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Evaluation of the impact of hydrogen-rich water on the quality attribute notes of butter

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Research Article

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Abstract

The effects of washing raw butter with hydrogen-rich water (HRW), prepared with hydrogen (H_2) and/or magnesium (Mg), on butter quality were investigated in this research paper. During the washing process, titratable acidity (TA) decreased by 12% for all washed samples. During the storage period, TA increased by 28% and 93% (control), 14% and 58% (H_2), and 10% and 66% (Mg) for the 60th and 90th days, respectively. Peroxide value (mEq O_2/kg) increased to 2.76 and 8.83 (control), 1.92 and 7.25 (H_2), and 2.02 and 8.12 (Mg) for the 60th and 90th days. HRW samples showed the lowest acid degree value (ADV) and the highest color notes (L*, C*, and h). The HRW treatment of raw butter has shown improving effects on the product without any harmful residuals in the final product or the environment.

Butter is a dairy product characterized by its emulsion system containing water and oil that provides its unique taste, smell, and consistency. The richness of butter is maintained by the unsaturated fatty acid content, however, this makes butter susceptible to different oxidation-related spoilage risks, shortening its shelf life. The oxidative reactions occurring in butter lead to the destruction of essential fatty acids, as well as vitamins A, D, E, and K. The first oxidative decomposition products formed in fatty produce such as butter are hydroperoxides that, with the progress of oxidation, are decomposed into low molecular weight compounds such as aldehydes, ketones, alcohols, and free fatty acids, causing rancidity (Akgül and Ayar, 1993; Koczoñ *et al.*, 2008). Additionally, an increase in microbiological and enzymatic activities can partially hydrolyze triglycerides, causing a decrease in the oxidative stability of the product (Koyuncu, 2010).

Previous reports have revealed the beneficial effects of plant extract incorporation on the quality and stability of butter. Vidanagamage and co-workers reported that cinnamon extract-incorporated butter exhibited low levels of peroxide and free fatty acids values when compared with control butter (Vidanagamage *et al.*, 2016). Further research in this field has determined that the ethanolic extracts of vidarikand (*Pueraria tuberosa*), shatavari (*Asparagus racemosus*), and ashwagandha (*Withania somnifera*) were effective in preventing the development of the peroxide value and conjugated diene in ghee (Pawar *et al.*, 2014). Similarly, butter with added green tea extract exhibited a lower peroxide value but presented a higher acid value after six weeks of storage when compared with control (Thakaeng *et al.*, 2020). The contents of α-tocopherol and β-carotene, polyunsaturated fatty acids and cholesterol in butter with added skullcap (*Scutellaria baicalensis Georgi*) flavones were shown to be higher than in control samples (Wojdyło *et al.*, 2005). Conversely, butter with added walnut kernel septum membranes (hydroalcoholic extract) showed low levels of free fatty acids (Mehdizadeh *et al.*, 2019). Traditional Tunisian butter (TTB) enriched with 400 mg of tomato processing by-products (TPB) extract/kg of TTB, revealed the lowest peroxide values. However, raw TTB and highly enriched TTB (800 mg of TPB extract/kg of TTB) showed higher lipid peroxidation (Abid *et al.*, 2017).

Hydrogen is the smallest, lightest, and most plentiful element found in the universe (Alwazeer *et al.*, 2021). Molecular hydrogen (H_2) is a colorless, odorless, tasteless, nonmetallic and nontoxic gas at room temperature (Alwazeer, 2019). Several studies have revealed the advantages of using hydrogen to improve the quality and extend the shelf-life of different food products such as white cheese (Alwazeer *et al.*, 2020), orange juice (Alwazeer, 2003; Alwazeer *et al.*, 2003; Cachon and Alwazeer, 2019), dried apple (Alwazeer, 2018), dried apricot (Alwazeer and Örs, 2019), strawberries (Alwazeer and Özkan, 2022), fresh and dried foods (Alwazeer, 2020), fat-free fermented milk (Ebel *et al.*, 2011), butter (Alwazeer *et al.*, 2022; Bulut *et al.*, 2022), non-fat yogurt (Martin *et al.*, 2011), acid skimmed milk gels (Martin *et al.*, 2009), and polyunsaturated fatty acids-enriched dairy beverage (Giroux *et al.*, 2008). These studies conclude that the beneficial effects of hydrogen on the quality of the products were related to its reducing capacity and antioxidative properties. Hydrogen-rich water

(HRW) can be used as a treatment in food production and is easily prepared by bubbling pure hydrogen gas or dissolving magnesium in water as well as water electrolysis (Russell *et al.*, 2021). HRW possesses antioxidant and reducing properties due to the low standard (25°C, pH 7) redox potential value (E°′) of hydrogen i.e. −414 mV (Jay *et al.*, 2020), and its electron-donating ability.

