gastronomy, Faculty of Tourism, Iğdır University, 76000, Iğdır, Turkey

^e Department of Gastronomy, Hatay Mustafa Kemal University, Hatay, Turkey

^f Department of Food Processing, Eşme Vocational School, Uşak University, 64000, Uşak, Turkey

^g Food Analysis Application and Research Center, Research Laboratories Center, Muğla Sıtkı Koçman University, 48000, Muğla, Turkey

^h Department of Food Processing, Keban Vocational School, Fırat University, 23700, Elazığ, Turkey

ⁱ Department of Food Engineering, Inönü University, 44280 Malatya, Turkey

ARTICLE INFO

Keywords:

Butter
Hydrogen gas
Nitrogen gas
Butylated hydroxytoluene
Quality attributes

ABSTRACT

Butter is susceptible to chemical and biological degradation. Hydrogen (H₂) and nitrogen (N₂) gases and BHT were incorporated into the butter, packaged into polyethylene laminated aluminum bags, and stored at 4 °C for 90 days. The microbial counts, peroxide value (PV), titratable acidity (TA), acidity degree value (ADV), and conjugated dienes and trienes (CD and CT) were examined. The samples' color and biogenic amines, amino acids, and volatile profiles were analyzed. At the end of storage, microorganisms grew slower in the H₂ samples than in the other groups. The highest values of fat deterioration were determined for the control, while the lowest values were for the H₂- and BHT-incorporated samples. All biogenic amines (BAs) showed the highest values in the control, while the lowest ones were observed in the BHT samples, followed by H₂ and N₂. H₂ incorporation into butter limited the spoilage microorganism growth, improved the color, restricted the formation of BAs (44–61%), increased the levels of some amino acids (Met and Lys), and didn't give an off-flavor. The physicochemical results showed that H₂ extended the shelf life of butter by 30 days. H₂ incorporation can help the dairy industry extend shelf life and maintain the product's quality and safety.

1. Introduction

Yayik butter is a high-fat product with an intense aroma and taste. However, the unsaturated fat and water content makes butter susceptible to many chemical and biological factors, especially oxidative reactions, that lead to the deterioration of color, texture, aroma, and taste, destruction of pigments and fat composition, and formation of off-flavors (Kong & Singh, 2016). Traditional butter production methods involve adding salt (1–3% salt) or LAB cultures to create a low-pH product and produce antimicrobial compounds. However, butter has a short shelf life as it contains many unsaturated fats, which are susceptible to oxidation. Therefore, there is a need to use a “green” preservation method to extend the shelf life of butter.

Most of the oxidation-susceptible fatty acids in butter are

monounsaturated (e.g., oleic, palmitoleic, myristoleic acids) and polyunsaturated (e.g., linoleic and linolenic acids), which make up around 30% of the total fatty acid composition (Pădureț, 2021). Synthetic antioxidants such as BHT and BHA have been proposed to protect the product against oxidative reactions (Carocho & Ferreira, 2013). However, these synthetic antioxidants are prohibited by many countries due to their potential health risks (Zhang et al., 2010). Additionally, the increasing consumer awareness of the health disorders caused by synthetic additives has prompted food producers to look for natural alternatives (Pateiro et al., 2018).

Hydrogen gas (H₂) is a natural antioxidant with some unique characteristics, such as small size, tastelessness, colorlessness, high diffusivity, inertness, non-toxicity, and anti-radical properties, as well as other specific chemical, physical, and biological properties (Alwazeer,

* Corresponding author. Department of Nutrition and Dietetics, Faculty of Health Sciences, Iğdır University, 76000 Iğdır, Turkey.

E-mail address: alwazeerd@gmail.com (D. Alwazeer).

<https://doi.org/10.1016/j.lwt.2024.116550>

Received 3 June 2024; Received in revised form 23 July 2024; Accepted 25 July 2024

Available online 30 July 2024

0023-6438/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC license (<http://creativecommons.org/licenses/by-nc/4.0/>).

2024a). These characteristics of H₂ have led researchers in the food sector to investigate the potential of using H₂ in food. In the dairy field, our team recently found that the use of H₂-rich water (HRW) in the washing process of raw cultured butter could improve the quality attributes (Ceylan, Bulut, Alwazeer, & Koyuncu, 2022), limit the formation of biogenic amines (Bulut, Çelebi Sezer, Ceylan, Alwazeer, et al., 2022), and decrease the heavy metal content (Alwazeer, Ceylan, Bulut, & Koyuncu, 2022) of butter. There is no study on the potential impact of H₂ incorporation into butter on the product's quality characteristics and safety aspects. Despite the numerous benefits of using hydrogen in food, safety concerns pose a serious challenge for the industry due to its explosive properties when combined with air (Alwazeer, 2024b).

This study aimed to evaluate the effects of the incorporation of H₂ on the microbiological, chemical, nutritional, and sensory properties, as well as the safety of butter, during 90 days of storage at 4 °C. The method of incorporating hydrogen into butter differs from the typical hydrogenation process of vegetable oils, which requires high temperatures and pressures and the presence of a catalyst responsible for forming trans fatty acids (Dhaka, Gulia, Ahlawat, & Khatkar, 2011). Thus, the effects of injecting H₂ compared with N₂ and BHT into butter were investigated on microbial counts, deteriorative indicators, sensory scores, biogenic amines, amino acids, and volatile compounds of butter.

2. Materials and methods

2.1. Materials

Fresh cow's milk was obtained from a local farmer (İğdır, Türkiye) in November 2022. Microbiological media were purchased from Merck (Darmstadt, Germany). All the amino acid standards, including glycine (Gly), alanine (Ala), serine (Ser), proline (Pro), valine (Val), threonine (Thr), cysteine (Cys), *trans*-4-hydroxyproline (Hyp), isoleucine (Ile), leucine (Leu), asparagine (Asn), aspartic acid (Asp), lysine (Lys), glutamine (Gln), glutamic acid (Glu), methionine (Met), histidine (His), phenylalanine (Phe), arginine (Arg), tyrosine (Tyr), and tryptophan (Trp) were purchased from Sigma-Aldrich (Steinheim, Germany). The remaining reagents were of analytical grade and were purchased from Merck (Darmstadt, Germany). The biogenic amines (histamine, tryptamine, tyramine, phenylethylamine, putrescine, and cadaverine) in HPLC-grade (purity 98–99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Yayık butter preparation

The milk was pasteurized (85 °C for 15 min) and cooled to 43 °C. The pasteurized milk was inoculated with yogurt starter culture (2%, v/v) (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) (Lactoferm Biochem, Italy) and incubated overnight at 43 °C to a pH 4. The yogurt formed was kept at 4 °C for 6 h. Then, the cold yogurt was diluted with drinking water at a 1:1 ratio (v/v) and cooled to 10 °C. The diluted yogurt was then placed in a churning machine (Minisan, Kayseri, Türkiye) and churned for 20 min. The resulting raw yayık butter was washed twice with cold water (4 °C) to remove undesirable flavor or aromas as well as residual buttermilk and milk solids (Bulut, Çelebi Sezer, Ceylan, Alwazeer, et al., 2022). The washed yayık butter was stored at 4 °C until the next step (Fig. S1).

2.3. Incorporation of hydrogen and nitrogen gases into butter

The washed butter was melted by incubation at 35 °C overnight. The melted butter was then placed in a water bath (40 °C) (DAIHAN, WB-22, Korea) until completely melted. The H₂ and N₂ gases were separately injected into the melted butter at a flow rate of 1 L min⁻¹ for 10 min using a sterile 1 mL pipette and a sterile hand mixer (Arçelik, Türkiye), which assisted in the distribution of the gas inside the butter. Similarly, BHT (200 ppm; Sigma-Merck) was added to another butter sample for

comparison purposes (this maximum value was used according to the Codex Alimentarius (Alimentarius, 2023)), and the medium was mixed for 10 min using a sterile hand mixer. Subsequently, 150 g of the gas-incorporated, BHT-containing, and untreated butter samples (control) were placed in sterile Ziplock bags made of laminated polyethylene (Plain Glossy, India) without leaving a headspace. An aluminum layer provides impermeability to gases and moisture in the packaging (Deshwal & Panjagari, 2020). The hermetically sealed butter bags were stored at 4 °C for 90 days (Fig. S1).

2.4. Analysis methods

2.4.1. Microbiological analyses

The microbial groups were counted using decimal dilution and bacterial colony counting methods (Bulut, Çelebi Sezer, Ceylan, Alwazeer, et al., 2022). The counts of microbial groups were performed using decimal dilution and bacterial colony count methods. The counts of total mesophilic aerobic bacteria (TMAB) and total psychrophilic aerobic bacteria (TPAB) were determined using Plate Count Agar and an incubation period of 37 °C for 18–24 h and 4 °C for 18–24 h, respectively. The yeast-mold counts were determined using Potato Dextrose Agar and incubated at 25 °C for 7 days. The bacterial counts of *S. thermophilus* and *L. delbrueckii* subsp. *Bulgaricus* were determined using M17 agar (aerobic conditions at 45 °C for 3 days) and MRS agar (anaerobic incubation at 45 °C for 3 days), respectively. The microbial counts were expressed as the decimal logarithm of colony-forming units per gram of butter (log CFU g⁻¹).

2.4.2. Chemical deteriorative parameters

The peroxide value (PV) was determined according to the AOCS Cd8 method (Anonymous, 1989b), and the PV was expressed as milliequivalents of oxygen contained in 1 kg of butter. The titratable acidity (TA) value was measured according to the AOCS Ca 5a-40 method (Anonymous, 1989a), and the TA value was expressed as a percentage of lactic acid (%). The acid degree value (ADV) was determined using the method of Anonymous (1991). Conjugated dienes (CD) and trienes (CT) were analyzed according to the official AOCS method Ch 5–91 (Anonymous, 1989c).

