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A Comparative Study on the Use of *Lactobacillus plantarum* and Water Kefir to Improve Fermented Sausage Quality

Nesreen Z. Eleiwa[1], Radwa A. Lela[2], Eman K. Fathalla[2]

[1]Food Hygiene Department, Animal Health Research Institute (AHRI), Dokki, Agricultural Research Center (ARC), Egypt; [2]Food Hygiene Department, Animal Health Research Institute (AHRI), Tanta Lab., Agricultural Research Center (ARC), Egypt.

Abstract | The continuous search for natural preservatives that improve the microbial quality, physicochemical characteristics, shelf life, and sensory attributes of food products has become an issue of interest to researchers and consumers alike. Lactic acid bacteria, especially Lactobacillus plantarum, have been frequently used as a starter culture for fermented sausage production. Water Kefir is another valuable product that causes sausage fermentation and enhances its flavor, quality, and shelf life. Therefore, this study was conducted to compare *Lactobacillus plantarum*, which acts as a control group, and different concentrations of Kefir 1%, 3%, and 5% in the fermentation process of oriental sausages. The results demonstrated that including water Kefir notably improved the sausages' quality. Specifically, water Kefir 3% and 5% concentration groups showed overall acceptability till the 15th and 17th days. In comparison, water Kefir 1% and the control group showed acceptability till the 11th and 7th day, respectively. In addition, a significant difference ($P<0.05$) in levels of total bacterial, psychrotrophic, and yeasts and molds counts, especially on the 7th day between the control group (7.78±.01a, 4.38±.01a and 4.96±.01a), water Kefir 1% (6.83±.01b, 3.73±.01band 4.66±.01b), 3% (5.69±.01c, 3.54±.01c and4.53 ±.01c) and 5% (5.60±.03d, 3.41±.01d and 4.50±.01d), respectively. It was observed that water Kefir 3% and 5% had significant differences ($P<0.05$) effects on color intensity, optimum acidity levels, increased water holding capacity, and reduced drip and cooking losses compared to 1% water Kefir and the control one. In conclusion, Water Kefir was preferred to be used in the fermented sausage as it not only enriched the flavor and texture of the sausages but also decreased total bacterial, total psychrotrophic, and total mycotic counts, enhanced the physicochemical characteristics, sensory qualities, and overall standard of the sausages.

Keywords: Sausage, Food safety, Water Kefir, *Lactobacillus plantarum*, Natural preservatives

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***Correspondence** | Nesreen Z. Eleiwa, Food Hygiene Department, Animal Health Research Institute (AHRI), Dokki, Agricultural Research Center (ARC), Egypt; **Email:** nesreenwow@gmail.com
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INTRODUCTION

Food safety is a critical issue in the food sector because of its significant and lasting impact on public health, especially when individuals eat food contaminated with harmful bacteria (Food and Drug Administration, 2020). Meat products are gaining popularity due to the fast-paced nature of modern life, as they are quickly served and have a pleasant taste at a suitable price (Maky *et al.*, 2020). Fermented sausage is one of the conventional meat products that have been consumed for centuries in various regions of the world (Alkazzaz *et al*., 2022a; Shan *et al*., 2023).

Fermentation is among the earliest techniques in food science. It is naturally eco-friendly, which results in products with an acceptable taste and more nutritional value. This process is proven by thousands of years of successful history in food preservation. It aligns with consumers preferences and expectations and is constantly gaining increasing attention and popularity (Alkazzaz *et al*., 2022b). In fermentation, starter cultures play a crucial role as they accelerate the aging process and improve preservation by lowering the pH, which gives the characteristic acidic flavor of fermented products (Leroy and de Vuyst, 2004). Lactic acid fermentation is one of the oldest and most commonly employed techniques for bioconservation. It is extensively utilized to preserve food, impart unique flavors and textures to foods, and provide health benefits (Sionek *et al*., 2023).

Lactobacillus plantarum is one of the widely used Lactic acid bacteria with promising characteristics to be applied in the commercial fermented meat industry; also, it has a significant antioxidant effect in the fermentation of sausage (Ayyash *et al*., 2019).

