Al-Muthanna J. For Agric Sci Online ISSN:2572-5149
Print ISSN: 2226-4086
Vol. 13 , Issue 1. 2026

https://muthjas.mu.edu.iq/ http://doi.org/10.52113/mjas04/13.1/11

Effect of liquid whey with ascorbic acid on some chemical and microbial properties of refrigerated sausage

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Abstract

This study sought to assess the impact of liquid whey combined with ascorbic acid on several chemical parameters, as well as the microbiological quality of processed and refrigerated beef sausage. The trial had five treatments: T1 (Sodium Nitrate 0.005%), T2 (Sodium Nitrate 0.005% + Liquid Whey 5 ml), T3 (Sodium Nitrate 0.002% + Liquid Whey 5 ml), T4 (Liquid Whey 5 ml), and T5 (Liquid Whey 5 ml + Ascorbic Acid 5%). Subsequent to production, the sausages were refrigerated, and physical, chemical, and microbiological analyses were performed during intervals of 1, 7, 14, and 21 days. The application of liquid whey or liquid whey combined with ascorbic acid led to favorable alterations in the produced sausages, notably a reduction in pH and a decline in thiobarbituric acid levels relative to the control treatment. The microbiological quality of the treatments was predominantly satisfactory. The results showed a substantial decrease ($p < 0.01$) in the total bacterial count in the treatments utilizing liquid whey or liquid whey with ascorbic acid compared to the control treatment. Escherichia coli (E. coli) remained undetected in all treatments throughout the storage durations of 1, 7, and 14 days, whereas it was detectable in minimal quantities throughout the 21-day storage period. A substantial elevation ($p < 0.01$) in lactic acid bacteria levels was noted in all treatments utilizing liquid whey in comparison to the control treatment.

Keywords : Ascorbic acid , Liquid Whey, pH , Sausage, TBA ,TPC

Introduction

Sausages rank among the most ancient food products crafted by humans. There are many different kinds of these products around the world. The type of meat used, the climate in each country, cultural and religious traditions, and the way the meat is processed all play a role (Carballo, 2021). Sausages are regarded as one of the most favored meat items among customers because of their substantial nutritious value (Holk et al. 2017).

Nonetheless, the manufacturing of these goods faces significant pressure from customers and nutritionists to fulfill supplementary criteria aimed at enhancing the nutritional content of processed meats. The requirements entail the elimination of detrimental additives to conform to the "clean label" trend (Cegiełka, 2020) and the reduction or eradication of nitrites or nitrates in processing (Karwowska et al., 2021). The lack of nitrites/nitrates in processed beef products poses certain technological obstacles due to the roles these additives fulfill in such goods. Nitrates and nitrites prevent bacterial proliferation, particularly *Clostridium botulinum*, hence restricting oxidation and enhancing color and flavor (Ma *et al*., 2018).

The increasing apprehension regarding the enduring detrimental impacts of nitrates has led to stringent regulations on their application in processed meat products in the majority of developed nations. The concurrent emergence of all-natural and clean-ingredient products has heightened the desire for healthier, superior-quality beef products. Due to health dangers, people favor natural ingredients over chemicals in processed meats. As a result, there has been an increase in research over the years focused on substituting the chemical nitrite additive with natural substances (Sindelar and Milkowski, 2011).

An alternative is the utilization of whey, a by-product of various dairy products such as yogurt, fresh and soft cheeses, and cream cheeses (Wherry et al. 2019). Whey typically comprises around 55% of the nutrients present in milk, encompassing 20% of the overall protein content and 85 to 95% of the original milk's volume (Guimaraes *et al.*, 2010; Walsh, 2014). Besediuk *et al*. (2024) asserted that whey possesses significant nutritional value across all metrics, is a low-calorie product, encompasses a diverse array of essential nutrients, offers extensive support for various bodily systems, exhibits anti-infective properties, and is abundant in lactic acid bacteria regarding physiological efficacy.

Recently, environmentally aware dairy companies have worked to develop sustainable whey utilization solutions by investing in and marketing it due to its antibacterial and antioxidant qualities (Chandrajith and Karunasena 2018). The addition of acidic whey to sausages (Wójciak *et al*. 2014) and dried steaks (Stadnik and Stasiac 2016) has been proven to give protection against oxidative damage without negatively impacting the physical, chemical, and sensory aspects of the meat (Burke and Monahan 2003). The incorporation of acidic whey in bacon and fermented pork resulted in a reduction of overall acidity and a decrease in microbial contamination. A greater concentration of lactic acid bacteria was noted in comparison to the control treatment. Following storage, the concentration of secondary oxidation products (TBARS) was comparable in samples treated with acidic whey and those treated with nitrite (Karageorgou *et al.* 2023).