2.4.3. Color analysis

The color traits (CIELAB), i.e., brightness (L*), red/green (a*), and yellow/blue (b*), of butter samples were measured using a colorimeter (CR-410, Konica Minolta, Japan) (Ceylan et al., 2022).

2.4.4. Determination of biogenic amines

A chromatographic method was used to determine the BA content according to the procedure outlined by (Bulut, Çelebi Sezer, Ceylan, & Alwazeer, 2022).

2.4.5. Analysis of free amino acids

The FAAs were conducted using UPLC-ESI-MS/MS according to (Çelebi et al., 2024).

2.4.6. Volatile compounds analysis

Volatile compounds (acids, alcohols, aldehydes, esters, ketones, terpenes, and miscellaneous compounds) of butter samples were determined using SPME-GC/MS according to (Çelebi et al., 2024).

2.4.7. Statistical analysis

One-way analysis of variance (One-way ANOVA) was performed to determine significant differences between groups ($p < 0.05$), and Duncan's test was used to assess the level of difference ($p < 0.05$) with the SPSS (version 20.0, SPSS Inc., Chicago, Illinois) package program. Triplicate experiments ($n = 3$) were performed, and results were expressed as mean $\pm$ SD. The data of volatile compounds in yayık butter (acids, alcohols, aldehydes, esters, ketones, terpenes, and miscellaneous

compounds) at the 0th and 90th days of storage were analyzed by using Principal Component Analysis (PCA; SPSS version 20) and heat mapping by R software (version 3.6.2 (2019-12-12)-CRAN).

3. Results and discussion

3.1. Microbiological evaluation

The progress in the counts of different microbial groups found in butter samples during storage is shown in Table 1. The evolution of the yogurt starters (*Lactobacillus delbrueckii* subsp. *Bulgaricus* and *Streptococcus thermophilus*) changed depending on the type of application. The two starter strains grew better in both the control and BHT samples during cold storage than in the other butter groups ($P < 0.05$), while N₂ and H₂-incorporated samples showed a stable trend (stationary phase) in their counts ($P > 0.05$). Similar results were reported for cultured butter washed with HRW, in which the counts of yogurt starter strains remained stable during 90 days of cold storage (Bulut, Çelebi Sezer, Ceylan, Alwazeer, et al., 2022).

Both the H₂- and N₂ butter samples were degassed (the air was removed) during the gas injection procedure, creating anaerobic conditions. Although yogurt starters are facultative anaerobes, they generally grow better and are more active in low oxygen environment (Gänzle, 2015). *S. thermophilus* grew better in H₂ butter samples compared to *L. delbrueckii* subsp. *Bulgaricus*. Bulut et al., 2023a reported that H₂-incorporated milk samples promoted the acidification (metabolization) of the mixed culture of *L. delbrueckii* subsp. *Bulgaricus* and *S. thermophilus*. Higher counts of yogurt starter bacteria were obtained in butter samples containing BHT. The number of TMAB increased in all butter groups during storage; however, at the end of storage, i.e., on the 90th day, both the control (13.66 log CFU g⁻¹) and N₂ (13.67 CFU g⁻¹) butter samples showed higher counts than those of H₂ (11.39 log CFU g⁻¹) and BHT (11.59 CFU g⁻¹) butter samples ($P < 0.05$) (Table 1). Both the H₂ and BHT butter samples are characterized by their low redox values (−222 mV and −246 mV, respectively), which may be the cause of these differences in microbial growth compared to the control and N₂ butter samples (+349 mV and +316 mV, respectively) (data not shown).

It has been reported that the reducing compounds can affect the growth and metabolism of microorganisms such as LAB (bib_waché_et_al_2002 Waché, Riondet, Diviès, & Cachon, 2002). Another report indicated that the combination of reducing conditions and low pH led to a drastic decrease in the growth parameters of *Bacillus cereus* (Le Lay, Bahloul, Sérino, Jobin, & Schmitt, 2015). This latter case is similar to the BHT and H₂ butter samples, where reducing conditions were created.

TPAB counts increased during 90 days of refrigerated storage. Similar to the yogurt starter bacteria, TPAB grew better in both the control and BHT samples, while their counts remained stable in the N₂ and H₂ butter samples ($P < 0.05$). The anaerobic conditions created during the degassing process (H₂ and N₂ injection) could be responsible for these differences, while aerobic conditions are favorable for many bacterial strains of TPAB. Psychrophilic bacteria have been reported to be the most important bacteria associated with aerobic spoilage of dairy products at refrigerated temperatures (Ebrahimian, Mehdiadeh, & Aliakbarlu, 2023). Furthermore, results comparable with the present study were obtained for Iranian butter, where both BHT- and tocopherol-containing samples showed an increase in TPAB counts by approximately 6 and 5 log CFU g⁻¹, respectively, while control samples showed approx. 7 log CFU g⁻¹ during 90 days of storage at 4 °C (Ebrahimian et al., 2023).

The number of the yeasts gradually increased in all butter groups during the 90-day cold storage. At the end of storage, yeast-mold counts were the lowest in H₂ butter samples (11.15 log CFU g⁻¹) ($P < 0.05$) compared with control (13.94 CFU g⁻¹), N₂ (13.09 CFU g⁻¹), and BHT (14.48 CFU g⁻¹) ones, without significant differences between the three later groups ($P > 0.05$) (Table 1). Similar results were reported in white

Table 1

Microbial counts of yayik butter samples during cold storage.

Microorganism	Group	Log (CFU/g)			
		Storage (day)			
		0	30	60	90
<i>Lactobacillus delbrueckii</i> subsp. <i>Bulgaricus</i>	Control	9.68 ± 0.45 ^{cB}	7.12 ± 0.05 ^{dB}	11.12 ± 0.17 ^{bA}	12.89 ± 0.27 ^{aA}
	N ₂	11.28 ± 0.13 ^{aA}	7.91 ± 0.12 ^{cAB}	10.55 ± 0.07 ^{bB}	10.84 ± 0.34 ^{abC}
	H ₂	10.39 ± 0.38 ^{aB}	6.86 ± 0.33 ^{bB}	9.96 ± 0.01 ^{aC}	10.39 ± 0.13 ^{aC}
	BHT	8.65 ± 0.07 ^{bC}	9.98 ± 1.52 ^{abA}	11.52 ± 0.34 ^{aA}	11.89 ± 0.05 ^{aB}
<i>Streptococcus thermophilus</i>	Control	8.40 ± 0.77 ^{bAB}	7.55 ± 0.49 ^{bBC}	11.15 ± 0.28 ^{aA}	11.55 ± 0.21 ^{aA}
	N ₂	9.97 ± 0.53 ^{aA}	9.38 ± 0.98 ^{aA}	10.23 ± 0.33 ^{aB}	10.65 ± 0.49 ^{aB}
	H ₂	8.56 ± 0.28 ^{aB}	7.02 ± 0.02 ^{cC}	10.24 ± 0.08 ^{aB}	10.39 ± 0.13 ^{aB}
	BHT	7.39 ± 0.54 ^{cB}	8.72 ± 0.24 ^{bAB}	11.61 ± 0.35 ^{aA}	12.20 ± 0.28 ^{aA}
TMAB (37 °C)	Control	8.67 ± 0.47 ^{bB}	7.15 ± 0.19 ^{cB}	14.75 ± 0.68 ^{aA}	13.66 ± 0.17 ^{aA}
	N ₂	11.22 ± 0.38 ^{bA}	8.07 ± 1.04 ^{cAB}	11.78 ± 0.14 ^{bB}	13.67 ± 0.26 ^{aA}
	H ₂	9.42 ± 0.19 ^{bB}	7.67 ± 0.71 ^{cAB}	10.63 ± 0.09 ^{aB}	11.39 ± 0.13 ^{aB}
	BHT	8.73 ± 0.38 ^{cB}	10.20 ± 1.36 ^{bcA}	14.89 ± 1.19 ^{aA}	11.59 ± 0.16 ^{bB}
TPAB (4 °C)	Control	8.56 ± 0.28 ^{cAB}	7.50 ± 0.14 ^{dBC}	11.03 ± 0.13 ^{bA}	11.54 ± 0.12 ^{aA}
	N ₂	9.84 ± 0.62 ^{bA}	9.29 ± 0.93 ^{bA}	11.70 ± 0.33 ^{aA}	9.50 ± 0.28 ^{bB}
	H ₂	8.68 ± 0.85 ^{bAB}	7.06 ± 0.49 ^{bC}	10.68 ± 0.56 ^{aA}	8.45 ± 0.21 ^{bC}
	BHT	7.20 ± 0.35 ^{cB}	8.87 ± 0.14 ^{bAB}	11.43 ± 0.65 ^{aA}	9.35 ± 0.49 ^{bB}
Yeasts and molds	Control	8.72 ± 0.28 ^{cB}	7.60 ± 0.36 ^{dB}	11.64 ± 0.36 ^{bB}	13.94 ± 0.06 ^{aAB}
	N ₂	10.42 ± 0.23 ^{bA}	7.77 ± 0.33 ^{cAB}	11.17 ± 0.74 ^{bB}	13.09 ± 0.55 ^{aB}
	H ₂	9.43 ± 0.13 ^{bAB}	7.29 ± 0.16 ^{cB}	11.59 ± 0.37 ^{aB}	11.15 ± 0.21 ^{aC}
	BHT	6.55 ± 0.71 ^{cC}	9.53 ± 0.20 ^{bA}	15.32 ± 0.65 ^{aA}	14.48 ± 0.52 ^{aA}

A–C: The difference between values expressed with different capital letters in different samples in the same column on the same storage days was statistically significant ($P < 0.05$).

a–d: The difference between values expressed in different lowercase letters in the same rows on different storage days in the same samples is statistically significant ($P < 0.05$).

cheese packaged under an H₂-included atmosphere (Alwazeer, Örs, & Tan, 2020). The authors found that H₂ can inhibit yeast molds in white cheese during cold storage.

