RESEARCH
ARTICLE

Can hydroponic forage affect the chemical and sensory properties of PDO buffalo Mozzarella cheese?

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The aim of this study was to evaluate the effect of hydroponic barley forage in the diets of lactating Italian Mediterranean buffaloes on Mozzarella cheese chemical and sensory properties. Thirty-six buffaloes were evenly assigned to three groups: control (C) with standard maize silage-based ration, and low hydroponic (LH) and high hydroponic (HH) barley forage substitution for 50% and 100% of maize silage, respectively. HH Mozzarella had lower hardness, reduced saturated fatty acids, increased oleic and α-linolenic fatty acids. Hydroponic forage could be an innovative system for sustainable livestock production with a positive impact on the Mozzarella cheese improving its nutritional value.

INTRODUCTION

The coastal and fertile plains of southern Italy are characterised by intensive agricultural and dairy activities, with a particular emphasis on buffalo herds for the production of Mozzarella di Bufala DOP (Masucci *et al*. 2016; Zicarelli *et al*. 2023). The majority of dairy buffalo farms adopt total mixed rations (TMR) and produce maize (*Zea mays* L.) for silage as main forage crop and, to a lesser extent, ryegrass or grass-legume as secondary fodder sources (Serrapica *et al*. 2020, 2022). While maize for silage remains a crucial forage crop in many intensive ruminant farming systems, its production necessitates significant inputs of land, fertiliser and especially water (Gallo *et al*. 2014; Tabacco *et al*. 2018). Additionally, the rising costs of production inputs and the unpredictability of water availability pose challenges to maize production efficiency (Altobelli *et al*. 2018; Bellingeri *et al*. 2019).

Recently, the hydroponic system has emerged as a soilless forage production system for growing fresh forage of consistent quality throughout the year, garnering attention for its application in intensive dairy systems (Ceci *et al*. 2023).

The hydroponic system is based on the indoor germination and growth of seeds for a short period (5–8 days), with barley being a particularly suitable species (Hassen and Dawid 2022). In addition to the short production cycle, hydroponic forage production offers advantages such as independence from agro-climatic conditions, reduced labour requirements, reduced use of resources (e.g. water, land) and optimisation of forage production space and time (Ahamed *et al*. 2023).

Replacing maize silage with fresh forage is also encouraged by the improved fatty acid composition of the resulting dairy products, such as higher levels of unsaturated fatty acids, including conjugated linoleic acids and vaccenic acid, and a lower omega-6/omega-3 ratio (Balivo *et al*. 2023b). Despite conflicting results in the literature regarding the effects of hydroponic forage on animal performance, a recent review concluded that its use may enhance milk yield, feed intake, feed efficiency and the health status of lactating animals (Terefe and Mengistu 2022). To confirm this, a study complementary to the present one (Masucci *et al*. 2024) observed a possible increase in buffalo milk production with the complete replacement

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of maize silage with hydroponic barley forage. This resulted in significant water savings, low energy efficiency and possible compensation of hydroponic forage-related production costs if the increase in milk yield is sufficiently high.

The sensory properties of cheese are influenced by several factors related to the cheesemaking process (such as heat treatments, use of starter cultures or native microbiota and curdling conditions) as well as by enzymatic reactions that occur during maturation. However, when these factors are constant, some characteristics of cheese can be linked to the animal diet (Coulon *et al*. 2004; Martin *et al*. 2005; Balivo *et al*. 2023b). In a recent work, the inclusion of hydroponic barley forage as a maize silage substitute was authenticated using E-nose analysis on raw buffalo milk samples, with a correct classification rate of 90% (Balivo *et al*. 2024). However, there is still a lack of knowledge about the effect of using hydroponic forage on the chemical and sensory characteristics of Mozzarella specifically, and cheese in general, although replacing silage with fresh forage or pasture may affect the texture, fatty acids and volatile organic compounds of Mozzarella (Uzun *et al*. 2018; Natrella *et al*. 2020; Sacchi *et al*. 2020). This knowledge is crucial for artisanal PDO products, such as buffalo Mozzarella cheese, where the stability of organoleptic characteristics plays an important role in strengthening consumer loyalty to the product (Vecchio *et al*. 2016; Serrapica *et al*. 2022).

This paper aimed to address this research gap by investigating the chemical, physical and sensory properties of PDO Mozzarella cheese produced from the milk of buffaloes fed hydroponic barley forage as a substitute for maize silage in a typical diet for lactating buffaloes.

MATERIALS AND METHODS

Mozzarella cheese samples

The procedures described in this experiment involving handling and treatment of animals have been approved by the Institutional Ethics Committee on Animal Use of the University of Naples Federico II (protocol code PG/0025485) and in compliance with the EU requirements concerning the protection of animals used for scientific purposes (Directive 2010/63/EU of the European Parliament) as implemented by the Italian legislation (d.lgs. n. 26, 4 March 2014).

A feeding trial was carried out at a water buffalo farm and the adjoining dairy (41°16′ N 14°27′ E, Campania region, southern Italy), which produces PDO Mozzarella cheese. The farm is equipped with a fully automated hydroponic forage production plant (EA-38*2, Eleusis International Sau, Spain) able to produce up to 6000 kg/day of fresh forage (about 1000 kg/ on a DM basis). For this experiment, hydroponic forage produced from barley seed has been used. Thirty-six lactating buffaloes (Italian Mediterranean type) homogenous for days in milk (57.1 ± 22.1 days) and milk yield (12.36 ± 3.42 kg/head/day) were

Table 1 Ingredients and chemical composition (% of dry matter unless otherwise stated) of the experimental diets fed to the buffalo cows

Item	Experimental diets[a]		
	C	LH	HH
Dietary ingredients			
Maize silage	27.7	15.3	–
Hydroponic barley forage	–	13.7	21.9
Alfalfa hay	26.8	26.3	27.0
Alfalfa wrapped silage	10.8	10.6	10.9
Mixed hay	5.3	5.2	10.7
Maize meal	19.3	18.9	19.4
Concentrate mix[b]	10.1	10.0	10.1
Chemical composition			
Dry matter (DM), %	53.97	42.59	40.52
Ash	7.24	6.87	6.91
Crude protein	14.56	15.14	15.47
Ether extract	2.95	3.05	3.03
NDF	39.66	38.51	39.19
ADF	24.05	23.70	24.66
ADL	3.73	3.48	3.53
Starch	23.51	20.95	17.52
Water-soluble carbohydrates	2.70	6.10	8.30
Nonfibre carbohydrates	35.60	36.40	35.40
NE_L, MJ/kg DM	6.18	6.18	6.11
Dry matter intake, kg/day	14.6	14.4	14.9

ADF, acid detergent fibre; ADL, acid detergent lignin; NDF, neutral detergent fibre; NE_L, net energy of lactation.