Materials and Methods

The study was conducted in the Meat Laboratory, Department of Animal Production, College of Agriculture, University of Basra .

1- Preparation of liquid whey

Liquid whey was obtained from fresh cow's milk using the procedure outlined by (Cipolat-Gotet *et al*.2013) ,which entails heating the milk to 60°C for thirty minutes. Subsequently, 5 cc of white vinegar was incorporated. Subsequent to reducing the milk temperature to 45°C, the milk was incubated at ambient laboratory conditions for 3 hours to finalize the curdling process. The whey was removed from the cheese using a sterile cloth, and the whey arising from the milk coagulation was collected in a clean glass container.

2- Manufacturing of Sausages

Five sausage mixes were prepared, and an electric mincing machine (Panasonic-3500w) made in Japan was used to mince the meat. The minced meat and other additives were mixed and packed inside the natural casings of the large intestine. Sodium nitrate, liquid whey, and ascorbic acid were added in different concentrations to the sausage mixes, as in Table (1).

Table 1. Formulation of sausage

Sausage Ingredients	T1	T2	T3	T4	T5
Minced Meat	80%	80%	80%	80%	80%
Fat	15%	15%	15%	15%	15%
Starch	5%	5%	5%	5%	5%
Salt	2%	2%	2%	2%	2%
Spices	1%	1%	1%	1%	1%
Sodium Nitrate	0.005%	0.005%	0.002%	–	–
Liquid Whey	–	5ml	5ml	5ml	5ml
Ascorbic acid	–	–	–	–	5%

3- pH estimation

The pH was estimated according to the method of Capita et al. (2006) by weighing 10 g of sausage sample and adding 90 ml of distilled water to it, and placing it in an electric mixer for 30 seconds to obtain a homogeneous mixture. After that, the pH was measured directly using a pH-meter.

4- Thiobarbutyric acid (TBA) estimation

The value of thiobarbituric acid was estimated according to the method of (Mehran (1976) and (Jalali Mousavi et al. (2015), where 5 g of sausage was weighed and dissolved in 10 ml of chloroform, placed in a water bath at 60°C for 5 minutes. 10 ml of 0.07% thiobarbituric acid solution (prepared with glacial acetic acid) was added, then the mixture was centrifuged at 1000 rpm for 5 minutes. The filtrate was taken and placed in a boiling water bath for 30 minutes. The optical absorbance of the filtrate was read at laboratory temperature at a wavelength of 532 nm. The value of thiobarbituric acid was calculated as mg Malondialdehyde/ (MD) kg of meat according to the following equation:

MD concentration (mg malondialdehyde/kg meat) = optical absorbance × 7.83 (Egan et al., 1981)

5- Total bacterial count (TPC)

0.1 ml of the appropriate dilutions were transferred to sterile Petri dishes, Nutrient Agar was added to them, and they were incubated at 37°C for 24 hours. The number of bacterial colonies in the dishes was counted using a colony counter, and the average for dishes was extracted. The results were expressed on the basis of colony-forming units/g according to the equation mentioned in the study of (Aderinola et al., 2014).

$$CFU=N\times 1/D$$

Where CFU : colony-forming unit, N : number of colonies, and D : dilution.

6- Estimation of *E. coli* bacteria in meat

The Pour-plate method mentioned in APHA (1978) was followed, using Petri dishes and the Pour Plate method, by placing (1) ml of the appropriate dilution of the sample in a sterile glass Petri dish, and adding about (15) ml of MacConkey agar culture medium and mixing by moving the dish gently clockwise and counterclockwise, then leaving it until it solidifies. The dishes were incubated upside down in the incubator at a temperature of (35°C) for (24) hours, and the total number of colonies per gram of the sample was estimated by multiplying the average number of colonies by the reciprocal of the dilution used.

7- Estimation of lactic acid bacteria

MRS medium was used and the method described by APHA (1984) was followed, and incubation was carried out at 39°C for 48 hours.

8- Statistical Analysis and Mathematical Model

The results were analyzed statistically using a completely randomized design (CRD). The data were analyzed statistically using the statistical program (Genstat), and the results were compared using the least significant difference (RLSD) at a probability level of (P<0.01) (Rawi and Khalaf Allah ,2000).