3.2. Peroxide value (PV)

Table 2 shows the PV of the butter samples during the 90-day cold storage period. The PV increased rapidly throughout storage for all butter groups. On the 0th day of storage, the PV was highest in the control samples (1.59 mEq O₂ kg⁻¹) ($P < 0.05$), while the lowest one was obtained for both BHT and H₂ butter samples (0.67 and 0.7 mEq O₂ kg⁻¹, respectively) without significant difference between them ($P > 0.05$). On the 30th day, the PV increased significantly to 7.51, 4.96, 3.67, and 3.46 mEq O₂ kg⁻¹ for the control, N₂, H₂, and BHT butter samples, respectively. The highest PV was found for the control and the lowest for BHT butter samples, followed by H₂ butter samples ($P < 0.05$). The PV of H₂ butter samples was the closest to BHT, followed by N₂ samples. Comparatively, the highest PV was shown for control samples (16.33 mEq O₂ kg⁻¹), and the lowest one was obtained for BHT (8.98 mEq O₂ kg⁻¹) ($P < 0.05$) butter samples after 60 days of storage. Again, comparable to day 30, the closest PV value to BHT butter samples was found for H₂ (9.73 mEq O₂ kg⁻¹) followed by N₂ (12.38 mEq O₂ kg⁻¹) ($P < 0.05$). At the end of storage (90th day), the highest PV was found for the control (18.88 mEq O₂ kg⁻¹) ($P < 0.05$), and the lowest one was shown for both BHT (10.93 mEq O₂ kg⁻¹) and H₂ (11.05 mEq O₂ kg⁻¹) butter samples without significant difference between them ($P > 0.05$) (Table 2).

The results show how important it is to remove the air and its component oxygen from the butter to limit peroxidation reactions. The unsaturated fats and oils are susceptible to oxidation reactions mainly due to oxidative factors such as free radicals, oxygen, and metal ions (Fig. 1). The ability of H₂ to scavenge the strongest free radicals, i.e., hydroxyl radicals (•OH) and peroxyxynitrite anions (ONOO⁻) (Alwazeer,

Liu, Wu, & LeBaron, 2021), could explain the low PV levels in H₂-incorporated butter samples. On the other hand, BHT has been reported to possess anti-radical scavenging activity (Hussein, 2011). BHT (100 mg L⁻¹) inhibits the peroxidation of linoleic acid emulsions by about 20% and scavenges hydrogen peroxide by 50% and hydroxyl radicals by 52%.

This beneficial result of PV could be achieved by injecting H₂ and N₂ into the butter, whereby the positive effect of H₂ was superior to that of N₂. These results show that adding H₂ to butter can lead to results comparable to or even superior to those of one of the powerful antioxidants used in fat-containing products, such as BHT. However, BHT is a synthetic food additive (E321) that was widely used in foods due to its high thermal stability and strong antioxidant capacity; however, its use in food products was prohibited due to the reports indicating its health risks (Wang et al., 2021); while H₂ is a “green” and natural antioxidant with many health benefits (Alwazeer, 2024a). The superiority of H₂ over BHT can be explained by the high diffusivity of H₂ and its small size. Similarly, our team found that the increase in PV was slowed when raw cultured butter was washed with HRW (Ceylan et al., 2022). A comparable inhibitory effect of H₂ on the progression of PV has been shown in other fatty products, such as crude olive pomace oil washed with HRW (Ceylan, Silgan, Elnasanelkasim, & Alwazeer, 2023) and rainbow trout fillets packaged under an H₂-included atmosphere (Bulut, Okutan, Alwazeer, & Boran, 2023). On the other hand, some synthetic antioxidants, e.g., BHT and BHA, or natural antioxidants, e.g., tocopherols, have been reported to slow down the formation of peroxides in oils or fats (Ozturk & Cakmakci, 2006).

3.3. Titratable acidity

The value of TA increased progressively during the storage period (Table 2). On the 0th day of storage, TA was obviously lower in both BHT and H₂ butter samples than in control and N₂ butter samples, with 2.66, 2.55, 3.32, and 3.19%, respectively ($P > 0.05$). On both the 30th and 60th day, H₂ butter samples showed the lowest TA values, followed by the BHT samples, while on the 90th day, BHT again had the lowest TA values, followed by H₂ ($P < 0.05$). On all storage days, the control samples had the highest TA value ($P < 0.05$).

Similar results were reported when raw cultured butter was washed with HRW, where TA was slowed down during 90 days of cold storage (Ceylan et al., 2022). In another study, the TA value of crude olive pomace oil decreased significantly when the product was washed with HRW (Ceylan et al., 2023).

The outcomes of the present and the previous studies showed an inhibitory effect of H₂ on the progress of the acidity of the product during storage (Fig. 1). Although this result could be obtained by injecting both H₂ and N₂ into the butter, the H₂ application showed a more pronounced effect than N₂. The exact mechanisms behind the inhibitory effect of H₂ on limiting acid development need further research. H₂ has a low redox potential (or ORP) value that can affect the metabolic activity of LAB (used in the cultured butter preparation), leading to modification in metabolite ratios such as organic acids (lactic and acetic acids) (Alwazeer, 2020). The ORP value of the medium/-product can affect the synthesis/stability of some fermentation enzymes of bacteria, e.g., *Escherichia coli* (Riondet, Cachon, Waché, Alcaraz, & Diviès, 2000).

3.4. Acid degree value

The acid degree values (ADV) of yayik butter samples are shown in Table 2. The ADV increased in all butter groups over the storage period. On the 0th day, the highest value was shown for the control (0.63%) and N₂ (0.60%) butter samples, while the lowest values were obtained for H₂ (0.24%) followed by BHT (0.40%) butter samples ($P < 0.05$). On the 30th day, BHT butter samples showed the lowest ADV values (0.83%), followed by H₂ (0.90%) and N₂ (1.05%) butter samples, while the

Table 2
Physicochemical parameters of yayik butter samples during cold storage.

Sample	Storage (day)			
	0	30	60	90
Peroxide value				
Control	1.59 ± 0.39 ^{aC}	7.51 ± 0.01 ^{aD}	16.33 ± 0.80 ^{bD}	18.88 ± 0.27 ^{cC}
N ₂	0.95 ± 0.26 ^{aB}	4.96 ± 0.64 ^{aC}	12.38 ± 0.10 ^{bC}	15.72 ± 0.26 ^{cB}
H ₂	0.70 ± 0.07 ^{aA}	3.67 ± 0.06 ^{aB}	9.73 ± 0.25 ^{bB}	11.05 ± 0.84 ^{cA}
BHT	0.67 ± 0.58 ^{aA}	3.46 ± 0.14 ^{aA}	8.98 ± 0.30 ^{aA}	10.93 ± 0.30 ^{dA}
Titratable acidity (%)				
Control	3.32 ± 0.19 ^{aA}	5.65 ± 0.03 ^{bD}	6.57 ± 0.04 ^{cC}	7.32 ± 0.09 ^{dD}
N ₂	3.19 ± 0.69 ^{aA}	5.40 ± 0.06 ^{bC}	6.41 ± 0.13 ^{cC}	7.06 ± 0.11 ^{cC}
H ₂	2.55 ± 0.03 ^{aA}	4.71 ± 0.02 ^{bA}	5.74 ± 0.04 ^{aA}	6.86 ± 0.04 ^{dB}
BHT	2.66 ± 0.06 ^{aA}	4.97 ± 0.08 ^{bB}	6.12 ± 0.09 ^{cB}	6.70 ± 0.11 ^{dA}
Acid degree value				
Control	0.63 ± 0.01 ^{aC}	1.77 ± 0.02 ^{bD}	2.19 ± 0.04 ^{cC}	2.56 ± 0.05 ^{dC}
N ₂	0.60 ± 0.01 ^{aC}	1.05 ± 0.01 ^{bC}	2.04 ± 0.07 ^{cB}	2.41 ± 0.03 ^{dB}
H ₂	0.24 ± 0.01 ^{aA}	0.90 ± 0.01 ^{bB}	1.83 ± 0.04 ^{cA}	2.05 ± 0.04 ^{dA}
BHT	0.40 ± 0.01 ^{aB}	0.83 ± 0.03 ^{bA}	1.93 ± 0.06 ^{cAB}	2.11 ± 0.01 ^{dA}
Conjugated diene value				
Control	0.59 ± 0.01 ^{aD}	1.23 ± 0.01 ^{bC}	1.44 ± 0.01 ^{cC}	1.65 ± 0.02 ^{dB}
N ₂	0.52 ± 0.01 ^{aC}	1.09 ± 0.02 ^{bB}	1.37 ± 0.01 ^{cB}	1.47 ± 0.03 ^{dA}
H ₂	0.18 ± 0.01 ^{aB}	0.89 ± 0.02 ^{bA}	1.33 ± 0.05 ^{cA}	1.45 ± 0.04 ^{dA}
BHT	0.09 ± 0.03 ^{aA}	1.06 ± 0.04 ^{bB}	1.31 ± 0.01 ^{cA}	1.46 ± 0.04 ^{dA}
Conjugated triene value				
Control	0.08 ± 0.01 ^{aB}	0.13 ± 0.00 ^{bB}	0.16 ± 0.01 ^{cC}	0.22 ± 0.01 ^{dB}
N ₂	0.07 ± 0.00 ^{aB}	0.13 ± 0.01 ^{bAB}	0.15 ± 0.00 ^{cC}	0.18 ± 0.01 ^{dA}
H ₂	0.03 ± 0.01 ^{aA}	0.12 ± 0.01 ^{bA}	0.13 ± 0.00 ^{bA}	0.17 ± 0.01 ^{cA}
BHT	0.04 ± 0.01 ^{aA}	0.12 ± 0.00 ^{bAB}	0.14 ± 0.00 ^{cB}	0.16 ± 0.00 ^{dA}

A – D For each storage, different superscript uppercase letters show differences between the samples ($P < 0.05$).

a – d Within the same yayik butter sample, different superscript lowercase letters show differences between the storage ($P < 0.05$).