[a]C = control diet; LH = 50% replacement of maize silage with hydroponic barley forage; HH = 100% replacement of maize silage with hydroponic barley forage.

[b]Commercial concentrate based on whole flaked soybean, maize meal, sodium bicarbonate, magnesium oxide, wheat middling, calcium carbonate, sodium chloride, Saccharomyces cerevisiae culture products, sugar cane molasse, vitamin and mineral supplements.

randomly assigned to three dietary groups. The control group (C) received a standard total mixed ration based on maize silage, which was substituted at a rate of about 50% and 100% by hydroponic barley forage (HBF) in the low (LH) and high (HH) groups, respectively. Table 1 presents the constituents, both as fed and on a dry matter (DM) basis, along with the chemical composition of the three diets. Table S1 reports the chemical composition of all dietary components. Due to the differing DM content of maize silage and HBF, 16 kg of maize silage in the control diet C was replaced by 16 kg of HBF in diet LH and by 25 kg of HBF plus 1 kg of mixed hay in diet HH. The adjustments were standardised on a DM basis, but the crude protein content was 1 percentage point higher in the HH diet than in the C diet. Given that HBF consists of germinated barley seeds, there was a notable reduction in starch content in the

Table 2 Chemical composition (LSM $\pm$ SEM) of milk and mozzarella cheese obtained from maize silage-fed buffaloes (control group, C) and hydroponic forage-fed buffaloes, with 50% (LH) and 100% (HH) silage replacement percentage.

Item	Milk					Mozzarella				
	C	LH	HH	SEM	*P*-value	C	LH	HH	SEM	*P*-value
Fat, %	9.14	9.01	8.80	0.13	0.66	22.97	22.88	22.90	0.14	0.86
Protein, %	4.37	4.39	4.53	0.01	0.73	15.58	15.50	15.48	0.12	0.78
Lactose, %	4.88	4.86	4.90	0.01	0.30	0.44	0.44	0.46	0.02	0.12
Salt (NaCl), %						0.77	0.76	0.77	0.003	0.94
pH						5.63	5.61	5.61	0.01	0.41

HH diet compared with the C diet. Conversely, water-soluble carbohydrate content was higher in the HH diet. However, overall non-fibre carbohydrate and energy content were comparable between the diets. A detailed account of the characteristics of the hydroponic system, animals and diets can be found in Masucci *et al*. (2024).

The experimental period consisted of a 2-week dietary adaptation, followed by 5 weeks of milk data recording and sample collection. Thereafter, three cheesemaking sessions were conducted on consecutive days using the refrigerated daily bulk milk (pooled milk from consecutive afternoon and morning milkings) produced by each treatment group, C, LH and HH. Table 2 reports the chemical composition (FoodScan™ Lab Dairy Analyser, Foss Electric, Hilleroed, Denmark) of milk and Mozzarella cheese as affected by the different diets. No significant differences in fat, protein and lactose content were observed between the samples ($P \leq 0.05$). Mozzarella was produced in small balls (about 50 g, 5.5 cm wide $\times$ 4 cm high) in separate vats for the three treatments, according to the traditional procedure previously described by Uzun *et al*. (2018). Mozzarella samples were collected from each production batch (about 3000 g) the same day of cheesemaking, for a total of $n = 9$ batches produced in 3 days for the three dietary groups, and were transported to the laboratory in separate polystyrene containers. Sensory and physical analyses were performed on fresh samples, which were then frozen at $-23°C$ for the subsequent analyses.

Sensory analysis
Mozzarella cheese samples were analysed for sensory properties on the same day of production. The sensory analysis tests were conducted by a panel of 11 trained assessors, aged between 25 and 50, who were recruited from the staff of the Department of Agricultural Sciences of the University of Naples Federico II based on their sensory acuity. Triangle test, a forced-choice ISO 4120:2004 sensory analysis methodology (ISO 2007), was conducted to compare the pairs of samples (C vs LH and C vs HH). Thus, two independent triangle tests were designed to determine sensory differences between treatments with 11 or 12 assessors. In a first

triangle test, panellists compared the samples from buffaloes fed maize silage (C) with the samples from buffaloes fed hydroponic barley forage as a 50% substitute for maize silage. In a second triangle test, they compared the C samples with the samples from buffaloes fed with 100% hydroponic barley forage. Each comparison was carried out on each day of the production of the samples of Mozzarella cheese. Each sensory test was divided into two sessions in order to randomise and balance the samples. Three samples, two of which were identical, were presented simultaneously to the panellists, in six possible randomised serving orders (ISO 2007). Panellists, without any information about the samples that could compromise or bias the test results, needed to identify the odd sample. All samples were coded with random three-digit numbers and blindly presented at 20°C.

Descriptive sensory analysis was also carried out. The gustatory, olfactory, visual and texture descriptors were developed by the panellists (Table 3) and quantified by quantitative descriptive analysis (QDA) methodology. Panel calibration and training sessions were conducted prior to the QDA. The intensity of each attribute was quantified with a continuous scale, anchored to extremes, from 0 (weak perception) to 10 (strong perception). Panellists were divided into two groups to randomise the presentation order of the samples. About 50 g of Mozzarella samples was placed in blind three-digit plastic plates and presented at 20°C.

Instrumental texture and colour analysis
The texture profile analysis (TPA) of the Mozzarella cheese was measured using a Texture Analyzer FRTS-50 N (IMADA, Toyohashi, Japan) equipped with a 50 N load cell according to Rehman *et al*.'s (2018) method at a temperature of 20°C. Ten small balls of Mozzarella were placed on a Petri plate, positioned vertically on the compression disc of the texture analyser and compressed to 40% of original height in two successive strokes (bites) with a test speed of 2 mm/s and a trigger system of 5 g force, using a cylindrical probe (5 mm diameter). The analysed parameters, obtained from the resultant force–time curve (Force Recorder Professional Software, version 1.03, IMADA,

Table 3 List of sensory descriptors evaluated in the quantitative descriptive analysis (QDA) of buffalo Mozzarella cheese samples