Although the effect of *Lactobacillus plantarum* on the quality of fermented meat products was evaluated before (Sun *et al*., 2016), recently, the Kefir grains could be used as a starter culture as they contain high levels of Lactic acid bacteria (LAB), which produced varying metabolites including lactic acid, acetic acid, diacetyl, free fatty acids, amines, and aldehydes which contributed to the texture and flavor of fermented meat products (Wu, 2014)

Kefir is an attractive alternative for the food industry. It can provide an alternative to starter cultures for sausage production that can provide sensory, technological, and nutritional advantages and contribute to the safety of the processed product (Carasi *et al*., 2014). It is a probiotic beverage originating in the Caucasus Mountains of Russia (Wu *et al*., 2018) and produced through a double fermentation process involving both lactic and alcoholic fermentation. This process produces a complex mixture of lactic, formic, succinic, and propionic acids, carbon dioxide, ethyl alcohol, aldehydes, isoamyl alcohol, acetone, and folic acids. In addition, Kefir contains a suspension of associated microorganisms, including acidophilic bacteria and yeast. These components provide Kefir's unique health benefits, attributed to its distinct probiotic properties (Magalhães *et al*., 2011).

Kefir has multiple positive technological effects. It prevents the growth of spoilage and pathogenic microorganisms, accelerates the drying process, and enhances the product's texture by hydrolyzing and coagulating proteins. In addition, it activates muscle proteins and contributes to the development of redness in the product through the formation of nitric oxide and nitrosylated myoglobin. (Bedia *et al*., 2011).

Despite the well-established benefits of traditional lactic acid bacteria such as *Lactobacillus plantarum* in sausage fermentation, there is limited research on using water Kefir as a starter culture. This study aims to explore its novel application in fermented sausage production, assessing its potential to enhance sensory, chemical, and microbiological properties compared to traditional lactic acid bacteria *Lactobacillus plantarum*.

MATERIALS AND METHODS

Preparation of sausage

Two kilograms of local chilled beef from a local slaughterhouse one day before sausage preparation. The sausage formulation was prepared following the formula outlined by Mejri *et al*. (2017) with slight modifications, using beef meat (75% w/w) and fat (25% w/w) instead of camel meat, mincing it with a meat mincer (MK-G20NR-W, Panasonic, Osaka, Japan). The ingredients added included 25g/ kg^{-1} NaCl, 10g/ kg^{-1} powdered garlic, 4g /kg^{-1} sucrose, and mixed spices 30g/ kg^{-1}. The prepared mixture was tested for *Salmonella* spp. and *Shigella* spp. before use and then stored overnight at 4°C.

Preparation of starter culture

Lactobacillus plantarum was a starter culture with a recommended dose (7 log cfu/mL) from the Media Unit, Food Hygiene Department, Animal Health Research Institute, Dokki, Giza, Egypt.

Water Kefir

Water Kefir was purchased from (Tayyiba Farms)[R], Egypt. According to the company's instructions, Kefir water was prepared from grains by pouring 1/4 cup sugar into the clean jar, then adding 4 cups hot water and dissolving the sugar well. The mixture was cooled at room temperature, and then the water Kefir grains package (5.4g) was closed in the jar and left in a warm place at 25°C for 24 hours; separate Kefir grains and discard the first ferment liquid. Place water Kefir grains again in the new batch of sugar water and leave it to ferment for 48 hours, then store in the refrigerator till use.

Before the experiment, water pH was measured by an electric pH meter (Bye version 6020, USA), and the count of lactic acid bacteria (LAB) was performed on de man rogosa and Sharpe agar media (MRS) (Oxoid) at 37°C in an anaerobic jar according to Laureys and De Vuyst (2014).

Experimental application

The prepared mixture was divided into four groups (500g for each group). The 1st group was inoculated with a starter culture (*L. plantarum* adjusted with a recommended dose of 10^7 CFU/mL) and kept as a control group. The 2nd group was inoculated with 1% water Kefir from the weight of the sample. The 3rd group was inoculated with 3% water Kefir from the weight of the sample. The 4th group was inoculated with 5% water Kefir from the weight of the sample. according to Wu *et al.* (2018), Jatupornpipat and Keatikumjorn (2007). After inoculation, all groups were stuffed into a hang using a handheld sausage filling machine.