BHT. BHT added yayik butter, H₂. Yayik butter with hydrogen, N₂. Yayik butter with nitrogen.

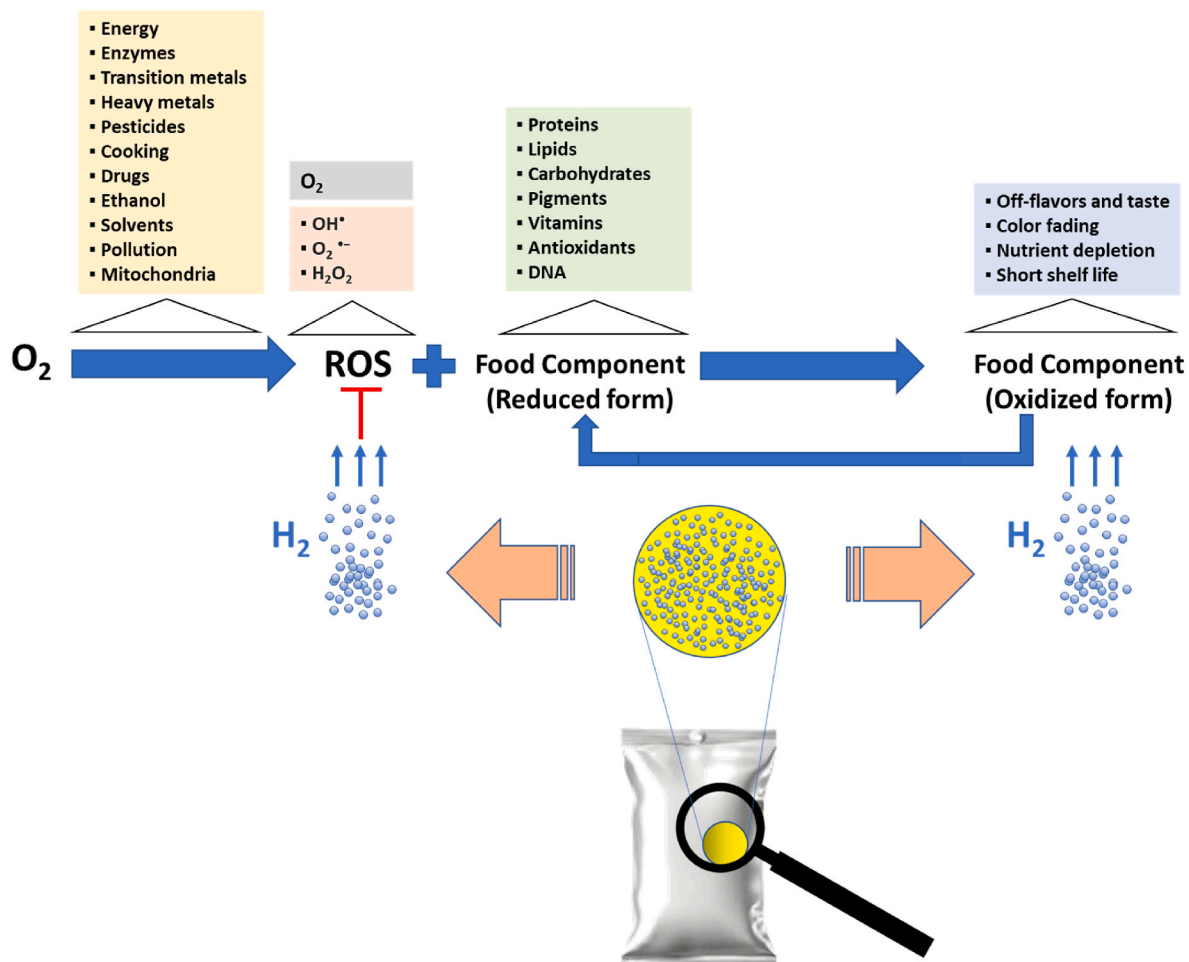


Fig. 1. Possible mechanisms of hydrogen protective effects on food quality and safety (Çelebi et al., 2024).

control (1.77%) exhibited the highest levels ($P < 0.05$). On the 60th and 90th day of storage, control samples kept their highest levels of ADV, while H₂ and BHT butter samples were at the lowest levels without significant differences ($P > 0.05$). Comparable results were obtained for cultured butter washed with HRW, which exhibited lower ADV levels (Ceylan et al., 2022).

The results showed that H₂ can limit the increase in acidity of the butter samples and that this ability is similar to or close to that of the synthetic antioxidant, i.e., BHT. The results also revealed the usefulness of removing air (oxygen) from butter during processing to keep lower acidity levels (Fig. 1). This result could be achieved by injecting H₂ and N₂ into the butter during the process, with H₂ showing a more substantial effect than N₂.

It is known that the ADV of fat-containing foods increases with storage time. Oxidation and lipolytic reactions are essential for improving ADV (Mallia, Escher, & Schlichtherle-Cerny, 2008). The results of the present study suggest that H₂ may limit the lipolytic reactions of butter, leading to limited progress of ADV. Reducing conditions prepared by bubbling H₂/N₂ (5/95, vol%) caused a decrease in the proteolytic activity of a mixture of two LAB strains (*Pediococcus pentosaceus* and *Weissella cibaria*) during sourdough fermentation (Capuani, Werner, Behr, & Vogel, 2014).

Further studies will reveal the mechanisms behind this inhibitory effect of H₂ on lipolytic reactions. However, H₂ can terminate the free radical reaction chains and scavenge the strongest radicals, i.e., hydroxyl radical and peroxynitrite (Fig. 1) (Alwazeer et al., 2021).

3.5. Conjugated diene-triene

The changes in conjugated diene (CD) and triene (CT) values in the butter samples are shown in Table 2. Results showed that the CD and CT values increased through the storage time. After the incorporation of gases (H₂ and N₂) and BHT into the butter was completed, i.e., 0th day, the highest CD values were observed for the control (0.59) followed by N₂ (0.52) butter samples, and the lowest ones were noticed for BHT (0.09) butter samples followed by H₂ (0.18) samples ($P < 0.05$). This trend of the inhibitory effect of H₂ and BHT continued during 60 days of storage, and at the end of storage, i.e., on the 90th day, each of the H₂, N₂, and BHT butter samples showed lower CD values than control ones ($P < 0.05$).

Peroxides, the primary degradation products of the oxidation reaction, are associated with conjugated diene structures (Ceylan & Basturk, 2022). Therefore, an increase in CD during storage can be associated with the formation of peroxides (Table 2).

Regarding the CT, its value increased with the storage time. On the 0th day, both control (0.08) and N₂ (0.07) butter samples showed the highest CT values, while the H₂ (0.03) and BHT (0.04) had the lowest values ($P < 0.05$), without significant difference between H₂ and BHT butter samples ($P > 0.05$). The inhibitory effect of H₂ on CT was more evident on both the 30th and 60th day of storage, showing a slight decrease in CT value, but this was significant compared to BHT ($P < 0.05$). On the 90th day, the two gases (H₂ and N₂) and BHT butter samples showed a significant decreasing effect on CT value ($P < 0.05$).

Components such as aldehydes and ketones, produced by the degradation of peroxides, increase the CT value (Hamed & Abo-Elwafa,

2012). Therefore, the fact that the butter samples have lower CD values, especially in the H₂ and BHT groups, means that the process of formation of volatile components such as aldehydes and ketones has been limited (Kalua et al., 2007). Fig. 1 shows the possible mechanisms of the protective effect of H₂ on quality attributes and product safety parameters.

3.6. Color properties

Table 3 shows the changes in the color parameters of the butter samples during cold storage. The L* value increased during storage for all butter groups ($P < 0.05$). After the incorporation process, the color analysis showed the highest L* values for BHT butter samples (58.39), followed by H₂ (57.34) and N₂ (56.99) ones, with the lowest values observed for the control (55.09) samples ($P < 0.05$). At the end of storage, all butter groups showed similar L* values ($P > 0.05$).

Regarding the a* value, on the 0th day, all butter groups showed slightly negative values, indicating a slightly green note. This value increased slightly during storage for all butter groups. At the end of storage, i.e., on day 90, all butter groups had similar values for a* ($P > 0.05$).

The values of b* were in the range of yellow coloration, i.e., positive b*, and gradually increased during the storage time. On the 0th day, the BHT butter samples showed the lowest value (13.17) compared to the other groups, i.e., control, H₂, and N₂ ones, with 16.54, 16.19, and 14.91, respectively ($P < 0.05$). On both the 30th and 60th day, control samples exhibited the lowest b* values compared to the other groups ($P < 0.05$). At the end of storage, i.e., 90th day, all butter groups showed similar b* values ($P > 0.05$).