Descriptor	Definition
Taste descriptors	
Salty	Fundamental taste associated with sodium chloride
Sour	Fundamental taste associated with citric acid
Sweet	Fundamental taste associated with sucrose
Olfactory descriptors	
Overall odour	Overall odour intensity
Overall flavour	Overal flavour intensity
Milk	Room temperature whole-fat milk aroma
Butter/Cream	Aroma of milk fat, lactones and coconut
Whey	Aroma of whey (e.g. ricotta)
Yoghurt	Natural whole yoghurt aroma
Visual descriptors	
Colour	Intensity of colour (from white to ivory)
Brightness	Light reflected intensity from the external surface
Smoothness	Uniformity of external surface
Texture descriptors	
Hardness	Minimum force required to chew mozzarella samples: the higher the force, the higher the hardness
Elasticity	Original shape restored degree after compression between the teeth
Juiciness	Moisture released during mastication (low: saliva is absorbed by the product; high: abundantly liquids release during mastication)
Cohesiveness	The degree to which a mozzarella sample holds together or adheres to itself while being chewed
Chewiness	Easiness to masticate the sample to a state pending swallowing
Screechy	Friction of the product against the teeth, typical of milk casein soon after hot water stretching

Toyohashi, Japan), were hardness, stickiness, cohesiveness, springiness, gumminess and chewiness.

The colour measurements of the buffalo mozzarella cheese samples were carried out on 10 small balls of Mozzarella using the Portable Colour Meter FRU® WR-10QC (Shenzhen Wave Optoelectronics Technology Co Ltd, Shenzhen, China) equipped with a photodiode array sensor with a sensor head of 8 mm in diameter. The intensity of L*, a* and b* values, corresponding to whiteness (100)/darkness (0), redness (+values)/greenness (−values) and yellowness (+values)/blueness (−values), respectively, were measured in the inner and outer surface of samples arranged in Petri plates (Uzun *et al.* 2018).

Analysis of fatty acids

Fat extraction, fatty acid methylation to produce fatty acid methyl esters and GC analysis were performed according to the procedure described by Romano *et al.* (2011). In brief, for each batch ($n = 9$), three small balls of cheese were taken and mixed. From the resulting mixture, 6 g of sample was mixed with 20 mL of a 25% (w/v) hydrochloric acid solution and 20 mL of 95% (v/v) ethyl alcohol. The cheese suspension was homogenised and cooled. After cooling, the fat was extracted with an *n*-heptane/diethyl ether mixture. The organic extracts were dried over anhydrous sodium sulphate, filtered with a cellulose filter and evaporated with a rotary evaporator.

A solution of 2 N potassium hydroxide in methanol was used for fatty acid methyl esters (FAMEs) preparation, then 1 μL of the upper organic phase was analysed by high-resolution gas chromatography (HRGC). A Perkin Elmer Auto-system XL model gas chromatograph equipped with a PTV (programmed temperature vaporiser), a FID (flame ionisation detector) and a fused silica capillary column (Supelco Bellofonte, USA) (100 m × 0.25 mm i.d.; 0.20 μm film thickness) was used. The oven, the PTV operating conditions and the FID conditions were carefully described in Romano *et al.* (2011). Fatty acid peaks in chromatograms were identified using the Supelco 37 Component FAME MIX (Supelco, Bellofonte, PA). Standards for CLA (C18:2 cis-9-trans-11) and trans-vaccenic acid (C18:1 trans-11) were obtained from NuChek Prep (Elysian, MN). Fatty acids (FA) were expressed as a percentage of total methylated fatty acids (g/100 g FAs). The sum of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) FA was reported. The atherogenic and spreadability index were calculated as reported by Couvreur *et al.* (2006).

Extraction and analysis of volatile organic compounds

For each sample batch ($n = 9$), three small balls of Mozzarella were immediately frozen for a total of nine replicates per type. The extraction of volatile organic compounds (VOCs) was performed using Headspace-SPME technique

according to the procedure described by Sacchi *et al.* (2020). In brief, 25 g of frozen sample was finely grated and transferred to a 100-mL Duran® glass bottle with a magnetic stirring bar and suspended with 25 mL of distilled water. Then, 6.25 g of sodium phosphate (NaH_2PO_4) (Sigma-Aldrich) and 50 µL of 2-methyl-3-heptanone (purity 99%, Sigma-Aldrich, St. Louis, MO, USA), used as an internal standard (530 mg/L, in water solution), were added. The bottle was conditioned at 50°C for 10 min without stirring, to allow the cheese to melt and homogenise. The sample was magnetically stirred (150 rpm) for 20 min at the same temperature, to isolate and favour the equilibrium of VOCs between the cheese matrix and the headspace. The adsorption of VOCs was performed by inserting a 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 2-cm fibre (Supelco, Bellefonte, PA) into the headspace of the bottle and exposing the polymer for 30 min at 40°C while stirring.

Hence, the VOCs were desorbed directly in the injector port of the GC kept at a temperature of 250°C in split mode with a 4:1 split ratio, for 10 min. Volatile compound analysis was performed on an Agilent 7890A GC System gas chromatograph coupled to an Agilent 5975C VL MSD with Triple-Axis-Detector mass spectrometer (Agilent Technologies, Inc., Palo Alto, CA, USA). GC was equipped with a Zebron ZB-WAX capillary column (60 m × 0.25 mm i.d. × 0.25 µm film thickness 100% polyethylene glycol; Phenomenex, USA). The carrier gas was helium with a flow of 1 mL/min. The temperature program was 40°C for 10 min, then raised at 5°C/min to 240°C and held for 11 min (Balivo *et al.* 2023a). Mass spectra were recorded at 70 eV. The source temperature was 230°C, the quadrupole temperature was 150°C, and the interface temperature was 250°C.

The identification of VOCs was performed by comparing retention times and mass spectra obtained by analysing pure reference compounds in the same conditions. All chemical standards were supplied by Sigma-Aldrich (St. Louis, MO, USA). The identification was confirmed by comparing mass spectra with those of the National Institute of Standards and Technology (NIST) database. The fibre was conditioned at 270°C for 1.5 h before the analysis. A blank test was performed before each analysis. The quantitative data of the volatile compounds of milk and Mozzarella were obtained by normalising the peak areas of each compound with respect to the peak area of the internal standard. Peak area data were processed by MSD ChemStation 5975 TAD Data Analysis software (Agilent Technologies, Palo Alto, CA, USA).