Butter is known for its susceptibility to oxidative deteriorations during storage, and the quality of washing water can significantly affect the quality of product. A recent study demonstrated that the use of hydrogen-rich water in washing raw sour cultured butter decreased the formation of biogenic amines during cold storage (Bulut *et al.*, 2022). Many components including polyphenols, carotenoids, unsaturated fatty acids and some vitamins (including vitamins C and E) are responsible for the sensory and nutritional quality of dairy products and sensitive to oxygen. We hypothesise that using HRW rather than normal washing water would reduce various destructive components (oxygen, free radicals) and protect oxygen-sensitive components during storage. With that hypothesis in mind, the present study evaluates the effect of using of HRW as reducing medium in washing raw cultured butter on the physicochemical and color properties of the final product during cold storage.

Materials and methods

Butter preparation

The cow milk and yogurt bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) used in the preparation of cultured butter were obtained from a local company (Yeni Yayla Dairy Products Company, Igdir, Turkey). The raw milk after a filtration step was preheated at 60°C for 5 min followed by a cream separation phase using a household manual cream separator. The cream was pasteurized at 68.8°C for 30 min followed by cooling to 13°C. The pasteurized cream was then inoculated with fresh yogurt (%2 v/v) and incubated for 7 d at +4°C. The cultured cream was then kneaded by hand, and buttermilk and unwashed raw cultured butter were obtained. The unwashed raw cultured butter was manually washed by hand with different cold (0–4°C) washing waters (normal drinking washing water, H_2 water, Mg water). The washed butter samples were then manually packaged hermetically inside a polyethylene-laminated aluminum package without keeping any air inside and stored at 4°C for 0, 30, 60, and 90 d (illustrated schematically in online Supplementary Fig. S1).

HRW preparation

Tap drinking water (normal water, pH 7.17, Eh + 321 mV) and two types of HRW i.e., hydrogen-bubbled (H_2 water, pH 7.15, and Eh −283 mV) and magnesium-incorporated (Mg water, pH 9.8, and Eh −344 mV) were used in the washing phase of raw butter. For the hydrogen bubbling method, H_2 gas was bubbled directly into tap drinking water at 1 l/min for 10 min using a hose equipped with a needle. The concentration of dissolved hydrogen in HRW samples and its stability were evaluated in preliminary experiments using the ORP electrode (Sensorex, USA). The hydrogen content was approx. 1.6 ppm. For preparing Mg water, 160 mg magnesium powder (particle size <0.1 mm) (Sigma-Aldrich, Germany) was dissolved in 1 l tap drinking water. The hydrogen content was also about 1.6 ppm. All water types used in the preparation of the HRW and washing process were previously cooled to 0–4°C.

Titratable acidity

The titratable acidity (TA) of the butter samples was carried out according to Marth (1978). The titratable acidity value was expressed as the percentage of lactic acid (%) in samples.

Peroxide value

Peroxide value was determined according to the AOCS Official Method Cd8-53 (Anonymous, 1989). Peroxide value expresses the amount of active milliequivalents oxygen (mEq O_2) in 1000 g of oil.

Acidity degree value

5 g butter samples were placed in special butyrometers. 20 ml of BDI solution (prepared by dissolving 30 g of Triton X-100 and 70 g of sodium tetraphosphate in 1 l of distilled water) was added to the butter sample, and the butyrometers were placed in a boiling water bath. The mixture was then centrifuged by Nova Safety Gerber Centrifuge (Funke, Germany) and aqueous methanol (1 : 1 water-methanol) was added until the level of oil layer to the neck of the butyrometer was reached. The samples were centrifuged again, as described above, and kept in a water bath at 57°C for 5 min. A sufficient amount (1.5–2 g) of oil was collected with a syringe from the upper part and 5 ml of solvent (petroleum ether/n-propanol; 4 : 1 v/v), containing 0.1 g/l thymol blue indicator was added. This was followed by titration with tetra n-butyl ammonium hydroxide (0.01 N). ADV values (%) were calculated according to the following formula (Anonymous, 1991).

$$\text{ADV} = \frac{(\text{A} - \text{B}) \times \text{N} \times 100}{\text{W}}$$

where:

A, Tetra n-butyl ammonium hydroxide (0.01 N) spent for the sample (ml)

B, Tetra n-butyl ammonium hydroxide (0.01 N) spent for the blank (ml)

N, Normality of tetra n-butyl ammonium hydroxide i.e. 0.01 N

W, Butter sample amount (g)