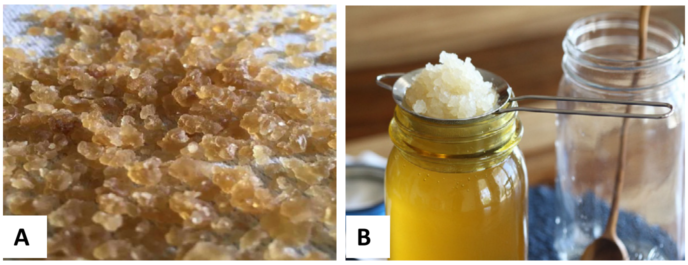

Figure 1: Preparation of Water Kefir from Kefir grains.

All the examined sample groups were placed in separate sterile polyethylene bags and stored in a domestic refrigerator at approximately ±4 °C. Each group was evaluated for sensory chemical and microbiological properties on 1st Day 1, 3rd, 5th, 7th, 9th, 11th, 13th,15th, 17th, and 18th day during storage or until the signs of spoilage appeared. This experiment was conducted in triplicate with the same sausage formula and conditions (storage and temperature) across all groups.

Sensory evaluation

It was carried out from the 1st day to the 18th day of the experiment, according to Pearson and Tauber (1984). A 9-point scale: 9 (Excellent), 8 (Very Very Good), 7 (Very Good), 6 (Good), 5 (Average), 4 (Fair), 3 (Poor), 2 (Very Poor), and 1 (Extremely Poor) was used to assess the overall acceptability.

Microbiological evaluation

Microbiological analyses were performed according to Wang *et al.* (2019). Samples were aseptically opened, and then 10g from each one was transferred into 90mL of 0.1% buffered peptone water (BPW) and stomached (model G-560E, Bohemia) for 1 minute. Ten-fold serial dilutions were made in BPW, and 1mL was poured on selective media.

Total bacterial counts were enumerated on plate count agar (PCA), incubating at 30°C for 48h in aerobic conditions. In aerobic conditions, total psychrotrophic counts on plate count agar at 7(C) for ten days. In aerobic conditions, total yeasts and molds on Sabouraud agar were at 25°C for three days. The results were expressed as log CFU/g^{-1} means ± standard error for all microbial counts.

Physico-chemical evaluation

Sausage groups were sent to Cairo University Research Park (CURP)/ Faculty of Agriculture for physicochemical evaluation.

Determination of color density according to Malle and Tao (1987)

The sausage sample's color was assessed according to (Malle and Tao, 1987) using a Chroma meter (Konica Minolta, model CR 410, Japan), calibrated with a white calibration plate and light trap provided by the manufacturer. Color measurements were expressed using the CIE L*, a*, and b* color system (CIE, 1976). Three spectral readings were taken for each sample. Lightness (L*) ranged from dark (0) to light (10). Redness (a*) values ranged from (+) reddish to (-) greenish. Yellowness (b*) values ranged from (+) yellowish to (-) bluish. Moreover, Chroma and Hue were measured.

Determination of water holding capacity (WHC)

With some modifications, water holding capacity (WHC) was assessed using low-speed centrifugation (Honikel and Hamm, 1994). Briefly, 5 g of whole meat samples were centrifuged at 10,000 x g and 5°C for 10 minutes in a 15 ml Falcon tube containing glass beads. After centrifugation, the sausage was removed with forceps, dried with filter paper, and reweighed. The change in WHC was calculated as the percentage of the difference in weight of the sausage before and after centrifugation.

Determination of drip loss percentage

The analysis of drip losses was performed using the method outlined by (Demirok *et al.*, 2013). The percentage of drip loss was designed as the variance between the first sausage weight measured at (initial frozen weight-weight after thawing)/ initial frozen weight) x100.

Determination of cooking loss percentage

Moisture content and cooking loss were determined according to the method specified by the Association of Official Analytical Chemists AOAC (2005). The moisture content of the sausage (25g) was assessed as the percentage weight loss after cooking in a water bath at 100°C for 60 minutes until a constant weight was achieved.

Chemical examination

Determination of pH corresponding to Pearson (2006)

An average of 10 g of sausage sample was blended with 10 mL of neutralized distilled water. The mixture was incubated at room temperature for 10 minutes with continuous shaking. The pH was measured using an electronic pH meter (Bye version 6020, USA). The pH meter was calibrated with buffer solutions at pH 7.0 (basic) and pH 4.0 (acidic). After calibration, the pH electrode was rinsed with neutral water and then inserted into the homogenate after adjusting the temperature correction device.