Statistical analyses

Three indipendent cheesemaking trials for each dietary treatment (C, LH and HH) were carried out on three consecutive days, for a total of *n* = 9 batches. For each batch (day of production), the analyses were conducted at least in triplicate. Results were expressed as the least squares mean ± standard deviation or standard error. Differences in instrumental and sensory variables were assessed by analysis of variance (ANOVA) with Tukey's HSD test, for a significance level set at $P \leq 0.05$. To assess the differences of the triangle tests, data were analysed by counting the number of correct responses (correctly identified 'different' sample) and the number of total responses. These numbers were compared with critical values as described by Meilgaard *et al.* (1999) to determine significant differences. Partial least squares regression (PLSR) analysis was performed using the jackknife (LOO) cross-validation method with a 95% confidence interval to investigate the correlativity between sensory and instrumental (VOCs, instrumental hardness, SFA, MUFA and PUFA) data and graphically illustrate them on a correlation plot. Statistical analysis and visualisation were carried out in XLStat environment (Version 2019 v.2.2), an add-in software package for Microsoft Excel (Addinsoft Corp., Paris, France).

RESULTS AND DISCUSSION

Sensory analysis

The sensory evaluation of Mozzarella produced with milk from buffaloes receiving maize silage (C) and with 50% hydroponic barley forage (LH) and 100% hydroponic barley forage (HH) as silage substitute, was replicated in three different sessions for each day of cheesemaking. The results of the triangle test for Mozzarella samples are shown in Table 4. Assuming a 95% confidence level for significance, the HH Mozzarella samples were always discriminated against the C Mozzarella samples, while LH against C only in one session. These results indicate that panellists were unable to differentiate between the control group and the LH experimental group, but when the buffalo diet included total silage replacement (HH group), the panel members were always able to distinguish HH samples from C samples.

In this regard, a QDA was conducted to understand the sensory descriptors responsible for these differences, and mean intensity score results are shown in Figure 1. The average intensity of olfactory and gustatory descriptors was not different among the three types of Mozzarella samples, C, LH and HH (Figure 1a). In general, the sensory aroma and taste differences between cheeses from fresh or conserved forage-based diets are often subtle compared with those detected in milk (Kilcawley *et al.* 2018; Natrella *et al.* 2020), due to the technological factors (enzymes, microbial fermentation) that influence the sensory properties of the product. However, Manzocchi *et al.* (2021) investigated the sensory differences of uncooked Cantal-type cheese, matured for 9 weeks, obtained from cows fed with forage belonging to the same grassland but integrated as fresh or preserved (hay and silage) and reported that

Table 4 Triangle test results of Mozzarella cheese from milk of maize silage-fed buffaloes (control group, C) and hydroponic forage-fed buffaloes, with 50% (LH) and 100% (HH) silage replacement percentage for each day of production

No. of session	Treatment comparison	No. of assessors	No. of correct answers	Significance
1	C vs LH	11	5	No
	C vs HH	11	7	Yes ($\alpha \square 0.05$)
2	C vs LH	12	4	No
	C vs HH	12	8	Yes ($\alpha \square 0.05$)
3	C vs LH	11	7	Yes ($\alpha \square 0.05$)
	C vs HH	11	8	Yes ($\alpha \square 0.01$)

C, control group sample, consisting of Mozzarella cheese from milk of buffaloes fed with a ration based on maize silage as a fodder source; HH, second treatment, consisting of Mozzarella cheese from milk of buffaloes fed with a ration based on hydroponic forage as a fodder source; LH, first treatment, consisting of Mozzarella cheese from milk of buffaloes fed with a ration based on maize silage and hydroponic forage (50:50) as a fodder source. An α values ≤ 0.05 was considered for statistical differences between sample pairs.

panellists perceived a greater intensity of barnyard and dry fruit/nuts aroma attributes in cheeses from cows fed fresh forage. Cheeses from preserved forages showed a higher intensity of lactic aroma (Manzocchi *et al*. 2021). Milk components, such as fatty acids, are important precursors of aroma compounds in cheese, which could cause greater differences in odour and aroma sensory descriptors during cheese maturation time (Balivo *et al*. 2023b).

Regarding the 'colour' sensory descriptor, a higher yellowness is usually reported in cheeses obtained from cows fed with fresh forage rather than preserved forage, that is silage-fed and especially hay-fed cows (Carpino *et al*. 2004; Faulkner *et al*. 2018; Manzocchi *et al*. 2021), and was not observed in this study, consistent with findings reported by

Uzun *et al*. (2018) for buffalo Mozzarella. This is because cattle, not buffaloes, accumulate high amounts of carotenoids in milk due to their lower hepatic efficiency in synthesising vitamin A (Noziere *et al*. 2006).

Some differences were found for visual and texture descriptors. The perceived 'hardness' of the HH Mozzarella cheese samples was lower than that of the C and LH samples, while that of the LH samples obtained an intermediate score (Figure 1b). In particular, the mean intensity of the 'hardness' descriptor for the HH Mozzarella samples was approximately 22% lower than for the C and LH Mozzarella samples, suggesting that less force was required to chew the Mozzarella in the first bite. The descriptors 'smoothness' and 'brightness', which indicated the uniformity of the external surface and the intensity of the light reflected from the external surface, respectively, obtained higher intensity scores in the HH samples and lower scores in the C samples, while the LH samples ranked intermediate. Similar results were previously reported by Uzun *et al*. (2018), where Mozzarella made from milk of buffaloes fed with fresh sorghum exhibited lower hardness and higher smoothness. Couvreur *et al*. (2006) found that variations in animal diet led to changes in milk fatty acid composition, resulting in decreased final melting temperature and solid fat content in butter, leading to a lower hardness in the mouth. Moreover, a higher percentage of polyunsaturated fatty acids in milk fat appears to be associated with the increased polarity of the fat globule membrane (Lopez *et al*. 2008; Manzocchi *et al*. 2021). This increased polarity reduces the cohesion of milk fat globule membrane, which will be more prone to disruption during cheese production, which could then affect the texture of the cheese.