Free fatty acid (FFA) profile determination: extraction

1 g of sample was mixed with 3 g of dehydrated sodium sulfate, 0.3 ml of sulfuric acid (2.5 mol/l), and 1 ml of internal standard (C5 : 0, 0.5 mg/ml; C13 : 0, 05 mg/ml, Sigma–Aldrich, Milwaukee, WI, USA). The mixture was extracted three times with a 3 mL ether-heptane solution of a 1/1 (v/v) ratio, and the solvent was transferred to a separate test tube. Before introducing the aminopropyl SPE column (500 mg; Agilent Technologies, Stockport, UK), the butter sample was conditioned by adding 10 ml heptane. Then, the solvent was transmitted through the aminopropyl column. The resulting eluent was transferred to the same column for a second time, thus fixing the free fatty acids onto the column. Neutral lipids were removed from the column by passing 10 ml ether/heptane (1/1 (v/v) ratio). Free fatty acids were processed with a solution containing 3 ml diethyl

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ether and 100 mL formic acid which was directly injected into a gas chromatography device (De Jong and Badings, 1990).

Free fatty acid (FFA) profile determination: identification

FFA profile analysis was carried out using a GC-MS system (Thermo Fisher Trace ISQ GC-MS, USA). Free fatty acids were separated using a capillary column (TRB-FFAP, 60 m × 0.25 mm × 0.25 µm; Teknokroma, Barcelona, Spain). The carrier gas was helium with a flow of 1 ml/min. The temperature program was set at 60°C for 2 min, then raised at 5°C/min to 120°C and held for 2 min, and raised at 15°C/min to 230°C and held for 30 min. The GC–MS transfer line temperature was 250°C. The MS operated in electron impact mode with an electron impact energy of 70 eV and collected data at a rate of 0.2 scans/s over m/z 40–450. FFAs were identified by Wiley 9 and Mainlib mass spectral libraries. Short-chain FFA calculations were based on the peak area of pentanoic acid, while medium- and long-chain FFAs were evaluated with tridecanoic acid peaks (Andic *et al.*, 2011).

Color analysis

The color values (CIELAB system) of butter samples were measured using Konica Minolta (CR-410, Japan) colorimeter to determine brightness (L*), red-green (a*), yellow-blue (b*), Chroma (C*), and Hue (h*) values.

Statistical analysis

Data obtained were subjected to Analysis of Variance (ANOVA). The differences between means were evaluated using Duncan's multiple comparison test and significance was accepted at $P < 0.05$ level. The statistical package in SPSS 20 computer program was used. All the analyses were carried out in duplicate for three samples ($n = 3$).

Results and discussion

Titratable acidity

The titratable acidity values (TA, %) that express the rancidity of the sample caused by free fatty acids are shown in Fig. 1a. During the washing process, the TA value (%) decreased for all butter-type samples. TA decreased significantly from 0.33% (unwashed raw butter) to 0.29% with a decreasing percentage of 12% for all washed samples ($P < 0.05$). On the other hand, during the storage period, TA values increased for all butter types. However, these changes were significant only on the 60th day (control) and 90th day (H_2 and Mg) of storage ($P < 0.05$).

On the 60th day of storage, the TA value of butter samples increased by 28% and 93% (control), 14% and 58% (H_2), and 10% and 66% (Mg) for the 60th and 90th day of storage, respectively. There was a significant difference between the control sample and both HRW-type butter samples (H_2 and Mg) only at 60-day and 90-day storage times ($P < 0.05$). The increase in the TA of butter during the storage stage was reported by different researchers (Simsek, 2011; Çakmakçi *et al.*, 2014). Titratable acidity represents free fatty acids that are not chemically esterified. The formation of TA leads to a decrease in the quality of fatty products (Kayahan, 2008). The hydrolysis of triglycerides, which constitutes more than 98% of milk fat, is the principal