Results show that injecting H₂ and N₂ and adding BHT to the butter could lead to a higher value of b* than control samples. This finding may be explained by protecting fat-soluble carotenoids from oxidative reactions. The electron-rich conjugated double bond structure of β -

carotene makes it unstable against oxidation (Hejri, Khosravi, Gharanjig, & Davarani, 2019). The oxidative reactions of fats and pigments are the primary reason behind the change in food color. The use of antioxidants is a common practice as a measure to prevent oxidative reactions in food products. It has been reported that BHT could improve the chemical stability of β -carotene while vitamin C decreased it (Hejri et al., 2019). The incorporation of H₂ into butter samples led to an increase in the b* value, which means the protection of yellow-related pigments such as carotenoids (Fig. 1). In a recent study, washing raw cultured butter with HRW decreased the changes in b* value during 90 days of cold storage (Ceylan et al., 2022).

Similarly, washing crude olive pomace oil with HRW has led to a higher value of b* than non-HRW water-washed samples (Ceylan et al., 2023). H₂ is a non-polar antioxidant with a solubility value three times higher in fat than in water (4.32 vs. 1.38 mg L⁻¹ at 40 °C) (Ceylan et al., 2022). This property may be advantageous in protecting lipophile pigments such as carotenoids.

Butter is rich in fat and contains β -carotene, riboflavin, vitamin A, and other pigments responsible for butter's color (Milovanovic et al., 2020). Therefore, the color of butter is susceptible to many processing and storage-related deterioration factors. The results of the color analysis performed in this study are consistent with those typically found in butter (Milovanovic et al., 2020). The change in butter color depends on many factors, including color and size of fat globules, process conditions, type of packaging, and storage temperature (Milovanovic et al., 2020).

3.7. Biogenic amines profile

Fig. 2 shows the progress in the level of different BAs, including histamine, tryptamine, tyramine, phenylethylamine, putrescine, and cadaverine, in different butter samples during 90 days of cold storage at 4 °C. All BAs increased significantly during storage time ($P < 0.05$).

On the 90th day, the highest levels of BAs were found in the control group samples, while the lowest levels were reached in the butter samples containing BHT ($P < 0.05$), followed by both H₂ and N₂ butter samples ($P > 0.05$). The outcomes of the BA analysis showed that the injection of N₂ and H₂ into the butter during the process could significantly limit the formation of BAs (Fig. 2). As a result, all BA values were lowered when the injection of H₂ and N₂ during butter production was taken into account. Although the lowest BA levels were found for BHT, including butter samples, many reports point out the adverse effects of synthetic antioxidants, e.g., BHT, on health (Wang et al., 2021).

Biogenic amines (BAs) are nitrogenous compounds consisting of one or more amine groups formed during the decarboxylation of food amino acids by spoilage or fermentation microorganisms (Alwazeer, 2023). Most BAs are bioactive compounds that can negatively affect the central nervous and vascular systems (Alwazeer, Bulut, & Çelebi, 2022).

Only a few studies have reported the effects of adding BHT on forming BAs in products. One study was performed to evaluate the impact of the addition of sumac extract as well as BHT on the BA content of Turkish fermented sausage (sucuk) during the ripening stage. The study results showed that the addition of BHT could limit the formation of putrescine, histamine, and tyramine (Bozkurt, 2006). The results of the latter study are consistent with those of the present study, in which BHT exhibited an inhibitory effect on BA formation.

Comparable to our results, washing raw cultured butter with HRW could considerably restrict the formation of BAs through cold storage (Bulut, Çelebi Sezer, Ceylan, Alwazeer, et al., 2022). Additionally, the inclusion of H₂ in the atmosphere package could limit the formation of BAs, i.e., histamine, tyramine, putrescine, and cadaverine in two fish types (rainbow trout and horse mackerel) by cold storage (Sezer, Bulut, Boran, & Alwazeer, 2022). Furthermore, the use of HRW in the preparation of pickles limits the formation of many BAs, including tyramine, 2-phenylethylamine, histamine, tryptamine, and putrescine in red beetroot during fermentation (Bulut, Çelebi Sezer, Ceylan, Alwazeer,

Table 3
Evolution of color of butter samples during cold storage.

Feature	Group	Storage (day)			
		0	30	60	90
L*	Control	55.09 ± 0.24 ^{cC}	59.68 ± 0.03 ^{bB}	59.62 ± 0.17 ^{bB}	90.11 ± 0.52 ^{aA}
	N ₂	56.99 ± 0.25 ^{cB}	58.92 ± 0.26 ^{cB}	87.96 ± 0.73 ^{bA}	91.85 ± 2.10 ^{aA}
	H ₂	57.34 ± 0.50 ^{bB}	60.93 ± 0.66 ^{bA}	85.66 ± 6.79 ^{aA}	89.28 ± 3.15 ^{aA}
	BHT	58.39 ± 0.06 ^{cA}	58.83 ± 0.49 ^{cB}	90.14 ± 0.45 ^{aA}	87.87 ± 0.64 ^{bA}
a*	Control	-4.36 ± 0.23 ^{bC}	-3.03 ± 0.02 ^{aA}	-3.68 ± 0.21 ^{aA}	-5.04 ± 0.34 ^{cA}
	N ₂	-3.91 ± 0.06 ^{aB}	-3.72 ± 0.16 ^{aB}	-4.75 ± 0.05 ^{bB}	-4.89 ± 0.03 ^{bA}
	H ₂	-3.87 ± 0.07 ^{aAB}	-3.74 ± 0.03 ^{aB}	-4.69 ± 0.12 ^{bB}	-5.12 ± 0.20 ^{cA}
	BHT	-3.52 ± 0.11 ^{aA}	-3.49 ± 0.16 ^{aB}	-4.70 ± 0.21 ^{bB}	-4.55 ± 0.07 ^{bA}
b*	Control	16.54 ± 1.44 ^{bA}	13.61 ± 0.00 ^{cB}	18.78 ± 0.54 ^{bB}	26.19 ± 1.17 ^{aAB}
	N ₂	14.91 ± 0.18 ^{dAB}	17.00 ± 0.04 ^{cA}	23.39 ± 0.52 ^{bA}	26.83 ± 0.19 ^{aA}
	H ₂	16.19 ± 1.04 ^{cA}	17.95 ± 1.12 ^{cA}	23.46 ± 0.16 ^{bA}	26.06 ± 0.57 ^{aAB}
	BHT	13.17 ± 0.37 ^{cB}	16.95 ± 1.59 ^{bA}	24.26 ± 0.29 ^{aA}	24.59 ± 0.15 ^{aB}

^{a-c}: The difference between values expressed with different capital letters in different samples in the same column on the same storage days was statistically significant ($P < 0.05$).

^{a-d}: The difference between values expressed in different lowercase letters in the same rows on different storage days in the same samples is statistically significant ($P < 0.05$).

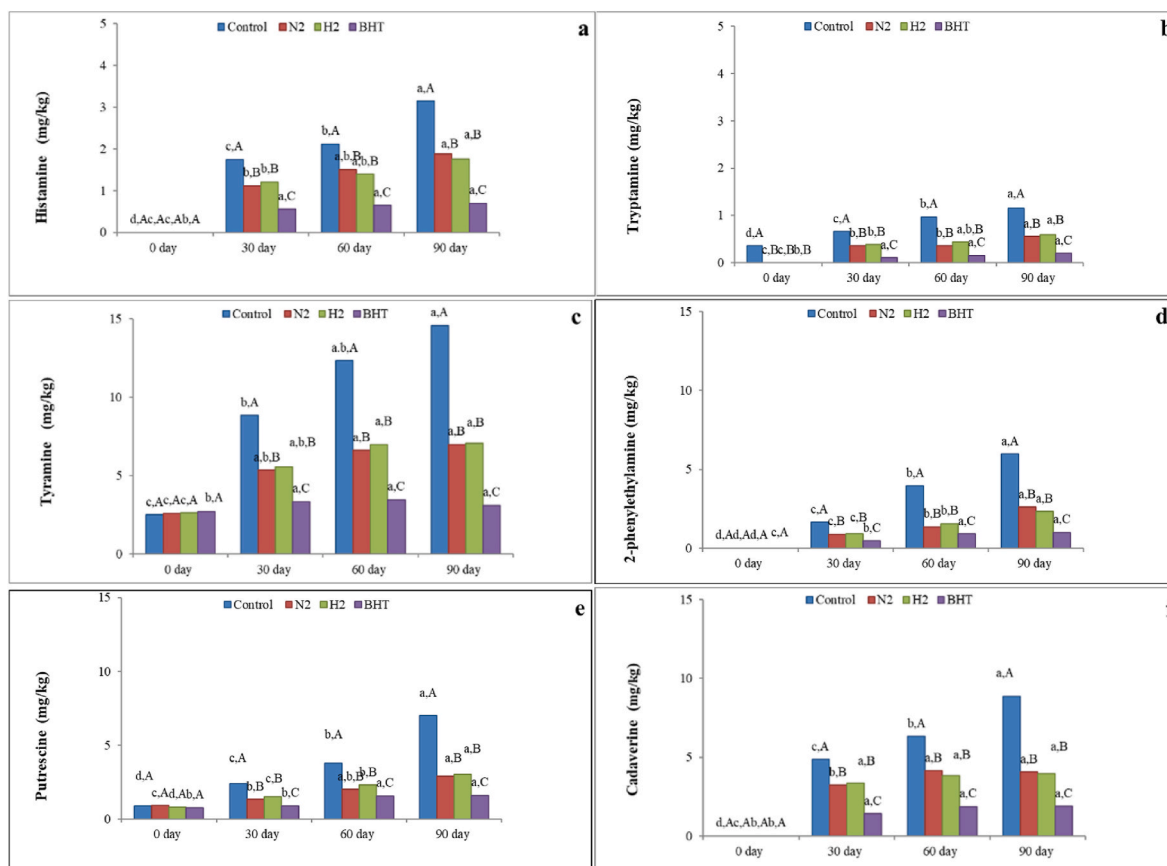


Fig. 2. Biogenic amine content (mg kg^{-1}) in yayik butter samples. a) Histamine, b) Tryptamine, c) Tyramine, d) Phenylethylamine, e) Putrescine, f) Cadaverine.

et al., 2022).