Instrumental analysis of texture and colour

The inclusion of hydroponic barley forage as a substitute for maize silage in the diet of lactating buffalos led to obtaining mozzarella cheese with a lower hardness, respectively of 3.2, 5.7 and 10.5 N for HH, LH and C samples (Table 5). This finding is in agreement with sensory
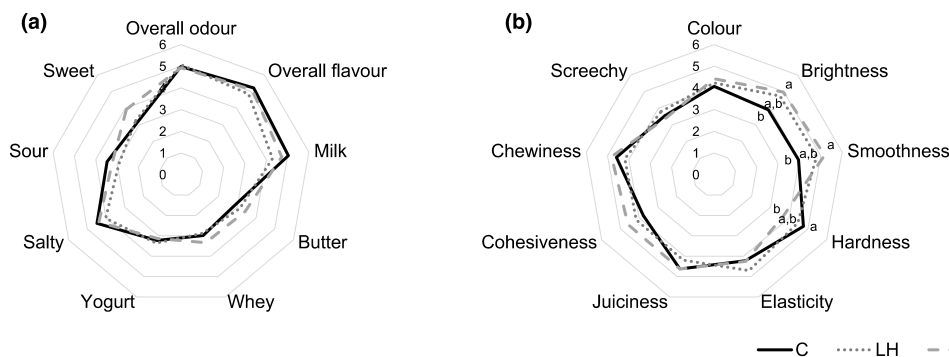
Figure 1 Mean intensity values of olfactory, taste (a), visual and texture (b) sensory descriptors perceived in the three different sessions (three differentiated cheesemaking trials) on Mozzarella cheese from maize silage-fed buffaloes (control group, C) and hydroponic forage-fed buffaloes, with silage replacement rates of 50% (LH) and 100% (HH). Different letters indicate statistically significant differences (*P* < 0.05).

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Table 5 Values of the texture and colour parameters of Mozzarella cheese samples made from maize silage-fed buffaloes (control group, C) and barley hydroponic forage-fed buffaloes, with 50% (LH) and 100% (HH) silage replacement percentage

	C	LH	HH
Texture			
Hardness (N)	10.48 ± 4.92 a	5.65 ± 1.33 b	3.18 ± 1.01 b
Stickiness (N)	0.06 ± 0.02 a	0.05 ± 0.02 ab	0.03 ± 0.02 b
Cohesiveness	0.44 ± 0.14 b	0.52 ± 0.09 ab	0.57 ± 0.09 a
Springiness (mm)	1.17 ± 0.34 a	1.00 ± 0.01 a	1.00 ± 0.03 a
Gumminess (N)	3.77 ± 1.23 a	2.95 ± 0.89 a	1.81 ± 0.58 b
Chewiness (N)	4.26 ± 1.26 a	2.95 ± 0.89 b	1.80 ± 0.58 c
Colour			
L* (lightness)	91.60 ± 2.47 a	91.25 ± 1.63 a	91.16 ± 2.34 a
a* (red-green index)	−1.44 ± 0.24 a	−1.48 ± 0.21 a	−1.35 ± 0.35 a
b* (yellowness)	7.33 ± 0.47 a	7.38 ± 0.62 a	7.19 ± 0.56 a

Values reported as mean of 10 replicates ± standard deviation. Different lowercase letters within the same row are statistically different ($P \leq 0.05$).

hardness, where a lower hardness for the HH Mozzarella samples was perceived by the panellists (Figure 1b). The lower hardness at room temperature could be due to a higher quantity of unsaturated fatty acids, which have a lower melting temperature and a lower solid fat content (O'Callaghan et al. 2016). Lower hardness, both sensory and instrumental, has previously been observed in dairy products from animals fed fresh forage rather than preserved foraged (Couvreur et al. 2006; Villeneuve et al. 2013; O'Callaghan et al. 2016, 2017). The stickiness (N) and chewiness (N), which indicate a measure of the force required to chew the food product to the point adequate for swallowing (Peleg 2019), were lower in experimental samples (HH and LH) than in control sample. On the contrary, the opposite behaviour was observed by cohesiveness, which was higher in the LH and HH Mozzarella samples (Table 5) and which describes how well the sample retains its form between the first and second compressions. Similar findings have been reported by O'Callaghan et al. (2017) in Cheddar cheese produced from cows fed fresh forage rather than silage, which had lower chewiness and that this textural parameter was positively correlated with hardness, as observed in our study.

The other parameters of the texture profile analysis were not different between the samples, as well as the instrumental colour parameters (Table 5). Cattle are the only ruminants that accumulate high levels of carotenoids, due to the lower hepatic efficiency of vitamin A biosynthesis, resulting in dairy products from cows fed fresh forage having a higher yellow intensity (Balivo et al. 2023b). Therefore, the lack of differences between samples for colour was expected and is consistent with previous results (Uzun et al. 2018) as buffalo milk does not contain detectable amounts of β-carotene, which is enzymatically converted into retinol (Cerquaglia et al. 2011). In fact, Mozzarella is an unseasoned fresh cheese with a moisture content of 52%–60%, which retains the typical white colour of buffalo milk (Arora and Khetra 2017).

Analysis of fatty acids

The average percentage values of fatty acids in Mozzarella cheeses in relation to the forage source (maize silage and hydroponic forage at 50% and 100% silage replacement) in the animal diet are shown in Table 6. Short and medium chain saturated fatty acids, from C4:0 (butyric acid) to C14:0 (myristic acid), were not statistically different between treatments. The inclusion of hydroponic barley forage in the buffalo diet resulted in a linear decrease in the quantity of saturated fatty acids (SFA), such as palmitic acid (C16:0), and an increase in the content of unsaturated fatty acids. Particularly, the amount of saturated fatty acids was reduced by 5.15% in HH vs C, while monounsaturated fatty acids (MUFA) increased by 12.85% and polyunsaturated (PUFA) by 22.28%. Higher unsaturated fatty acid content in buffalo Mozzarella when fresh forage replaced silage have been previously reported (Uzun et al. 2018; Sacchi et al. 2020). This improvement in the fatty acid profile is widely demonstrated in dairy products from cows fed with fresh forages (White et al. 2001; Villeneuve et al. 2013; Corazzin et al. 2019; Riuzzi et al. 2021).

Total replacement of maize silage with hydroponic barley forage increases rumenic acid (CLA) and vaccenic acid (VA) content by one and a half times. Rumen biohydrogenation of dietary unsaturated fatty acids, such as linoleic acid, leads to the formation of CLA and VA (Doreau et al. 2010). Rumen biohydrogenation also involves the production of saturated fatty acids, including stearic acid. However, stearic acid can be desaturated in the mammary gland by stearoyl-CoA desaturase reforming CLA, and this enzymatic activity was found to be greater in animal diets with fresh

Table 6 Fatty acid composition (% weight of total methyl esters) of Mozzarella cheese from milk of maize silage-fed buffaloes (control group, C) and hydroponic forage-fed buffaloes, with 50% (LH) and 100% (HH) silage replacement percentage