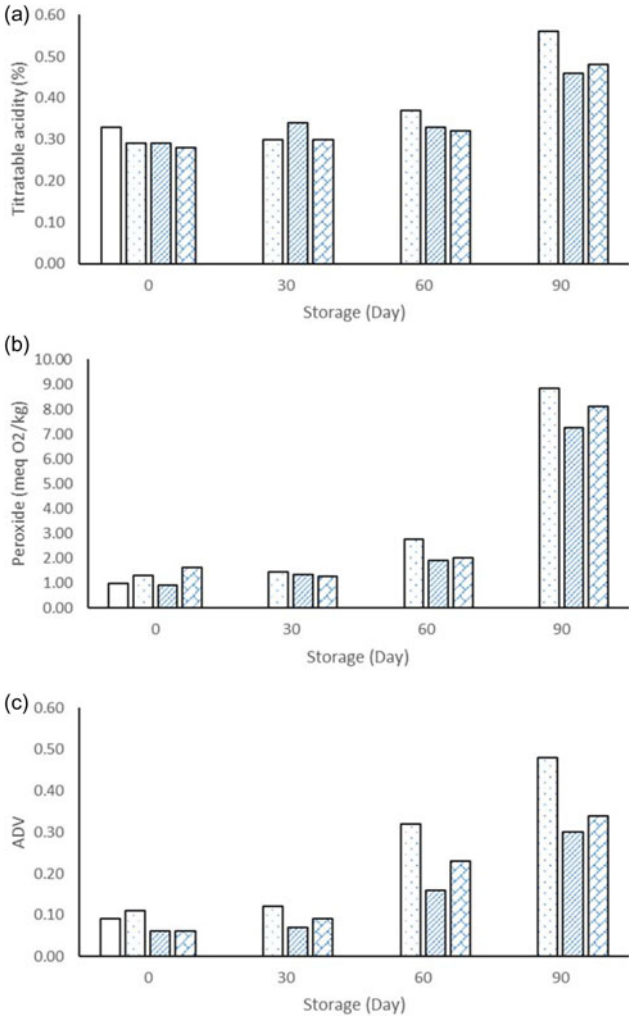

Fig. 1. The effect of different conditions (before washing (▭), normal water (▭), H_2 water (▭), and Mg water (▭)) on titratable acidity (a), peroxide value (b), and acid degree value (c) of butter samples during storage.

biochemical transformation of fat, leading to the production of free fatty acids (FFAs), di- and mono-glycerides, and possibly glycerol. Depending on the amount of small molecule fatty acids released by hydrolysis, aroma disorders characterized as rancidity were observed in oils (Kayahan, 2008).

The titratable acidity level in oils or fats tends to increase during storage due to chemical or enzymatic hydrolysis. In the chemical hydrolysis, with the presence of water, a monomolecular reaction occurs between water and glycerides and the esters causing the free acidity in oils or fats to arise spontaneously. Enzymatic hydrolysis, on the other hand, occurs when the lipases break down the fat, as they would in the presence of water, increasing titratable acidity and quality defects in the product. We assume that the non-polar property of molecular hydrogen (H_2) with a solubility rate in butter oil three times that of water (4.32 *vs.* 1.38 mg/l at 40°C) (Young, 1981) may have affected the hydrolytic and enzymatic degradation of fat, which may explain the lower titration acidity values of HRW-washed butter samples (Cadwallader and Singh, 2009).

Many reports have correlated the inhibitory effects of plant extracts, phenolics, and flavonoids upon lipases with the antioxidant activity of these extracts/compounds. As molecular hydrogen

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possesses antioxidant activity, we can relate the low titratable acidity, and acidity and peroxide values to this antioxidant activity of hydrogen (Nonaka *et al.*, 1991; Moreno *et al.*, 2003; Sreerama *et al.*, 2012; Kim *et al.*, 2016; Vangoori *et al.*, 2019; Huang *et al.*, 2020; Akhlaghi and Najafpour-Darzi, 2021; Alwazeer *et al.*, 2021).

Peroxide value

Hydroperoxides are the primary products formed during lipid oxidation. It is possible to determine the amount of these primary products which are formed in the initial stage of lipid oxidation, by determining the peroxide value (Ceylan, 2020). Figure 1b shows the peroxide values of different butter sample types during 90 d of storage. During the washing process of raw butter, the peroxide value (mEq O_2/kg) did not significantly change. It increased from 0.99 (unwashed raw butter) to 1.32 (normal water) and 1.64 (Mg water), while it decreased insignificantly to 0.91 (H_2 water) in butter samples ($P > 0.05$). During the storage period, the peroxide value increased for all butter sample types. This increase was significant for 60-d and 90-d storage times for all samples ($P < 0.05$). Peroxide value (mEq O_2/kg) increased to 2.76 and 8.83 (control), 1.92 and 7.25 (H_2), and 2.02 and 8.12 (Mg) for the 60th and 90th days, respectively, with significant differences noted between control and both HRW butter types at each timepoint ($P < 0.05$).

The results show that washing raw butter with HRW (H_2 and Mg waters) hindered the peroxidation of butter during storage. This property could be related to the reducing capacity of molecular hydrogen dissolved in HRW. HRW was also reported to retard the peroxidation process in alfalfa (Cui *et al.*, 2014), kiwifruit (Hu *et al.*, 2014), and edible mushroom *Hypsizygus marmoreus* (Zhang *et al.*, 2017).