Results and Discussion

pH: The table (2) displays the pH values obtained from the sausage treatments during the chilled storage duration. Table (2) indicates that, during a 1-day period, the pH readings exhibited no significant differences across the treatments. The control treatment T1 documented a value of 5.72, whereas treatments T2, T3, T4, and T5 documented values of 5.69, 5.70, 5.70, and 5.69, respectively, for the same duration. The results indicated distinct significant differences between the control treatment T1 and the other treatments for the remaining periods. The minimum pH value of 5.31 was observed in treatment T5 (Liquid Whey 5 ml + Ascorbic Acid 5%) during the 21-day refrigeration period, followed by treatment T3 (Sodium Nitrate 0.002% + Liquid Whey 5 ml), which exhibited a pH of 5.41 during the same duration, in contrast to the control treatment T1 (Sodium Nitrate 0.005%), which recorded a pH of 5.55 over the 21-day storage period. The decline in pH in sausage samples treated with liquid whey or liquid whey combined with ascorbic acid is mostly attributable to the composition of the liquid whey. Its presence facilitates the incorporation of lactic acid bacteria into the product, hence enhancing the sausage's acidity. This is deemed a significant element in suppressing the proliferation of harmful germs, as indicated by the USDA (2022). The findings align with the research conducted by Karwowska *et al.* (2022), which indicated that the incorporation of acidic liquid whey resulted in heightened acidity in fermented sausages at 7, 14, and 21 days relative to the control sample. A comparably low pH level was also recorded in the research undertaken by Dolatowski (2017) and Karwowska et al. The results indicated a notable reduction in pH value with prolonged storage duration across all treatments, peaking after one day of storage before thereafter declining. The storage duration continues until it attains its minimum point within the 21-day period.

Table (2) : pH in sausage treatments during a 21-day storage period at 4°C

Treatments	Day				Mean
	1	7	14	21	
T1	5.72	5.96	5.65	5.55	5.72
T2	5.69	5.66	5.45	5.42	5.56
T3	5.70	5.65	5.59	5.41	5.59

T4	5.70	5.56	5.60	5.55	5.60
T5	5.69	5.64	5.52	5.31	5.54
Mean	5.70	5.69	5.56	5.45	
L.S.D.	0.019				0.021

T1- Sodium Nitrate 0.005% ; T2- Sodium Nitrate 0.005%+Liquid Whey 5ml ; T3- Sodium Nitrate 0.002%+Liquid Whey 5ml , T4- Liquid Whey 5ml ; T5- Liquid Whey 5ml+ Ascorbic acid 5%. LSD value at ($p \leq 0.01$).

Thiobarbituric acid(TBA) : Table (3) presents the thiobarbituric acid values for beef sausages refrigerated. A notable reduction ($P<0.01$) in thiobarbituric acid levels was recorded in treatment T5 (Liquid Whey 5ml + Ascorbic acid 5%), which attained 0.51 Malon Dialdehyde/kg after 1 day of refrigeration. This was not significantly different from treatment T2 (Sodium Nitrate 0.005% + Liquid Whey 5ml), which measured 0.52 Malon Dialdehyde/kg throughout the same timeframe. Treatment T1 (Sodium Nitrate 0.005%) exhibited the highest thiobarbituric acid value of 1.76 Malon Dialdehyde/kg during the 21-day refrigeration period. There are notable discrepancies between the transactions and across various storage durations. Table (3) indicates that in treatment T5, the thiobarbituric acid value diminished relative to all other treatments over storage periods of 1, 7, 14, and 21 days, registering 0.51, 0.71, 1.01, and 1.59 Malondialdehyde/kg, respectively. Subsequently, treatment T2 (Sodium Nitrate 0.005% + Liquid Whey 5 ml) yielded acid values of 0.52, 0.74, 1.10, and 1.62 Malondialdehyde/kg for the specified times, respectively. The reduction in thiobarbituric acid levels in samples treated with liquid whey may result from the whey creating a transparent coating on the meat, inhibiting the transfer of moisture and oxygen, which are contributing factors to oxidation. Consequently, the whey treatment inhibits oxygen from accessing the meat. This decreases oxidation in meat (Belgheisi *et al.*, 2016). Ascorbic acid functions to inhibit oxidation by neutralizing free radicals via electron donation, so halting the propagation of free radicals and safeguarding meat from damage (Ma *et al*., 2012).