	C	LH	HH	SE	P value
Fatty acid*					
C4:0 (butyric)	3.43	3.42	3.58	0.13	0.63
C6:0 (caproic)	1.90	2.04	1.86	0.25	0.87
C8:0 (caprylic)	0.93	1.04	1.09	0.08	0.42
C10:0 (capric)	2.06	1.91	1.92	0.09	0.47
C11:0 (undecylic)	0.21	0.21	0.20	0.01	0.82
C12:0 (lauric)	3.16	3.00	3.12	0.10	0.44
C13:0 (tridecylic)	0.23	0.25	0.21	0.01	0.11
C14:0 (myristic)	12.79	12.92	12.5	0.30	0.6
C15:0 (pentadecylic)	0.94 b	1.18 a	1.09 ab	0.04	0.02
C16:0 (palmitic)	35.9 a	35.4 a	33.6 b	0.39	0.014
C17:0 (margaric)	0.49 b	0.49 b	0.58 a	0.02	0.01
C18:0 (stearic)	11.7 a	10.34 b	10.31 b	0.21	0.006
C20:0 (arachidic)	0.19	0.20	0.20	0.02	0.67
Σ SFA	73.8 a	72.18 ab	70.09 b	0.55	0.0092
C14:1n9 (myristoleic)	0.64	0.77	0.86	0.04	0.05
C16:1 n9 (palmitoleic)	1.41	1.35	1.44	0.11	0.87
C17:1 (heptadecenoic)	0.19 b	0.19 b	0.25 a	0.01	0.01
C18:1 n9t (elaidic)	0.32	0.38	0.33	0.03	0.5
C18:1 n11t (vaccenic)	0.7 b	0.9 a	1.04 a	0.04	0.004
C18:1 n9c (oleic)	19.06 b	20.3 ab	21.3 a	0.50	0.05
Σ MUFA	22.33 b	23.91 ab	25.2 a	0.44	0.01
C18:2 n9t, 12t (linolelaidic)	0.21ab	0.25 b	0.37 a	0.03	0.03
C18:2 n9c, 12c (linoleic)	2.09	1.91	2.15	0.15	0.54
C18:3 n3c, n6c, n9c (α-linolenic)	0.56 b	0.6 b	0.75 a	0.02	0.0005
CLA n9c, n11t (conjugated linoleic)	0.48 b	0.59 ab	0.7 a	0.03	0.009
C20:3 n8c, 11c, 14c (dihomo-γ-linolenic)	0.17 b	0.16 b	0.34 a	0.02	0.015
C20:3 n11c, 14c, 17c (eicosatrienoic)	0.16	0.19	0.21	0.02	0.48
Σ PUFA	3.86 b	3.90 ab	4.72 a	0.19	0.03
Omega 6/omega3	3.81	2.94	2.98		
Atherogenic index	3.45	3.24	2.90		
Spreadability index	1.88	1.75	1.58		

Data are shown as mean and standard error (SE). Different letters indicate statistically differences between the samples ($P < 0.05$). * = g/100 g of total FAs.

Atherogenic index = [C12:0 + (4 × C14:0) + C16:0]/(Σ MUFA + Σ PUFA).

Spreadability index = (C16:0/C18:1).

MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

forage inclusion (Wiking *et al.* 2010; Tudisco *et al.* 2019). These trans-11-based fatty acids, typical of ruminant fat, are associated with positive implications for human health, such as anti-inflammatory and immunomodulatory effects (Balivo *et al.* 2023b).

The different composition of the fatty acids has consequences on the physical properties of the Mozzarella cheese. The greater amount of MUFA and PUFA rather than SFA in Mozzarella LH and HH compared with C influences the melting temperature of the milk fat. Couvreur *et al.* (2006) used the ratio between palmitic acid (16:0; melting point of 62.9°C) and oleic acid (cis-9 18:1; melting point of 13–14°C), the two most abundant fatty acids, saturated and monounsaturated respectively, in milk fat to define the spreadability index of butterfat. In our study, palmitic acid was higher and oleic acid was lower in C, while the opposite trend was exhibited by Mozzarella LH and HH, where the latter had the least amount of palmitic and the greatest amount of oleic acid. Therefore, the spreadability index decreased with increasing hydroponic forage rate in the

buffalo diet (Table 6), and these results were corroborated by both the sensory (Figure 1b) and instrumental hardness (Table 5) previously discussed.

Regarding PUFA, while linoleic acid (ω-6) was not different between the three treatments, α-linolenic (ω-3) and dihomo-γ-linolenic acid (ω-6) were higher in Mozzarella HH, in agreement with Uzun *et al*. (2018) who explored the replacement of maize silage with fresh sorghum forage in the buffalo diet. Again, in agreement with the authors, the nutritional indicators of fats, such as the omega-6/omega-3 ratio and the atherogenic index, were lower in the Mozzarella samples in which the buffaloes received hydroponic barley forage, respectively, 3.81 in C and 2.98 in HH for the omega-6/omega-3 ratio and 3.45 in C and 2.90 in HH for the atherogenic index. Compared with maize silage, which is rich in linoleic acid (ω-6), fresh forages are richer in α-linolenic acid (C18:3 n-3), leading to higher amounts of these fatty acids in dairy products (Glasser *et al*. 2013).

Analysis of volatile compounds

The volatile compounds identified and quantified in the Mozzarella cheese samples, with the information relating to the odour descriptors found in the literature, are listed in Table 7.

A total of nine aldehydes, seven ketones, nine alcohols, eight acids and four esters have been identified in Mozzarella cheese samples. Differences in volatile compounds between samples C, LH and HH were only quantitative. Among the 37 VOCs identified, only seven were statistically different ($P < 0.05$). The HH samples had a higher quantity of aldehydes than volatile fatty acids, in agreement with the findings of Sacchi *et al*. (2020) when the buffalo's diet contained fresh forage rather than silage.

Overall, the C Mozzarella samples had a higher quantity of volatile compounds in the headspace than the LH and HH samples. Acetophenone was quantitatively higher in C samples, while octanal was more abundant in LH and HH samples. A higher quantity of acetophenone has recently been found in Mozzarella from buffaloes that have received wrapped ryegrass silage (Sabia *et al*. 2020). Straight-chain aldehydes, such as octanal, with a 'cut grass' odour, can derive from the lipoxygenase pathway of unsaturated fatty acids (Collins *et al*. 2003; Ianni *et al*. 2020), which were found in greater quantities in Mozzarella obtained from buffaloes fed with hydroponic barley forage, as revealed by the analysis of fatty acids (Table 6). The amount of 1-octen-3-ol increased with increasing amount of hydroponic forage replacing the maize silage. 1-octen-3-ol, a secondary alcohol with a mushroom and earthy odour typical of the aroma of water buffalo milk and Mozzarella cheese (Moio *et al*. 1993), can result from the metabolism of unsaturated fatty acids (Curioni and Bosset 2002), which were higher in the HH and LH samples (Table 6).