The low progress of BAs in the BHT butter samples, as well as H_2 and N_2 ones, can be discussed in the light of the low value of the redox potential in these three sample groups with -222 , -246 , and $+316$ mV, respectively, compared with that of control samples ($+349$ mV) (non-shown data). The injection of both N_2 and H_2 into the medium decreased the redox value, especially in the case of H_2 , thanks to its ability to reduce potential. However, H_2 is a small and light antioxidant that can be leaked during storage (Alwazeer et al., 2021). Thus, its reducing effect on the medium can be lost over time. At the same time, BHT is a synthetic compound with high stability and strong antioxidant capacity (Wang et al., 2021), and its reducing effect can continue for longer times. This finding may explain the superiority of BHT in restricting BA formation over H_2 . The different mechanisms behind the inhibitory effect of H_2 on the formation of BAs in food were discussed in a recently published review (Alwazeer, 2023).

On the other hand, BA-producing psychrophilic and psychrotrophic bacteria have been reported to have BA-production ability that can continue through the storage period (Benkerroum, 2016). This finding may explain the highest increase in BA formation in the control butter samples in which the number of psychrophilic aerobic bacteria (TPAB) was also highest. Similarly, the increase in TMAB and TPAB counts in control butter samples may be associated with the increased formation of BAs in these samples. Many milk and dairy-related spoilage and starter microorganisms were reported for their decarboxylase activity (Burdychova & Komprda, 2007).

3.8. Free amino acids profile

All essential and non-essential amino acids were detected in all yayik butter groups. However, the butter samples did not contain Gly, Ser, Cys, Hyp, and Asp. In the control samples, the contents of all amino acids

increased during storage, except for Ala, Pro, and Val, which decreased. The same trend was shown in BHT samples, except that Ile and Leu were also reduced. In H_2 and N_2 butter samples, the same increasing trend was also demonstrated except for Ile and the three others (Ala, Pro, and Val), which decreased (Table S1).

On the 0th day, the control samples showed the highest levels of most FAA among other groups, except for Leu, Phe, Tyr, Trp, Phe, Tyr, Trp, and Arg. On the 90th day, the control sample had the highest levels of Ala, Pro, and Val, while the highest levels of Met and Lys were found in the N_2 butter samples, followed by H_2 and BHT ones. The increase in the amino acid levels of the butter samples through the storage period may be associated with proteolysis. The increase in the levels of some BAs can be linked with the rise in the levels of their precursor, i.e., amino acids. For example, the levels of histamine, tryptamine, tyramine, 2-phenylethylamine, putrescine, and cadaverine were increased in the control samples. On the 90th day, the increase in amino acid levels, e.g., Try (244%), Arg (2156%), and Tyr (237%), correlated with the increase in the corresponding biogenic amines, i.e., tryptamine (328%), putrescine (793%), and tyramine (582%). The effect of both low redox value and H_2 on the decarboxylases was one of the possible mechanisms for the inhibitory effect of reducing agents (H_2 and BHT) on the formation of BAs (Alwazeer, 2023).

3.9. Volatile compounds profile

Analysis of the volatile compounds by GC-MS using the SPME technique revealed a total of 52 volatile compounds, including 22 esters, 8 alcohols, 6 aldehydes, five ketones, three acids, three terpenes, and five miscellaneous compounds (Table S2). It is worth noting that the volatile compound levels in all samples increased during storage (Fig. 3a). However, based on the heat map results of the data, it is evident that the control sample was distinct from the other samples at the end of the

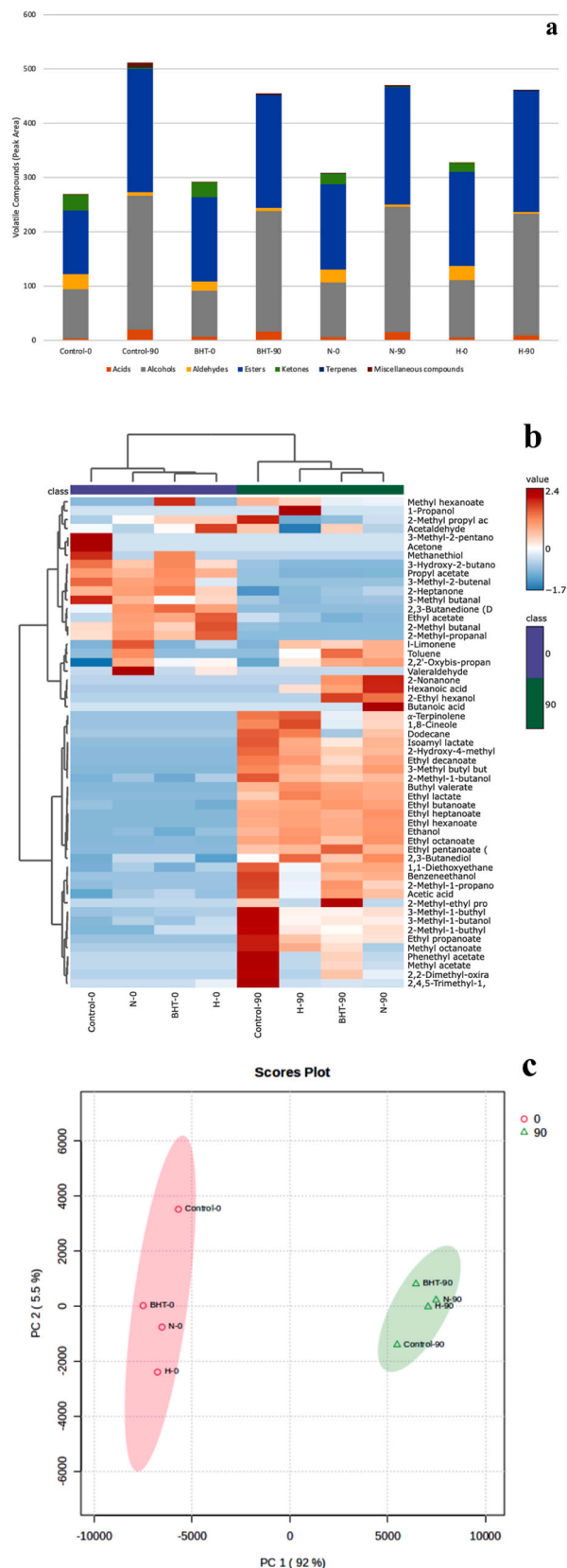


Fig. 3. Volatile compounds detected in yayik butter. a) Volatile chemical groups in yayik butter samples, b) Heat map of volatiles of yayik butter samples, c) Results of principal component analysis (PCA) of volatile compounds of yayik butter.

storage period (Fig. 3b). These findings are consistent with the results of the microbiology and spoilage parameter tests (PV, TA, ADV, CD, and CT). While the quantity of volatile compounds increased during storage in all groups, it is crucial to recognize that some compounds contribute to the desired flavor. In contrast, others may result in undesirable odors. Microbial growth, lipid oxidation, and lipolysis levels during butter storage can lead to the development of off-flavors (Mallia et al., 2008).

Esters were the most abundant group in terms of the number of volatile compounds (Table S2), while alcohols and esters were the most important compound groups in all sample treatments in terms of the amount (peak area) of volatile compounds (Fig. 3a). Esters, which are flavoring compounds in fermented milk products, provide flavor balance at low concentration, but at high concentrations, they can cause undesirable fruity odors (Tahmas-Kahyaoglu, Cakmakci, & Hayaloglu, 2022). Gundogdu, Cakmakci, and Hayaloglu (2020) reported that the type of starter, raw material, and storage time influenced the concentrations of esters in cream butter and yogurt butter samples. The formation of alcohols is related to the oxidation of unsaturated fatty acids or the degradation of aldehydes (Oussou et al., 2022). The decreasing amount of aldehydes and the increasing amount of alcohols during storage also support this statement. Alcohols can also be produced by amino acid catabolism and glycolysis (Sarhir, Amanpour, Bouseta, & Selli, 2021).

Ethanol and ethyl acetate were the compounds with the highest amounts among all the compounds (Table S2). They were distinct from all the other compounds, as evident in the heat map results (Fig. 3b). Additionally, ethyl hexanoate, ethyl lactate, and 3-methyl-1-butanol were the compounds that separated from the other butter samples (Fig. 3b). The amount of ethyl acetate remained high during storage. Still, ethyl lactate and ethyl hexanoate increased significantly in all samples at the end of the storage period. The formation of ethyl esters can help reduce bitterness and pungency resulting from acids and amines (Tahmas-Kahyaoglu et al., 2022). Some lactic acid bacteria (LAB) possess aldehyde and alcohol dehydrogenase enzymes that catabolize glucose to acetaldehyde and ethanol (Gundogdu et al., 2020). The significant increase in ethanol during storage and the presence of acetaldehyde, the most prominently detected compound among aldehydes, can be attributed to LAB activation (Table 1).