Statistical analysis

The results were expressed as mean±SD and analysis of variance (ANOVA) was performed. Variations were considered statistically significant when $P \leq 0.05$. Statistical analysis was performed according to (Petrie and Watson 1999) and computerized using (SPSS 20, 2011).

RESULTS AND DISCUSSION

Worldwide, people are increasingly aware of the connection between health and nutrition. As consumers have begun to use the terms probiotics and prebiotics, the symbiotic product market size is expected to grow significantly over the next five years (Cosme *et al*., 2022). Beyond previously disclosed ancestral practices, consumption of water Kefir has risen in recent years, supported by numerous scientific studies documenting that water Kefir is a source of probiotic microorganisms and metabolites with health benefits (Zavala *et al*., 2016; Romero-Luna *et al*., 2020; Lynch *et al*., 2021).

The results in (Table 1) revealed that all groups initially reflected high sensory scores. However, the control sample declined sharply to a score of 4 by the 7th day, followed by the 1% water Kefir group, which declined slightly more slowly, reaching a fair score of 4 by the 9th day. In contrast, the 3% and 5% water Kefir groups maintained scores of 6 (good) and 7 (very good) by the 9th day, respectively. Furthermore, the 3% and 5% water Kefir groups demonstrated overall acceptability until the 15th and 17th days, with both groups receiving a fair score of 4 at those respective times. This agrees with Jatupornpipat and Keatikumjorn (2007), who found that the Kefir starter enhanced Thai fermented sausage's sensory quality and overall acceptability.

Table 1: Sensory evaluation of treated sausage groups with various concentrations of Water Kefir during cold storage at 4°C.

	Time/days									
Groups	**1st**	**3rd**	**5th**	**7th**	**9th**	**11th**	**13th**	**15th**	**17th**	**18th**
Control	9	8	6	4	Spoiled					
Kefir										
1%	9	8	7	6	4	Spoiled				
3%	9	8	8	6	6	5	4	4		
5%	9	8	8	7	7	6	6	5	4	Spoiled

Excellent=9 Very very good=8 Very good=7 Good=6 Medium=5 Fair=4. Score system for sensory evaluation (Pearson and Tauber, 1984) score system.

Table 2 shows that the control group had the most total bacterial counts (TBC) increase. The control group started on the 1st day with 6.55 log CFU/g and slightly decreased to 6.50 log CFU/g on the 5th day, then showed an increase in TBC, reaching 7.78 log CFU/g on the 7th day, then got spoiled. While the 1% water Kefir group started with 6.52 log CFU/g then, the bacterial count decreased over time, reaching 6.27 log CFU/g on the 5th day, and on the 9th day, the count increased to 7.07 log CFU/g until spoiled at 11th day. In contrast, 3% and 5% water Kefir groups maintained lower microbial counts for longer, potentially extending the product's shelf-life till the 15th and 17th days with 6.83 log CFU/g and 5.75log CFU/g, respectively. These results agreed with Garrote *et al*. (2000) and Oliveira *et al*. (2021), who claimed that Kefir has antibacterial activity *in vitro* against a broad spectrum of Gram-positive and Gram-negative bacteria.

During the fermentation of water, Kefir produces various antimicrobial compounds such as bacteriocins, hydrogen peroxide, and lactic acid bacteria metabolites (LAB), which create an unfavorable environment for the growth of spoilage and harmful bacteria (Shen *et al*., 2018).

Results in Table 3 declared that the control sample's total psychrotrophic bacteria count (TPC) increased from 3.84 log CFU/g on the 1st day to 4.38 log CFU/g on the 7th day, exceeding the standard limit of 10^3 CFU/g for sausage

Table 2: Total bacterial counts (log cfu/g) of treated sausage samples with various concentrations of Water Kefir during cold storage at 4°C.