The straight-chain and branched short-chain volatile fatty acids, that is acetic acid, propanoic acid, 2-methyl propanoic and 2-methylbutanoic acid, were more abundant in Mozzarella samples produced from the milk of buffaloes fed maize silage. The extensive lipolysis of forage triglycerides during ensiling (Elgersma *et al*. 2003; Glasser *et al*. 2013) may have contributed to an increased quantity of volatile fatty acids in Mozzarella obtained from buffaloes fed with maize silage. In addition, lactic acid bacteria participating in the fermentation during maize silage production produce free short-chain fatty acids, including propionic and butyric acids, from carbohydrates. Such volatile fatty acids are typical of dairy products aroma, having cheesy, buttery, sweaty and rancid odours.

Therefore, the different animal diet, by influencing the composition of milk, particularly the quantity of fatty acids in milk fat, is responsible for the formation, starting from these precursors, of a different quantity of some VOCs, although some VOCs may derive directly from the feed (Balivo *et al*. 2023b). These differences in VOCs could be implicated in the perception of different aromatic nuances, especially in medium and long-ripened cheeses, as discussed above regarding sensory analysis. During the maturation period, reactions catalysed by enzymes and microorganisms on cheese components, as well as reactions between different VOCs, such as the interaction between free fatty acids and alcohols present in the cheese to produce esters, lead to the evolution of the flavour profile (Bertuzzi *et al*. 2018). In a recent study, incorporating cocoa bean shells to partially substitute concentrates such as corn in the diet of lactating sheep resulted in cheeses with higher levels of MUFA, without altering the sensory characteristics of aroma and odour, as evaluated by QDA, associated with the typical profiles of ewe cheese (Caccamo *et al*. 2024). These results were corroborated by few significant differences in VOCs between cheeses, although from the Gas Chromatography-Olfactometry (GC/O) analysis, it emerged that some compounds could be correlated with the perception of particular odour notes, such as rancid for acids, herbaceous for aldehydes and fruity/orange for some esters and ketones. However, this relationship between the odour activity of particular VOCs in cheese in relation to the animal diet, and also how this profile influences the human sensory perception, needs to be better explored.

Partial least squares regression analysis was used to summarise the results and explore the relationship between sensory descriptors and instrumental data (Figure 2) in C, LH and HH Mozzarella samples. For the PLSR analysis, the mean values of three replicates for the three different cheesemaking trials were used, indicated by the Numbers 1, 2 and 3 in Figure 2. Moreover, the instrumental data were selected as the X variables and the sensory descriptor values as Y variables. Overall, slight differences were observed. However, increasing the amount of hydroponic barley

Table 7 Quantitative data of volatile organic compounds identified in Mozzarella cheese samples obtained from maize silage-fed buffaloes (control group, C) and barley hydroponic forage-fed buffaloes, with low 50% (LH) and high 100% (HH) silage replacement percentage

Volatile compound	C	LH	HH	Key odour
Ketones				
2-Pentanone	2.0 ± 0.7a	1.7 ± 0.7a	1.6 ± 0.7a	Sweet, fruity
Diacetyl	3.7 ± 1.6a	3.1 ± 1.2a	3.8 ± 1.4a	Buttery[a]
2-Heptanone	16.1 ± 14.5a	13.3 ± 7.4a	10.1 ± 5.0a	Animals, blue cheese, mouldy[a]
Acetoin	16.6 ± 6.9a	12.7 ± 4.2a	18.5 ± 6.9a	Buttery, creamy[a]
2-Hydroxy-3-pentanone	0.6 ± 0.5a	0.4 ± 0.1a	0.5 ± 0.1a	Truffle, earthy, nutty
2-Nonanone	4.6 ± 3.2a	3.6 ± 1.3a	2.7 ± 0.3a	Hot milk, fruity
Acetophenone	0.9 ± 0.3a	0.3 ± 0.0b	0.5 ± 0.2b	Floral
Aldehydes				
2-Methylpropanal	7.7 ± 2.6a	4.8 ± 2.4a	6.8 ± 4.7a	
2-Methylbutanal	17.8 ± 8.1a	10.5 ± 7.1a	13.5 ± 10.2a	Musty, malty, fermented
3-Methylbutanal	7.6 ± 5.9a	3.9 ± 3.8a	3.8 ± 2.7a	Green, fruity
Pentanal	2.6 ± 1.3a	1.6 ± 0.7a	1.6 ± 0.7a	Grassy, fruity, green[b]
Hexanal	5.7 ± 1.6a	4.9 ± 1.3a	4.9 ± 1.3a	Green, cut-grass[a]
Heptanal	5.2 ± 3.8a	3.2 ± 1.0a	3.8 ± 0.7a	Herbaceous, green, oily[b]
Octanal	0.4 ± 0.2b	0.8 ± 0.6a	0.5 ± 0.1ab	Green, fatty[b]
Nonanal	8.2 ± 3.2a	6.2 ± 3.2a	5.7 ± 2.3a	Fatty, grassy, animal[a,b]
Benzaldheyde	0.9 ± 0.6a	0.9 ± 0.6a	0.7 ± 0.6a	Almond[b]
Alcohols				
Ethanol	28.6 ± 6.5a	23.8 ± 11.3a	23.0 ± 10.4a	Alcoholic
1-Propanol	15.4 ± 9.2a	12.2 ± 5.7a	9.5 ± 2.7a	Pungent[a]
1-Butanol	0.6 ± 0.5a	0.4 ± 0.2a	0.4 ± 0.1a	Medicinal, floral, fragrant[a]
3-Methyl-1-butanol	52.8 ± 35.5a	30.2 ± 24.7a	29.1 ± 20.6a	Fruity, banana
1-Pentanol	0.6 ± 0.3a	0.4 ± 0.2a	0.5 ± 0.2a	Sweet, fruity, plastic[b]
2-Pentanol	0.8 ± 1.1a	0.6 ± 0.4a	0.6 ± 0.2a	Green, fermented
1-Hexanol	2.1 ± 1.3a	1.2 ± 0.5a	1.8 ± 0.4a	Green[b]
1-Octen-3-ol	0.4 ± 0.2b	0.6 ± 0.4ab	0.8 ± 0.1a	Mushroom, earthy[b]
2-Phenylethanol	1.0 ± 0.9a	0.5 ± 0.2a	0.4 ± 0.1a	Floral, rose-like
Acids				
Acetic acid	5.4 ± 3.7a	1.7 ± 0.3b	1.7 ± 1.2b	Vinegar-like, pungent[a]
Propanoic acid	7.3 ± 5.4a	3.4 ± 1.4b	2.6 ± 1.4b	Pungent, cheesy
2-Methylpropanoic acid	0.7 ± 0.4a	0.5 ± 0.2ab	0.3 ± 0.2b	Dairy, buttery, rancid
Butanoic acid	6.0 ± 3.5a	3.7 ± 1.3a	3.1 ± 0.9a	Sweet, sweaty, cheesy[a]
2-Methylbutanoic acid	2.1 ± 2.0a	0.7 ± 0.3b	0.8 ± 0.5b	Pungent, cheesy
Hexanoic acid	24.5 ± 22.0a	13.4 ± 6.1a	15.3 ± 1.4a	Pungent, goaty, cheesy[a]
Octanoic acid	17.2 ± 9.8a	12.7 ± 4.0a	11.5 ± 1.3a	Fatty, musty, camphor, nutmeg[a]
Decanoic acid	4.0 ± 1.8a	4.1 ± 1.3a	3.4 ± 1.1a	Grassy, fatty, goaty, sour[b]
Esters				
Ethyl acetate	25.1 ± 9.9a	21.1 ± 20.2a	36.6 ± 25.8a	Fruity, sweet, green
Ethyl buatanoate	2.3 ± 0.5a	1.9 ± 1.1a	1.4 ± 0.7a	Sweet, fruity[a], apple-like[b]
Isopropyl isovalerate	0.4 ± 0.1a	0.4 ± 0.1a	0.4 ± 0.1a	Fuity, apple, pieapple
Ethyl hexanoate	0.3 ± 0.1a	0.4 ± 0.3a	0.3 ± 0.2a	Sweet, unripe fruity[b]