Lipid peroxidation, or 'oxidative deterioration', is a chain reaction resulting in the oxidative degradation of lipids. This reactive cascade occurs when preferentially polyunsaturated fatty acids (PUFAs) are oxidized by free radicals such as reactive oxygen species (ROS), a process termed autoxidation. The presence of pro-oxidants such as iron and copper ions can accelerate autoxidation, as can the presence of oxygen, and the exposure to light (Feiner, 2016). The increase in peroxide value and the formation of free fatty acids are considered the best indicators of fat deterioration and the extent of spoilage within oils. Peroxides are decomposition products that occur at the beginning of the oxidative cascade. The presence and number of allyl groups (-C = C-) in fatty acids is an important factor in the oxidation mechanism. Some factors such as air, temperature, and light cause ionization in the hydrogen atoms attached to the carbons adjacent to the allyl group, conferring activity to the radical to which they are attached (Belitz and Grosch, 1999; Kayahan, 2008).

Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG), and tert-butylhydroquinone (TBHQ) are widely used as food additives to increase the shelf life, especially of fat and lipid-containing products, which act by retarding the process of lipid peroxidation. On the other hand, many reports have shown that natural and plant extracts can scavenge hydrogen peroxide and decrease the rate of peroxide formation as a result of their antioxidant activity (Basaga *et al.*, 1997; Duh, 1999; Salariya and Habib, 2003; Bera *et al.*, 2006). Thus, we can correlate the low levels of peroxide value found in H_2-washed butter with the reducing capacity, and anti-radical and antioxidant properties of molecular hydrogen, with H_2 reducing the autoxidation of unsaturated fatty acids through neutralization of oxidative agents.

Acid degree value

Figure 1c shows the change in acid degree value (ADV) values of butter samples during storage. During the washing process, ADV values (%) of raw butter increased insignificantly from 0.9 (unwashed butter) to 1.1 (control) ($P > 0.05$), but it decreased significantly to 0.6 for both H_2 and Mg ($P < 0.05$). During the storage period, the ADV value increased significantly on the 60th day for control (191%) and H_2- washed (167%) samples, while it increased on the 30th day for Mg samples ($P < 0.05$). At the end of storage (90th day), the lowest ADV value was shown for the H_2 samples (3.0%), while the highest ADV value was found for the control ones (4.8%). For 0- and 30-d storage periods, both HRW types i.e., Mg and H_2 butter samples showed the lowest value of ADV, while for 60- and 90-d storage periods, only H_2 samples had the lowest ADV values ($P < 0.05$) (Fig. 1c).

ADV typically increases during storage as a result of the activity of lipolytic enzymes and microorganisms. Like any type of food with high-fat content, lipolytic (enzymatic hydrolysis by lipases and esterases) and oxidative (chemical) changes are likely to occur in dairy products (Mallia *et al.*, 2008). Lipases in dairy products can originate from six possible sources: (1) the milk, (2) rennet preparation (rennet paste), (3) starter culture, (4) adjunct starter, (5) non-starter bacteria and, possibly, (6) their addition as exogenous lipases (Collins *et al.*, 2003). Milk contains a potent endogenous lipoprotein lipase (LPL), which is completely inactivated at 78°C for 10 s (Blaya *et al.*, 2018). Short- and medium-chain fatty acids are preferentially released by LPL (Collins *et al.*, 2003). As the majority of released fatty acids found in this study were medium- and long-chain fatty acids, we can assume that the lipolytic activity found in this study is not related to LPL but may be attributed to the microbial growth with lipolytic activity.

Lactic acid bacteria (LAB) possess esterases and exhibit lipolytic activity, due mainly to intracellular enzymes capable of hydrolyzing fat in milk and dairy products (Blaya *et al.*, 2018). Lipolytic enzymes of LAB are mainly intracellular, thus, they must be released upon autolysis to be exposed to their substrates (Blaya *et al.*, 2018). Meyers and co-workers found that whole intact cells of *S. thermophilus* F410 possess lipolytic activity towards high carbon saturated triacylglyceride substrates (C18 : 0, C16 : 0, C14 : 0, and C12 : 0) when compared with low carbon saturated triacylglyceride substrates (Meyers *et al.*, 1996). This study has found also that seven strains of *Streptococcus thermophilus* possess lipolytic activity towards butter oil in whole and intracellular form. Thus, we can correlate the high levels of long-chain fatty acids found in the present study with the lipolytic activity of starters i.e., *S. thermophilus,* added during the preparation process. Additionally, these results assume that HRW (H_2 and Mg) could slow down the lipolytic bacterial activity through the reducing and antioxidant properties of molecular hydrogen found in HRW, as discussed above. A future study will be needed to confirm this hypothesis.