Groups	Time/days								
	1st	**3rd**	**5th**	**7th**	**9th**	**11th**	**13th**	**15th**	**17th**
Control	6.55±.01^{a}	6.52±.01^{a}	6.50±.02^{a}	7.78±.01^{a}	Spoiled				
Kefir									
1%	6.52±.01^{ab}	6.34±.01^{c}	6.27±.01^{b}	6.83±.01^{b}	7.07±.01^{a}	Spoiled			
3%	6.52±.01^{b}	6.47±.02^{ab}	5.77±.01^{d}	5.69±.01^{c}	5.17±.01^{b}	5.06±.33^{a}	5.82±.01^{a}	6.83±.02^{a}	Spoiled
5%	6.50±.01^{b}	6.44±.01^{b}	5.90±.01^{c}	5.60±.03^{d}	5.11±.04^{c}	4.30±.05^{b}	4.04±.01^{b}	4.50±.01^{b}	5.75±.01^{a}

Table 3: Total psychotrophic counts (log cfu/g) of control and treated samples with various concentrations of Water Kefir during cold storage at 4 °C.

Groups	Time/days								
	1st	3rd	5th	7th	9th	11th	13th	15th	17th
Control	3.84±.01[a]	3.79±.02[a]	4.39±.01[a]	4.38±.01[a]	Spoiled				
Kefir									
1%	3.77±.01[b]	3.78±.01[a]	3.77±.01[b]	3.73±.01[b]	4.38±.01[a]	Spoiled			
3%	3.69±.01[c]	3.69±.01[b]	3.60±.01[c]	3.54±.01[c]	3.43±.01[c]	3.27±.01[a]	3.42±.01[a]	4.79±.01[a]	Spoiled
5%	3.69±.02[c]	3.60±.01[c]	3.30±.02[d]	3.41±.01[d]	3.60±.01[b]	2.90±.01[b]	2.72±.01[b]	3.81±.01[b]	4.57±.01[a]

Table 4: Total mycotic counts (log cfu/g) of control and treated samples with various concentrations of Water Kefir during cold storage at 4 °C.

Group	Time/days								
	1st	3rd	5th	7th	9th	11th	13th	15th	17th
Control	4.85±.01[a]	4.81±.01[a]	4.69±. 1[a]	4.96±.01[a]	Spoiled				
Kefir									
1%	4.84±.02[a]	4.77±.01[b]	4.69±.01 [a]	4.66±.01[b]	5.74±.01a	Spoiled			
3%	4.845±.01[a]	4.69±.01[c]	4.60±.01[a]	4.53 ±.01[c]	3.88±.01c	3.90±.01a	4.06±.08a	4.67±.01a	Spoiled
5%	4.69±.01[b]	4.64±.01[d]	4.56±.01[a]	4.50±.01[d]	3.95±.01[b]	3.30±.01[b]	3.00±.05[b]	3.47±.02[b]	3.92±.01[a]

Table 5: pH evaluation of control and treated samples with various concentrations of Water Kefor during cold storage at 4 °C.

Groups	Time/days								
	1st	3rd	5th	7th	9th	11th	13th	15th	17th
Control	5.52±.01[a]	5.61±.01[a]	5.58±.01[a]	6.3±.01[a]	Spoiled				
Kefir									
1%	5.51±.01[a]	5.45±.01[b]	5.37±.01[a]	5.31±.01[b]	5.9±.01[a]	Spoiled			
3%	5.48±.01[b]	5.46±.02[b]	5.44±.02[b]	5.22±.01[c]	5.19±.01[b]	5.3±.01[a]	5.72±.01[a]	5.8±.01[a]	Spoiled
5%	5.45±.01[c]	5.45±.01[b]	5.39±.01[c]	5.18±.01[d]	5±.01[c]	5.2±.01[b]	5.5±.01[b]	5.6±.01[b]	5.7±.01[a]

according to Al-Faydi (1996). The TPC for the treated group with 1% water Kefir exceeded the permissible limit, reaching 4.38 log CFU/g by the 9th day. In contrast, 3% and 5% water Kefir treated groups initially reduced TPC to 3.42 log CFU/g and 2.72 log CFU/g, respectively, on the 13th day with significant difference ($p< 0.05$), then increased to 4.79 and 4.57 log CFU/g on the 15th and 17th days, respectively. These findings are consistent with Majeed *et al.* (2002), who demonstrated that adding lactic acid bacteria to pastrami significantly ($p\leq0.05$) reduced psychrotrophic bacterial counts. Similarly, Majeed *et al.* (2007) found that incorporating 1-2% *L. acidophilus* metabolites into minced meat refrigerated for up to 5 days led to an average reduction of 0.80% in psychrotrophic bacteria. Additionally, Al-Qureshi *et al.* (2022) observed that sausages cooled for 10 days had notably fewer psychrotrophic bacteria when lactic acid bacteria were included, whereas the control samples showed increased psychrotrophic bacteria along the 10 days of storage.