Concentration expressed in µg/Kg as the mean of the values of the three replicates for each cheesemaking period (*n* = 9 for C, 9 for LH and 9 for HH), followed by the standard deviation. Different letters correspond to statistically significant differences (*P* ≤ 0.05).
[a]Sacchi *et al.* (2020).
[b]Moio *et al.* (1993).

forage in the buffalo diet led to more pronounced differences, as in the case of HH Mozzarella cheese. It is interesting to note that increasing the replacement of maize silage with hydroponic forage (from C to HH samples) increased the amount of unsaturated fatty acids. This is correlated with the development of specific volatile compounds, such as
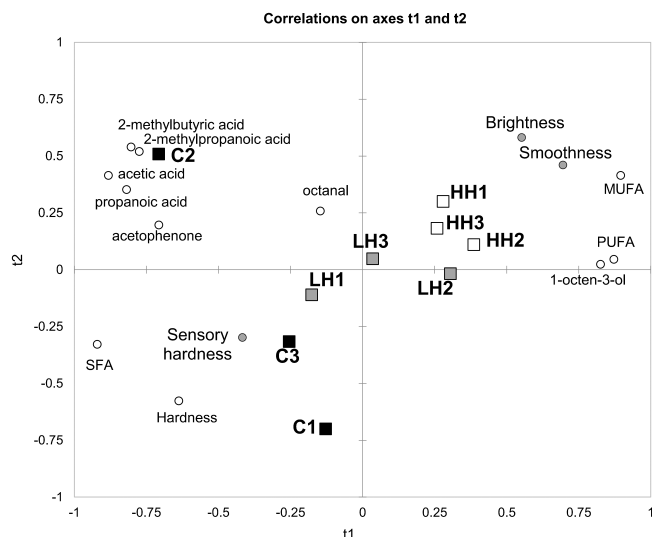
Figure 2 Partial least squares regression loading plot (t1 vs t2) for statistically significant ($P < 0.05$) volatile organic compounds, instrumental hardness, total saturated fatty acids (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) (white circles) and sensory descriptors (grey circles) of Mozzarella cheese from maize silage-fed buffaloes (control group, C) and hydroponic forage-fed buffaloes, with silage replacement rates of 50% (LH) and 100% (HH). The Numbers 1, 2 and 3 denote the three separate cheesemaking trials (average value of three replicates for each trial).

1-octen-3-ol and octanal, which have a mushroom and herbaceous odour and are derived from the catabolism of unsaturated fatty acids. Additionally, a good relationship was observed between hardness and the amount of saturated fatty acids. Figure 2 shows that the samples from the control group (C) and the 50% silage replacement group (LH) were more similar to each other than to the samples from the 100% silage replacement group (HH). As discussed earlier, C and LH were correctly identified as different in the triangle test only once, in the third session (Table 4), and, thus, this similarity was supported by sensory, texture and volatile compound analyses.

CONCLUSIONS

The results of this study showed that the inclusion of hydroponic barley forage as a substitute for maize silage in the buffalo diet resulted in slight differences in sensory and volatile compound profile of Mozzarella cheese. Some important differences found were a lower sensory and instrumental hardness, and a higher content of octanal and 1-octen-3-ol in the Mozzarella obtained from buffaloes fed with hydroponic forage. These findings could be explained by the lower amount of saturated fatty acids (such as palmitic and stearic fatty acids) and the higher amount of unsaturated fatty acids (such as oleic and α-linolenic fatty

acids). This fatty acid profile affects the physical properties by lowering the melting temperature of milk fat, resulting in a lower cheese hardness, and the formation of specific volatile compounds derived from the catabolism of unsaturated fatty acids. Furthermore, these modifications in fatty acid composition determined the reduction in the atherogenic index and omega-6/omega-3 ratio, improving the nutritional properties of the mozzarella cheese.

It is interesting that the results indicated no major changes in the organoleptic characteristics of buffalo Mozzarella DOP, because sensory properties drive consumer preferences of traditional products. As this is the first study to investigate the chemical and sensory properties of dairy products obtained from hydroponic forages, further work, including the use of different forage species, is recommended to reinforce the positive role of hydroponic forages in animal nutrition.

AUTHOR CONTRIBUTIONS

Andrea Balivo: Investigation; formal analysis; data curation; writing – original draft; visualization. **Felicia Masucci:** Project administration; conceptualization; supervision; writing – review and editing. **Sonia Parlato:** Investigation; visualization. **Francesco Serrapica:** Investigation; writing – review and editing. **Raffaele Romano:** Investigation; visualization. **Antonio Di Francia:** Conceptualization; methodology. **Alessandro Genovese:** Project administration; conceptualization; data curation; supervision; resources; writing – review and editing.

CONFLICT OF 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 STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

The following supporting information is available for this article:

Table S1. Chemical composition (% dry matter unless noted) of ingredients used in the formulation of the experimental diets.