The free fatty acid (FFA) profile

The results of the free fatty acid (FFAs) profile at 90-d storage are shown in Table 1. Results showed that free fatty acid concentration increased during 90-d storage. Eight saturated and three unsaturated fatty acids were identified. The primary saturated and unsaturated fatty acids of all butter samples were palmitic acid and oleic acid, respectively. Similar data were reported by

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Table 1. The effect of different conditions (before washing, normal water, H_2 water, and Mg water) and storage period (days) on free fatty acid concentrations (mg/kg) of butter samples (mean ± standard deviations. $n = 2$)

Fatty acids	Water type	0	90th day
C:4	Normal water	0^{Ab}	3.56 ± 0.47^{Aa}
	H_2 water	3.66 ± 0.74^{Aa}	3.33 ± 0.38^{Aa}
	Mg water	2.97 ± 2.35^{Aa}	2.97 ± 0.26^{Aa}
C:6	Normal water	3.82 ± 0.90^{Ab}	5.96 ± 0.80^{Aa}
	H_2 water	5.36 ± 0.18^{Aa}	5.65 ± 0.48^{Aa}
	Mg water	3.92 ± 0.01^{Ab}	5.47 ± 0.04^{Aa}
C:8	Normal water	8.90 ± 2.10^{Aa}	10.33 ± 0.50^{Aa}
	H_2 water	10.50 ± 0.84^{Aa}	10.35 ± 1.32^{Aa}
	Mg water	11.03 ± 0.71^{Aa}	10.31 ± 0.81^{Aa}
C:10	Normal water	70.23 ± 5.29^{Bb}	107.94 ± 3.36^{Aa}
	H_2 water	118.17 ± 13.70^{Aa}	118.70 ± 22.02^{Aa}
	Mg water	116.50 ± 1.81^{Aa}	125.58 ± 3.89^{Aa}
C:12	Normal water	205.46 ± 101.75^{Aa}	213.35 ± 9.41^{Aa}
	H_2 water	201.76 ± 20.80^{Aa}	205.37 ± 20.22^{Aa}
	Mg water	196.42 ± 13.00^{Aa}	217.89 ± 3.78^{Aa}
C:14	Normal water	422.28 ± 129.30^{Aa}	482.97 ± 13.09^{Aa}
	H_2 water	445.98 ± 76.40^{Aa}	465.87 ± 79.26^{Aa}
	Mg water	431.98 ± 16.36^{Ab}	522.00 ± 2.67^{Aa}
C:16	Normal water	1621.28 ± 107.57^{Aa}	1318.28 ± 114.79^{Aa}
	H_2 water	1311.31 ± 270.43^{Aa}	1232.42 ± 163.90^{Aa}
	Mg water	1324.94 ± 19.30^{Aa}	1360.88 ± 14.60^{Aa}
C:16.1	Normal water	111.17 ± 57.44^{Aa}	122.95 ± 18.61^{Aa}
	H_2 water	118.43 ± 33.01^{Aa}	118.63 ± 18.14^{Aa}
	Mg water	121.52 ± 8.06^{Aa}	127.29 ± 11.70^{Aa}
C:18	Normal water	911.27 ± 84.11^{Aa}	531.34 ± 22.46^{Ab}
	H_2 water	501.10 ± 126.56^{Ba}	500.30 ± 77.44^{Aa}
	Mg water	508.12 ± 42.65^{Ba}	525.73 ± 23.32^{Aa}
C:18.1	Normal water	1098.29 ± 280.42^{Ab}	1633.92 ± 79.31^{Aa}
	H_2 water	1456.09 ± 225.15^{Aa}	1461.02 ± 397.55^{Aa}
	Mg water	1423.61 ± 67.30^{Aa}	1581.71 ± 20.49^{Aa}
C:18.2	Normal water	84.13 ± 10.93^{Ab}	125.83 ± 5.90^{Aa}
	H_2 water	96.10 ± 32.36^{Aa}	111.23 ± 12.72^{Aa}
	Mg water	83.85 ± 2.42^{Ab}	116.47 ± 2.07^{Aa}
SFA	Normal water	3243.21 ± 31.07^{Aa}	2673.71 ± 162.32^{Ab}
	H_2 water	2597.82 ± 254.69^{Ba}	2541.97 ± 364.26^{Aa}
	Mg water	2595.87 ± 47.77^{Bb}	2770.82 ± 18.47^{Aa}
MUFA	Normal water	1199.45 ± 208.82^{Aa}	1756.87 ± 97.92^{Aa}
	H_2 water	1574.52 ± 192.14^{Aa}	1579.65 ± 415.70^{Aa}
	Mg water	1545.13 ± 73.36^{Aa}	1709.00 ± 32.19^{Aa}
PUFA	Normal water	84.13 ± 10.93^{Ab}	125.83 ± 5.90^{Aa}
	H_2 water	96.10 ± 32.36^{Aa}	111.23 ± 12.72^{Aa}
	Mg water	83.85 ± 2.42^{Ab}	116.47 ± 2.07^{Aa}

SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids.
[A,B]For each storage, different superscript uppercase letters show differences between the samples ($P < 0.05$).
[a,b]Within the same butter sample, different superscript lowercase letters show differences between the storage ($P < 0.05$).