Table 4 shows that the total mycotic counts (TMC) for the control sample decreased from 4.85 log CFU/g on 1st day to 4.69 log CFU/g on the 5th day, then increased to 4.96 log CFU/g by the 7th day, surpassing the permissible limit of 10^4 CFU/g for molds and yeasts according to Al-Zobaie (2010). For the 1% water Kefir, TMC fell from 4.84 log CFU/g on the 1st day to 4.66 log CFU/g on the 7th day but rose significantly to 5.74 log CFU/g on the 9th day. The 3% water Kefir concentration showed a decrease from 4.84 log CFU/g on the 1st day to 4.53 log CFU/g on the 7th day and then dropped further 3.90 log CFU/g on the 11th day before rising to 4.68 log CFU/g on the 13th day. The 5% water Kefir concentration continued to decline until 3.00 log CFU/g on the 13th day, then rising to 3.93 log CFU/g on the 17th day. These findings align with Roseiro *et al.* (2010), who reported that molds and yeasts in Portuguese fermented sausage were reduced during aging. Kefir inhibited *Candida albicans* and various molds like *Aspergillus, Penicillium, and Fusarium* (Rodrigues *et al.*, 2005). Also, the low water activity in the fermented sausages inhibited the growth of molds and yeasts. Bacteriocins, hydrogen peroxide, and organic acids make the environment unsuitable for fungal growth (Soyer *et al.*, 2005).

The results in Table 5 showed that the pH values of fermented sausages made with water Kefir were lower than those in the control one. The pH values of the control group started at 5.52 on the 1st day and reached 6.30 on the 7th day. For the 1% water Kefir group, the pH value on the 1st day was 5.51 and decreased till it reached 5.31 on the 7th day and then increased on the 9th day till it reached 5.9. The 3% water Kefir group showed a decrease from 5.84 on the 1st day to 5.19 on the 9th day, then pH increased to 5.8 on the 15th day, while the pH value of 5% water Kefir group started by 5.45 on the 1st day and decreased till reached to 5.00 at 9th day and then increased till reached 5.7 at 17th day. These results agreed with Jatupornpipat and Keatikumjorn (2007), who recorded that the pH values decreased (from 5.5-5.7 to 5.1-4.8). This may be attributed to the production of organic acids by the bacteria (Lucke, 1994). At the end of the experiment, pH values increased for all sausage treatments, possibly due to proteolytic processes in the meat, which resulted in the production of non-protein nitrogen (Chen *et al*., 2017).

Table 6 details the water holding capacity (WHC) of control and treated samples with various concentrations of water Kefir during cold storage at 4 °C. The control group shows an initial WHC of 6.23, which increased slightly on the 3rd day but then declined to 6.14 by the 7th day. The 1% water Kefir group began with a WHC of 7.22 and reached 8.21 on the 5th day before dropping significantly to 6.7 by the 9th day. The 3% water Kefir group maintains a relatively consistent WHC, starting at 7.38 and peaking at 8.25 on the 5th day, then declining gradually to 6.25 by the 15th day. In comparison, the 5% water Kefir group consistently exhibited the highest WHC throughout the storage period, starting at 7.50 and reaching a peak of 8.55 on the 7th day; even by the 17th day, it retains a WHC of 6.26, demonstrating superior moisture retention capabilities.

The data in Table 7 shows the effects of control and different concentrations of water Kefir on cooking loss in samples. The control group exhibited a relatively stable cooking loss with values ranging from 1.11 to 1.24 on the 7th day and samples treated with 1% water Kefir showed a decrease in cooking loss over the first three days, reaching the lowest value of 0.83 on 3rd day but began to increase again towards 9th day reached to 1.31, while the 3% concentration maintained lower cooking loss values compared to the control and 1% group throughout the storage duration, the cooking loss decreased from 0.91 at 1st day reaching to 0.81 at 3rd day and increased again reaching to 1.41 at 15th day. The 5% water Kefir group consistently demonstrated the lowest cooking loss values, with the most significant reduction observed on the 5th day and 7th day, reaching 0.80 and 0.86, respectively, then began to increase towards the 17th day, reaching 1.49.