Another compound separated from other compounds in the heat map graph is 3-hydroxy-2-butanone (acetoin) (Fig. 3b). Acetoin imparts a buttery odor, and LAB can produce it by reduction of diacetyl or conversion of lactose; pyruvate, and citrate (Sarhir et al., 2021). Acetoin, formed by diacetyl reduction, can be reduced during storage and transformed into 2,3-butanediol and other compounds (Tahmas-Kahyaoglu et al., 2022). The decreasing acetoin content during storage is consistent with the findings of Tahmas-Kahyaoglu et al. (2022) obtained in cow butter. Acetic acid increased in all samples during storage, with the most significant increase in the control sample. This result is consistent with the titratable acidity and acidity degree value results. Carboxylic acids are generated due to the breakdown of the triglycerides butter ripening, and this degradation is a result of the enzymatic activities of microbial lipolytic flora (Sarhir et al., 2021).

3.9.1. Principal component analysis (PCA)

PCA was applied to the data of the respective groups to differentiate the effects of control, treatment (BHT, H₂, N₂), and storage times (day 0 and 90) on flavor compounds of all butter samples (Fig. 3c). Principal components (PC 1 and PC 2) explained 62.4% and 15.0% of the variance in volatile compounds (acids, alcohols, aldehydes, esters, ketones, terpenes, and miscellaneous compounds) derived from all butter samples. In Fig. 3, the distribution of the components (PC 1 and PC 2) was examined; in this plot, the H₂, N₂- or BHT-treated samples at day 90 were grouped in the exact location and correlated well with positive values of PC2 and negative values of PC1. Conversely, C90 (control sample on the 90th day) correlated with positive values of PC1 and PC2. The control sample at day 90 is clearly distinguished from other applications. When the data associated with the heat map (Fig. 3b) control

samples at day 90 are characterized with high concentrations of some esters and alcohols including methyl acetate, 2-methyl propyl acetate, phenethyl acetate, isoamyl acetate, ethyl propanoate, methyl octanoate, 2-methyl-1-butyl acetate, benzene ethanol, 2-methyl-1-octanol, 2-methyl-1-butanol, and 3-methyl-1-butanol.

Although PCA is a powerful tool for large amounts of data and is often used to analyze large chromatographic data, it has some limitations, e.g., PCA is sensitive to the choice of variables and the order of observations. Researchers can, therefore, obtain different results if they select or arrange the data differently. Therefore, they should always justify the choice of variables and make sure that the data are randomly ordered before applying PCA. Also, PCA is not robust to missing values, i.e., if the data has gaps or errors, PCA may not work properly or give inaccurate results. Usually, PCA assumes that the relationship between the variables is linear. PCA will return incorrect results if the data is embedded in a non-linear branch.

4. Conclusion

Butter is susceptible to chemical and biological degradation due to its unsaturated fat and water content. The present study aimed to evaluate the effect of hydrogen gas incorporation into butter on its microbial and chemical stability, safety, and quality attributes. Antioxidants are a technological necessity to protect this product from oxidative reactions. Synthetic antioxidants were used for this purpose before the appearance of toxicological reports highlighting their health risks. Hydrogen gas has been recently suggested to preserve many foods' quality attributes, safety, and nutritional properties. These advantageous effects were linked to the antioxidant and anti-radical activity of hydrogen. Its non-toxicity and inertness make hydrogen an excellent candidate for use as a natural antioxidant in food products. This study demonstrated that injecting hydrogen (H_2) into butter can improve microbial and chemical stability, enhance color (increasing yellowness), minimize the formation of biogenic amines (BAs), and produce no off-flavor or undesirable volatile compounds. These improvements could lead to a longer shelf life. The increase in the content of some amino acids forms an additional nutritional advantage of this method. The rise in the levels of many desirable volatile compounds ensured the high aromatic and flavor characteristics. The results of the physicochemical parameters showed that the values of the butter samples incorporated with H_2 were similar to those of the control samples 30 days before. This outcome shows that the method of H_2 incorporation can significantly extend the butter's shelf life while maintaining the product's sensory quality and safety. These promising outcomes of H_2 were compared with the use of BHT, which has long been considered a robust synthetic antioxidant and was used in many food products before prohibiting decisions by legislation agencies. Although BHT was described as a potent and stable antioxidant, H_2 application showed results similar to those of BHT. Again, H_2 has the advantage of being a natural antioxidant with many beneficial health effects without risks to consumers or the environment. This work is the first study to look at the potential benefits of using H_2 butter on its quality attributes, sensory traits, nutritional quality, and safety. Incorporating molecular hydrogen into butter as a safe and natural alternative to synthetic preservatives can be considered a new strategy that helps the dairy industry to extend shelf life and maintain the quality and safety of the product during storage. These findings can benefit butter manufacturers who want to produce high-quality products with a longer shelf life, leading to economic advantages. One of the study's limitations is the need to conduct a large-scale experiment to assess the feasibility and economics of hydrogen integration on an industrial scale. The storage temperature (4°C) may not represent all real-world storage conditions, so other storage temperatures must be investigated. The analysis of volatile compounds was limited to specific chemical groups, and a more comprehensive aroma analysis could be beneficial in this context. Future research should be performed to optimize the process conditions at a pilot and industrial scale. Additionally, for safety reasons, safe and non-

explosive hydrogen gas mixtures containing less than 4% (%vol) H_2 , such as H_2/N_2 (4/96, %vol), may also be considered in future studies.

CRediT authorship contribution statement

Duried Alwazeer: Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Menekşe Bulut:** Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Mehmet Murat Ceylan:** Writing – original draft, Methodology, Investigation, Formal analysis. **Yasemin Çelebi:** Writing – original draft, Methodology, Investigation, Formal analysis. **Enes Kavrut:** Methodology, Investigation, Formal analysis. **Yunus Çetintaş:** Writing – original draft, Methodology, Investigation, Formal analysis. **Ali Tekin:** Writing – original draft, Methodology, Investigation, Formal analysis. **Ali Adnan Hayaloğlu:** Writing – review & editing, Writing – original draft, Visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This research was not funded by any external funds.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2024.116550>.

References

- Alimentarius, C. (2023). *Codex general standard for food additives (GSFA) online database*.
- Alwazeer, D. (2020). Importance of consideration of oxidoreduction potential as a critical quality parameter in food industries. *Food Research International*, 132, Article 109108. <https://doi.org/10.1016/j.foodres.2020.109108>
- Alwazeer, D. (2023). Restrictive effect of molecular hydrogen on the formation of biogenic amines: Possible mechanisms and perspectives. *Process Biochemistry*, 132, 255–262. <https://doi.org/10.1016/j.procbio.2023.07.015>
- Alwazeer, D. (2024a). Consumption of hydrogen-treated foods provides nutritional and health benefits. In J. Slezak, & B. Kura (Eds.), *Molecular hydrogen in health and disease* (pp. 319–337). Nature Switzerland: Springer. https://doi.org/10.1007/978-3-031-47375-3_19.
- Alwazeer, D. (2024b). Use of hydrogen extraction in the food industry. In *Reference module in chemistry, molecular sciences and chemical engineering*. Elsevier. <https://doi.org/10.1016/b978-0-443-15978-7.00010-2>.
- Alwazeer, D., Bulut, M., & Çelebi, Y. (2022). Hydrogen-rich water can restrict the formation of biogenic amines in red beet pickles. *Fermentation*, 8(12). <https://doi.org/10.3390/fermentation8120741>. s Note: MDPI stays neutral with regard to jurisdictional claims in published ...
- Alwazeer, D., Ceylan, M. M., Bulut, M., & Koyuncu, M. (2022). Evaluation of the impact of hydrogen-rich water on the deaccumulation of heavy metals in butter. *Journal of Food Safety*. <https://doi.org/10.1111/jfs.13005>
- Alwazeer, D., Liu, F. F.-C., Wu, X. Y., & LeBaron, W. T. (2021). Combating oxidative stress and inflammation in COVID-19 by molecular hydrogen therapy: Mechanisms and perspectives. *Oxidative Medicine and Cellular Longevity*. <https://doi.org/10.1155/2021/5513868>
- Alwazeer, D., Örs, B., & Tan, K. (2020). Reducing atmosphere packaging as a novel alternative technique for extending shelf life of fresh cheese. *Journal of Food Science and Technology*, 57(8), 3013–3023. <https://doi.org/10.1007/s13197-020-04334-4>
- Anonymous. (1989a). Official method Ca 5a-40. Free fatty acid. In *Official methods and recommended practices of the American oil chemists' society* (4th ed.). Champaign, IL, USA: AOCS.
- Anonymous. (1989b). Official method Cd 8-53. Peroxide value. In *Official methods and recommended practices of the American oil chemists' society* (4th ed.). Champaign, IL, USA: AOCS.