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Erkaya *et al.* (2015); Fındık and Andiç, (2017); Koyuncu and Tuncturk, (2017). When comparing the results of 90-d with the starting ones, we find that butyric acid (C4 : 0) increased only in control, caproic acid (C6 : 0) increased in both control and Mg, capric acid (C10 : 0) increased in control, myristic acid (C14 : 0) increased in Mg, both stearic acid (C18 : 0) and oleic acid (C18 : 1) increased only in control, linoleic acid (C18 : 2) increased in both control and Mg, saturated fatty acids (SFA) decreased in control and increased in Mg without change in H_2, and polyunsaturated fatty acids (PUFA) increased in both control and Mg butter samples without change in H_2 (Table 1). Our results agree with those of Erkaya *et al.* (2015) who reported that the major free fatty acids in normal butter and butter prepared with *Bifidobacterium bifidum* or *Lactobacillus acidophilus* were, in descending order, as follows: saturated fatty acids (SFA), C16 : 0, mono-unsaturated fatty acids, and C18 : 1.

During the washing process, both stearic acid and SFA values were lower in HRW samples (H_2 and Mg) than in control ones, while capric acid increased ($P < 0.05$). After 90-d of storage, the profile of free fatty acids had changed according to the washing water type. Haddadian et al. (2016) reported that highly positive Eh values of milk increased deterioration by inducing oxidative reactions, such as lipid peroxidation. Production of undesirable oxidized/metallic flavors and shorter shelf life of dairy products are the consequence of such reactions. The Eh values of the H_2- and Mg-washed butter samples were decreased to negative values (reducing range) (unpublished data). We assume that the reason behind the low levels of stearic acid in butter washed with HRW (H_2 and Mg) could be the inhibition of lipolytic degradation of butter lipids in reducing conditions i.e., negative Eh values such as H_2 and Mg butter samples.

Color parameters

The color results of the butter samples are shown in Table 2. During the washing process, the L* value of raw butter for all butter types (normal water, H_2 water, and Mg water) improved. During the storage period, L* value significantly began to decrease from 60-d storage. At 60-d storage, both normal and H_2 butter samples showed the highest L* value, while at 90-d storage both HRW (H_2 and Mg) showed the highest L* value without a significant difference between H_2 and Mg butter samples ($P > 0.05$). The reason behind the decrease in the L*value of normal water-washed butter could be attributed to the oxidation of the color

Table 2. The effect of different conditions (before washing, normal water, H_2 water, and Mg Water) and storage period (days) on the color values of butter

	0	30th day	60th day	90th day
L*				
Before washing	87.31 ± 0.19^{C}			
Normal water	93.56 ± 0.07^{aB}	93.62 ± 1.61^{aA}	72.68 ± 3.14^{bA}	69.64 ± 0.49^{bB}
H_2 water	95.42 ± 0.35^{aA}	96.98 ± 1.07^{aA}	72.97 ± 0.90^{cA}	76.38 ± 0.13^{bA}
Mg water	94.95 ± 0.53^{aA}	95.53 ± 0.10^{aA}	62.60 ± 4.31^{cB}	75.18 ± 0.52^{bA}
a*				
Before washing	-3.29 ± 0.01^{D}			
Normal water	-3.49 ± 0.01^{abC}	-3.81 ± 0.23^{aA}	-3.22 ± 0.04^{bB}	-2.66 ± 0.02^{cC}
H_2 water	-3.57 ± 0.01^{abB}	$-3.84u \pm 0.19^{aA}$	-3.11 ± 0.09^{cB}	-3.42 ± 0.06^{bA}
Mg water	-3.75 ± 0.01^{aA}	-3.50 ± 0.02^{bA}	-2.58 ± 0.01^{dA}	-3.21 ± 0.04^{cB}
b*				
Before washing	28.71 ± 0.33^{B}			
Normal water	30.07 ± 0.08^{aA}	32.52 ± 1.01^{aAB}	21.16 ± 1.27^{bAB}	23.59 ± 1.05^{bA}
H_2 water	30.10 ± 0.12^{bA}	33.78 ± 0.18^{aA}	23.42 ± 1.07^{cA}	23.83 ± 1.20^{cA}
Mg water	30.17 ± 0.16^{cA}	31.09 ± 0.95^{cB}	18.94 ± 0.81^{aB}	22.36 ± 0.65^{bA}
C*				
Before washing	51.88 ± 0.88^{A}			
Normal water	40.80 ± 0.93^{aC}	32.74 ± 1.99^{bB}	21.40 ± 1.25^{cAB}	23.74 ± 1.05^{cA}
H_2 water	45.93 ± 0.76^{aB}	38.56 ± 1.27^{bA}	23.63 ± 1.08^{cA}	24.08 ± 1.18^{cA}
Mg water	42.34 ± 1.54^{aC}	34.07 ± 0.90^{bAB}	19.12 ± 0.80^{dB}	22.61 ± 0.61^{cA}
h*				
Before washing	97.07 ± 0.12^{AB}			
Normal water	96.95 ± 0.06^{bB}	96.64 ± 0.93^{bA}	98.66 ± 0.62^{aA}	96.44 ± 0.23^{bB}
H_2 water	97.26 ± 0.15^{abAB}	96.50 ± 0.35^{bA}	97.57 ± 0.12^{aA}	98.18 ± 0.55^{aAB}
Mg water	97.38 ± 0.09^{abA}	96.38 ± 0.09^{bA}	97.77 ± 0.29^{aA}	98.49 ± 0.78^{aA}