The results in Table 8 showed that the control group started with a high DL of 22.42, which decreases slightly over time, reached 20.33 by the 7th day before spoilage occurs, and the 1% water Kefir group shows an initial DL of 21.96, which decreases steadily to 19.55 by 9th day. The 3% group exhibits a more consistent and significant decrease in DL, starting at 20.84 and dropping to 18.43 by the 15th day, while the 5% water Kefir group consistently shows the lowest DL values, starting at 20.66 and decreasing to 18.24 by the 17th day.

Table 6: Water holding Capacity (WHC) of control and treated samples with various concentrations of Water Kefir during cold storage at 4 °C.

Groups	Time/days								
	1st	3rd	5th	7th	9th	11th	13th	15th	17th
Control	6.23±.01^{d}	7.25±.01^{d}	7.15±.01^{c}	6.14±.01^{d}	Spoiled				
Kefir									
1%	7.22±.01^{c}	7.84±.01^{c}	8.21±.01^{b}	7.95±.01^{c}	6.7±.01^{c}	Spoiled			
3%	7.38±.01^{b}	7.98±.02^{b}	8.25±.02^{b}	8.05±.01^{b}	7.70±.01^{b}	7.10±.01^{b}	6.88±.01^{b}	6.25±.01^{b}	Spoiled
5%	7.50±.01^{a}	8.01±.01^{a}	8.43±.01^{a}	8.55±.01^{a}	8.00±.01^{a}	8.56±.01^{a}	7.39±.01^{a}	6.86±.01^{a}	6.26±.01^{a}

Table 7: cooking loss (CL) of control and treated samples with various concentrations of Water Kefir during cold storage at 4 °C.

Groups	Time/days								
	1st	3rd	5th	7th	9th	11th	13th	15th	17th
Control	1.11±.02^{a}	0.98±.01^{a}	1.12±.01^{a}	1.24±.01^{a}	Spoiled				
Kefir									
1%	0.97±.01^{b}	0.83±.01^{b}	0.97±.01^{b}	1.13±.01^{b}	1.31±.01^{a}	Spoiled			
3%	0.91±.01^{b}	0.81±.02^{b}	0.90±.02^{c}	1.05±.03^{c}	1.14±.01^{b}	1.26±.01^{a}	1.33±.01^{a}	1.41±.01^{a}	Spoiled
5%	0.89±.01^{b}	0.80±.01^{b}	0.86±.01^{d}	1.00±.01^{c}	1.06±.01^{c}	1.23±.01^{a}	1.30±.01^{b}	1.39±.01^{a}	1.49±.01^{a}

Table 8: Dripping loss (DL) of control and treated samples with various concentrations of Water Kefir during cold storage at 4 °C.

Groups	Time/days								
	1st	3rd	5th	7th	9th	11th	13th	15th	17th
Control	22.42±.01^{a}	21.60±.01^{a}	20.66±.01^{b}	20.33±.01^{b}	Spoiled				
Kefir									
1%	21.96±.01^{a}	21.33±.01^{b}	21.06±.01^{a}	20.55±.01^{a}	19.55±.01^{a}	Spoiled			
3%	20.84±.01^{a}	20.55±.02^{c}	20.20±.02^{c}	19.43±.01^{c}	19.27±.01^{c}	19.05±.01^{b}	18.56±.01^{b}	18.43±.01^{b}	Spoiled
5%	20.66±.01^{a}	20.49±.01^{d}	19.35±.01^{d}	19.11±.01^{d}	19.45±.01^{b}	19.34±.01^{a}	18.90±.01^{a}	18.77±.01^{a}	18.24±.01^{a}

Table 9: Color attributes (L*, a*, b*) of control and treated samples with various concentrations of Water Kefir during cold storage at 4 °C.