- Anonymous. (1989c). *Official method Ch 5- 91. Specific extinction of oils and fats, ultraviolet absorption*. AOCS.
- Anonymous. (1991). *Routine methods for determination of free fatty acids in milk*. Bulletin of the IDF.
- Benkerroum, N. (2016). Biogenic amines in dairy products: Origin, incidence, and control means. *Comprehensive Reviews in Food Science and Food Safety*, 15(4), 801–826. <https://doi.org/10.1111/1541-4337.12212>
- Bozkurt, H. (2006). Investigation of the effect of sumac extract and BHT addition on the quality of sucuk (Turkish dry-fermented sausage). *Journal of the Science of Food and Agriculture*, 86(5), 849–856. <https://doi.org/10.1002/jsfa.2431>
- Bulut, M., Alwazeer, D., & Tunçtürk, Y. (2023a). Effects of the incorporation of hydrogen and nitrogen into milk on the reducing and acidification capacities of yoghurt bacteria. *Journal of Food Processing and Preservation*, 1–9. <https://doi.org/10.1155/2023/7462909>, 2023.
- Bulut, M., Çelebi Sezer, Y., Ceylan, M. M., Alwazeer, D., & Koyuncu, M. (2022). Hydrogen-rich water can reduce the formation of biogenic amines in butter. *Food Chemistry*, 384, Article 132613. <https://doi.org/10.1016/j.foodchem.2022.132613>
- Bulut, M., Okutan, G., Alwazeer, D., & Boran, G. (2023b). Hydrogen inclusion in modified atmosphere extends the shelf life of chilled rainbow trout fillets. *Turkish Journal of Fisheries and Aquatic Sciences*, 23(6). <https://doi.org/10.4194/TRJFAS22515>
- Burdychova, R., & Komprda, T. (2007). Biogenic amine-forming microbial communities in cheese. *FEMS Microbiology Letters*, 276(2), 149–155. <https://doi.org/10.1111/j.1574-6968.2007.00922.x>
- Capuani, A., Werner, S., Behr, J., & Vogel, R. F. (2014). Effect of controlled extracellular oxidation-reduction potential on microbial metabolism and proteolysis in buckwheat sourdough. *European Food Research and Technology*, 238(3), 425–434. <https://doi.org/10.1007/s00217-013-2120-9>
- Carocho, M., & Ferreira, I. C. (2013). A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food and Chemical Toxicology*, 51, 15–25. <https://doi.org/10.1016/j.fct.2012.09.021>
- Çelebi, Y., Kavrut, E., Bulut, M., Çetintaş, Y., Tekin, A., Hayaloğlu, A. A., et al. (2024). Incorporation of hydrogen-producing magnesium into minced beef meat protects the quality attributes and safety of the product during cold storage. *Food Chemistry*, 139185. <https://doi.org/10.1016/j.foodchem.2024.139185>
- Ceylan, M. M., & Basturk, A. (2022). Investigation of the effects of uckun (*Rheum ribes* L.), quinoa (*Chenopodium quinoa* Willd.), and propolis extracts on the thermal oxidation of palm olein oil during the deep-frying process. *Journal of Food Processing and Preservation*, 46(2), Article e16210.
- Ceylan, M. M., Bulut, M., Alwazeer, D., & Koyuncu, M. (2022). Evaluation of the impact of hydrogen-rich water on the quality attribute notes of Butter. *Journal of Dairy Research*. <https://doi.org/10.1017/S0022029922000681>
- Ceylan, M. M., Silgan, M., Elnasanelkasim, M. A., & Alwazeer, D. (2023). Impact of washing crude olive pomace oil with hydrogen-rich water and incorporating hydrogen into extraction solvents on quality attributes and phytochemical content of oil. *Journal of Food Measurement and Characterization*, 17(2), 2029–2040. <https://doi.org/10.1007/s11694-022-01801-8>
- Deshwal, G. K., & Panjagari, N. R. (2020). Review on metal packaging: Materials, forms, food applications, safety and recyclability. *Journal of Food Science and Technology*, 57(7), 2377–2392. <https://doi.org/10.1007/s13197-019-04172-z>
- Dhaka, V., Gulia, N., Ahlawat, K. S., & Khatkar, B. S. (2011). Trans fats—sources, health risks and alternative approach - a review. *Journal of Food Science and Technology*, 48(5), 534–541. <https://doi.org/10.1007/s13197-010-0225-8>
- Ebrahimian, M., Mehdizadeh, T., & Aliakbarlu, J. (2023). Chemical and microbiological stability and sensorial properties of traditional Iranian butter incorporated with pomegranate peel extract. *International Journal of Dairy Technology*, 76(1), 178–186. <https://doi.org/10.1111/1471-0307.12906>
- Gänzle, M. G. (2015). Lactic metabolism revisited: Metabolism of lactic acid bacteria in food fermentations and food spoilage. *Current Opinion in Food Science*, 2, 106–117. <https://doi.org/10.1016/j.cofs.2015.03.001>
- Gundogdu, E., Cakmakci, S., & Hayaloglu, A. A. (2020). Effects of starter culture and storage on volatile profiles and sensory characteristics of yogurt or cream butter. *Mljekarstvo*, 70(3), 184–200. <https://doi.org/10.15567/mljekarstvo.2020.0305>
- Hamed, S. F., & Abo-Elwafa, G. A. (2012). Enhancement of oxidation stability of flax seed oil by blending with stable vegetable oils. *Journal of Applied Sciences Research*, 8(10), 5039–5048.
- Hejri, A., Khosravi, A., Gharanjig, K., & Davarani, M. M. (2019). Effect of edible antioxidants on chemical stability of β -carotene loaded nanostructured lipid carriers. *Lebensmittel-Wissenschaft und -Technologie*, 113, Article 108272. <https://doi.org/10.1016/j.lwt.2019.108272>
- Hussein, M. A. (2011). A convenient mechanism for the free radical scavenging activity of resveratrol. *International Journal of Phytomedicine*, 3(4), 459.
- Kalua, C. M., Allen, M. S., Bedgood, D. R., Bishop, A. G., Prenzler, P. D., & Robards, K. (2007). Olive oil volatile compounds, flavour development and quality: A critical review. *Food Chemistry*, 100(1), 273–286. <https://doi.org/10.1016/j.foodchem.2005.09.059>
- Kong, F., & Singh, R. P. (2016). Chemical deterioration and physical instability of foods and beverages. In *The stability and shelf life of food* (pp. 43–76). Elsevier. <https://doi.org/10.1016/B978-0-08-100435-7.00002-2>
- Le Lay, J., Bahloul, H., Sérino, S., Jobin, M., & Schmitt, P. (2015). Reducing activity, glucose metabolism and acid tolerance response of *Bacillus cereus* grown at various pH and oxydo-reduction potential levels. *Food Microbiology*, 46, 314–321. <https://doi.org/10.1016/j.fm.2014.07.007>
- Mallia, S., Escher, F., & Schlichtherle-Cerny, H. (2008). Aroma-active compounds of butter: A review. *European Food Research and Technology*, 226(3), 315–325. <https://doi.org/10.1007/s00217-006-0555-y>
- Milovanovic, B., Djekic, I., Miocinovic, J., Djordjevic, V., Lorenzo, J. M., Barba, F. J., et al. (2020). What is the color of milk and dairy products and how is it measured? *Foods*, 9(11), 1629. <https://doi.org/10.3390/foods9111629>
- Oussou, K. F., Guclu, G., Sevidik, O., Starowicz, M., Kelebek, H., & Selli, S. (2022). Comparative elucidation of aroma, key odorants, and fatty acid profiles of ivoryan shea butter prepared by three different extraction methods. *Separations*, 9(9). <https://doi.org/10.3390/separations9090245>
- Ozturk, S., & Cakmakci, S. (2006). The effect of antioxidants on butter in relation to storage temperature and duration. *European Journal of Lipid Science and Technology*, 108(11), 951–959. <https://doi.org/10.1002/ejlt.200600089>
- Pădureț, S. (2021). The effect of fat content and fatty acids composition on color and textural properties of butter. *Molecules*, 26(15), 4565. <https://doi.org/10.3390/molecules26154565>
- Pateiro, M., Barba, F. J., Domínguez, R., Sant'Ana, A. S., Khaneghah, A. M., Gavahian, M., et al. (2018). Essential oils as natural additives to prevent oxidation reactions in meat and meat products: A review. *Food Research International*, 113, 156–166. <https://doi.org/10.1016/j.foodres.2018.07.014>
- Riondet, C., Cachon, R., Waché, Y., Alcaraz, G., & Diviès, C. (2000). Extracellular oxidoreduction potential modifies carbon and electron flow in *Escherichia coli*. *Journal of Bacteriology*, 182(3), 620–626. <https://doi.org/10.1128/JB.182.3.620-626.2000>
- Sarhir, S. T., Amanpour, A., Bouseta, A., & Selli, S. (2021). Fingerprint of aroma-active compounds and odor activity values in a traditional Moroccan fermented butter “Smen” using GC – MS – olfactometry. *Journal of Food Composition and Analysis*, 96, Article 103761. <https://doi.org/10.1016/j.jfca.2020.103761>
- Sezer, Y.Ç., Bulut, M., Boran, G., & Alwazeer, D. (2022). The effects of hydrogen incorporation in modified atmosphere packaging on the formation of biogenic amines in cold stored rainbow trout and horse mackerel. *Journal of Food Composition and Analysis*, 112, Article 104688. <https://doi.org/10.1016/j.jfca.2022.104688>
- Tahmas-Kahyaoglu, D., Cakmakci, S., & Hayaloglu, A. A. (2022). Changes during storage in volatile compounds of butter produced using cow, sheep or goat's milk. *Small Ruminant Research*, 211, Article 106691. <https://doi.org/10.1016/j.smallrumres.2022.106691>
- Waché, Y., Riondet, C., Diviès, C., & Cachon, R. (2002). Effect of reducing agents on the acidification capacity and the proton motive force of *Lactococcus lactis* ssp. cremoris resting cells. *Bioelectrochemistry*, 57(2), 113–118. [https://doi.org/10.1016/S1567-5394\(02\)00051-8](https://doi.org/10.1016/S1567-5394(02)00051-8)
- Wang, W., Xiong, P., Zhang, H., Zhu, Q., Liao, C., & Jiang, G. (2021). Analysis, occurrence, toxicity and environmental health risks of synthetic phenolic antioxidants: A review. *Environmental Research*, 201, Article 111531. <https://doi.org/10.1016/j.envres.2021.111531>
- Zhang, Y., Yang, L., Zu, Y., Chen, X., Wang, F., & Liu, F. (2010). Oxidative stability of sunflower oil supplemented with carnosic acid compared with synthetic antioxidants during accelerated storage. *Food Chemistry*, 118(3), 656–662. <https://doi.org/10.1016/j.foodchem.2009.05.038>