$^{A–C}$The difference between values expressed with different capital letters in different samples in the same column on the same storage days is statistically significant ($P < 0.05$).
$^{a–c}$In the same samples, the difference between the values expressed in different lowercase letters in the same rows on different storage days is statistically significant ($P < 0.05$).

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pigments of butter during the storage period (Kaya, 2000; Gonzalez *et al*., 2003). This shows that the use of H_2, which has reducing and antioxidant properties, in the washing process of butter could preserve the lightness of the product.

During the washing process, the a* value (red-green) of all methods of water-washed raw butter samples increased (less negative) significantly, meaning the green pigment levels decreased. During the storage period, the lowest a* value was shown in the butter sample washed with Mg water on the 60th day (−2.58 ± 0.01), whereas the highest one was in the butter washed with H_2 water on the 30th day (−3.84 ± 0.19). At the end of the storage (90-d), the normal water sample showed the lowest a* value (less negative), whereas the H_2 sample had the highest one (more negative) ($P < 0.05$). Thus, we can say that the greenness notes of butter (-a*) were better preserved in H_2 and Mg applications compared to normal water. The color has shifted towards red in the control butter sample which could be related to the oxidation properties of air.

During the washing process, b* values of butter washed with different water types (normal water, H_2 water, and Mg water) were significantly increased (more positive) ($P < 0.05$). During the storage period, at 30-d and 60-d, the lowest b* values (less yellow) were shown in Mg-washed water butter ($P < 0.05$). It was reported that the color of butter changed from yellow to light yellow (low b* values) due to the oxidation of chromophores at the end of storage (Kaya, 2000). The b* value for butter color is an important parameter that differentiates the analyzed samples (Pădureț, 2021). It has been stated that the yellowness (b* parameter) was directly related to trans-β-carotene, and the yellow color of milk fat was caused by vitamin A, carotene, and other color pigments (Kontkanen *et al*., 2011; O'Callaghan *et al*., 2016).

Regarding the C* value, it decreased along the storage time for all butter types starting from the 30-d storage. For 0-, 30-, and 60-d storage times, the H_2 butter samples showed the highest C* value compared with both control and Mg samples ($P < 0.05$). However, at the end of storage (90 d), there was a non-significant difference between all-butter sample types ($P > 0.05$). The lowest C* value was determined in butter washed with Mg water on the 60th day (19.12 ± 0.80) whereas the highest one was found in the unwashed raw butter sample (51.88 ± 0.88) followed by the sample washed with H_2 water on day 0 (45.93 ± 0.76). This shows the pigments found in raw butter were partially lost during the washing process (0-d) at a low rate for the H_2 sample and a high rate for normal and Mg samples. During the storage period, H_2 water could better protect the pigments when compared with control and Mg samples. Here, the presence of Mg ions in Mg butter samples may have facilitated the degradation of pigments. In comparing H_2 and Mg butters we also need to take into account that, whilst we were careful to establish that hydrogen levels were similar in the two butters, we cannot exclude the possibility that small differences were present.

Regarding the h (hue values), we found no change after the washing step. At the end of storage, HRW butter samples (H_2 and Mg) showed the highest h value compared with control ($P < 0.05$). In a previous study, we showed the beneficial impact of hydrogen in preserving the antioxidant activity and color of white cheese during storage under a hydrogen-rich atmosphere (Alwazeer *et al*., 2020).

In conclusion, we have shown that the reduction capacity of H_2 dissolved in HRW plays an important role in the protection of physicochemical properties and color notes of butter samples during 90-d of cold storage. The main advantage of the proposed method is its use of a novel gaseous antioxidant (H_2), recognized as a food additive (E949) by the European Commission Regulation (EC) No 1333/2008 (European Parliament and the Council of the European Union, 2008), as well as its many beneficial biologic activities in health fields. HRW treatment is also a non-toxic and eco-friendly method of food preservation that leaves no harmful residuals.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029922000681.

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