	Days	1st	3rd	5th	7th	9th	11th	13th	15th	17th
L*	Control	49.3±.01^{c}	48.9±.01^{d}	48.6±.01^{c}	48.2±.01^{d}	Spoiled				
	Kefir1%	50.3±.01^{b}	49.5±.01^{c}	49.5±.01^{b}	48.7±.01^{c}	49.9±.01^{a}	Spoiled			
	Kefir 3%	50.4±.01^{b}	49.7±.01^{b}	49.9±.01^{a}	49.4±.01^{b}	49.7±.01^{b}	49.9±.01^{b}	50.0±.01^{b}	50.0±.01^{b}	Spoiled
	Kefir 5%	51.3±.01^{a}	50.1±.02^{a}	49.9±.03^{a}	49.7±.04^{a}	49.9±.01^{a}	50.0±.02^{a}	50.1±.01^{a}	50.5±.05^{a}	50.2±.01^{a}
a*	Control	5.1±.02^{d}	4.9±.02^{d}	4.8±.04^{d}	4.4±.02^{d}	Spoiled				
	Kefir 1%	5.2±.01^{c}	5.43±.01^{c}	5.0±.03^{c}	4.9±.01^{c}	4.4±.04^{c}	Spoiled			
	Kefir 3%	5.4±.03^{b}	5.6±.02^{b}	5.2±.03^{b}	5.1±.01^{b}	4.9±.01^{b}	4.6±.01^{b}	4.4±.01^{b}	4.3±.01^{b}	Spoiled
	Kefir 5%	5.6±.02^{a}	5.7±.01^{a}	5.6±.01^{a}	5.5±.01^{a}	5.1±.01^{a}	5.1±.01^{a}	4.7±.02^{a}	4.4±.02^{a}	4.3±.02^{a}
b*	Control	9.7±.01^{b}	9.8±.01^{c}	9.±.02^{c}	8.6±.03^{c}	spoiled				
	Kefir 1%	9.7±.03^{ab}	9.7±.01^{c}	9.1±.01^{c}	8.8±.02^{b}	8.6±.02^{c}	Spoiled			
	Kefir 3%	9.8±.01^{ab}	10.±.01^{b}	10.±.01^{b}	9.9±.01^{a}	9.5±.01^{b}	8.9±.04^{b}	8.6±.01^{b}	8.4±.04^{b}	Spoiled
	Kefir 5%	9.8±.01^{a}	10.2±.02^{a}	10.2±.03^{a}	10.1±.04^{a}	10.0±.01^{a}	9.9±.02^{a}	9.3±.01^{a}	8.9±.05^{a}	8.4±.01^{a}

Water in meat and meat products is a crucial element that significantly affects the raw materials sensory, structural, and mechanical properties and the quality and shelf life of the finished food products ready for consumption (Kudryashov and Kudryashova, 2023).

Table 9 shows that color attributes (L*, a*, b*) can help maintain product consistency and ensure that sausages meet the desired color specifications. Sausages with higher Kefir concentrations (3% and 5%) show significantly higher b* and a* values than lower Kefir concentrations (1%) and control ones. The highest b* and a* values are seen in the 5% Kefir treatment. This difference is statistically significant compared to the control and 1% water Kefir group. Increasing Kefir concentration correlates with increased L* values, indicating greater lightness. The 5% Kefir treatment has the highest L* value, statistically significant compared to the control and lower Kefir concentrations. This finding could be useful for improving the visual appeal of fermented sausages and may have implications for consumer acceptance and product marketing. These results align with Sirini *et al.* (2020) and Zhang *et al.* (2023).

CONCLUSIONS AND RECOMMENDATIONS

The study demonstrated that incorporating water Kefir into the production of fermented sausages significantly enhanced the quality. Specifically, it extended the shelf life to 17 days at 4°C, compared to just 7 days for the control group. There were clear significant differences ($P < 0.05$) between *Lactobacillus plantarum* (the control group) and the 1%, 3%, and 5% water Kefir groups in total bacterial count, total psychrotrophic count, and total mycotic count. In addition, water Kefir positively influenced the sausage's physicochemical properties, sensory attributes, and overall quality. These improvements were due to water Kefir's dual role as a starter culture and a bio-preservative. Based on these findings, using water Kefir in meat fermentation is recommended for producing high-quality, safe, and consumer-friendly sausages, as it accelerates the drying process and enhances the product's texture by hydrolyzing and coagulating proteins. Furthermore, it activates muscle proteins and contributes to the product's redness development.

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NOVELTY STATEMENT

Water Kefir as a starter culture and a bio-preservative which can be used in fermented sausage.

AUTHOR'S CONTRIBUTION

EKF and RAL conducted the study, laboratory investigations and analyzed the data. NZE supervised the work. All the team members wrote, revised the original draft approved the final manuscript and agree to the conditions outlined in the copyright assignment form.

Conflict of interest

The authors have declared no conflict of interest.

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