The maximum concentration of thiobarbituric acid was seen in treatment T1 (Sodium Nitrate 0.005%) over all specified storage durations, attaining values of 0.54, 0.85, 1.22, and 1.76 malondialdehyde/kg, respectively. These results may be ascribed to the elevated levels of malondialdehyde resulting from prolonged storage, a consequence of fat oxidation in meat and meat products due to peroxide degradation (Al-Alwani, 2017). The table demonstrates a notable impact on thiobarbituric acid values across various storage durations. It was minimal during the 1-day interval, subsequently rising throughout the subsequent periods, ultimately attaining its peak in the 21-day interval. The observed increase may be ascribed to the elevated levels of malondialdehyde resulting from prolonged storage, a consequence of fat oxidation in meat and meat products due to peroxide decomposition (Al-Alwani, 2017). These findings align with those of the research conducted by Karwowska et al. (2017).

Table (3) : Thiobariotric acid (malon dialdehyde/kg) in sausage samples during a 21-day storage period at 4°C

Treatments	Day				Mean
	1	7	14	21	
T1	0.54	0.85	1.22	1.76	1.09
T2	0.52	0.74	1.10	1.62	1.00

T3	0.54	0.78	1.14	1.70	1.04
T4	0.54	0.84	1.20	1.74	1.08
T5	0.51	0.71	1.01	1.59	0.96
Mean	0.53	0.78	1.13	1.68	
L.S.D.	0.009				0.010

T1- Sodium Nitrate 0.005% ; T2- Sodium Nitrate 0.005%+Liquid Whey 5ml ; T3- Sodium Nitrate 0.002%+Liquid Whey 5ml , T4- Liquid Whey 5ml ; T5- Liquid Whey 5ml+ Ascorbic acid 5%. LSD value at ($p \leq 0.01$).

Total Bacterial Count (TPC) : Table (4) presents the total bacterial count (cfu/g) of sausages held under refrigeration for durations of 1, 7, 14, and 21 days. The data presented in Table (4) indicate a substantial reduction at a probability level ($p \leq 0.01$) in the total bacterial count for treatment T5 (Liquid Whey 5 ml + Ascorbic acid 5%) during the 1-day storage period, which measured 29.99 (cfu/g), in contrast to treatment T1 (Sodium Nitrate 0.005%), which exhibited the highest total bacterial count of 299.66 (cfu/g) over the 21-day storage period under refrigeration. Significant disparities existed between the treatments and the various storage durations. Table (4) illustrates that treatment T5 exhibited a substantial reduction in total bacterial count at a probability level ($p < 0.01$) throughout all treatments and storage durations (1, 7, 14, and 21 days), yielding counts of 29.99, 110.33, 168.03, and 240.33 (cfu/g), respectively. Moreover, the control treatment T1 (Sodium Nitrate 0.005%) exhibited a substantial elevation in the total bacterial count throughout all storage durations (1, 7, 14, and 21 days) in comparison to the treatments incorporating liquid whey or liquid whey with ascorbic acid, where the total bacterial counts were recorded at 34.91 and 123.66, 217.98 and 299.66 (cfu/g), respectively. This phenomenon is due to the presence of lactic acid bacteria in the whey, specifically Lactobacillus, which predominates and suppresses the proliferation of harmful bacteria. The findings align with Al-Marshaidy’s (2017) study, which indicated a reduction in total bacterial count in refrigerated burgers made with liquid whey compared to the control group.

The table(4) indicates substantial variations ($p < 0.01$) in the overall bacterial count during the refrigerated storage duration. The lowest bacterial count was observed after day 1, then it began to increase as the storage period progressed, reaching its highest level after day 21. This conclusion was corroborated by Al-Alwani (2017).

Table (4) : Total bacterial count (cfu/g) in sausage treatments during a 21-day storage period at 4°C

Treatments	Day				Mean
	1	7	14	21	
T1	34.91	123.66	217.98	299.66	169.05
T2	30.66	112.66	167.91	254.66	141.47
T3	32.66	119.66	175.66	266.33	148.58
T4	32.00	124.79	200.33	279.66	159.20
T5	29.99	110.33	168.03	240.33	137.17
Mean	32.04	118.22	185.98	268.13	
L.S.D.	0.678				0.758

T1- Sodium Nitrate 0.005% ; T2- Sodium Nitrate 0.005%+Liquid Whey 5ml ; T3- Sodium Nitrate 0.002%+Liquid Whey 5ml , T4- Liquid Whey 5ml ; T5- Liquid Whey 5ml+ Ascorbic acid 5%. LSD value at ($p \leq 0.01$)

lactic acid bacteria : Table (5) presents the counts of lactic acid bacteria in chilled beef sausages during 1, 7, 14, and 21 days. The study's results indicated that none of the treatments documented lactic acid bacteria numbers on the initial day of storage. The results indicated a substantial increase at a probability level ($p < 0.01$) in the quantity of lactic acid bacteria across all treatments utilizing liquid whey, in comparison to treatment T1 (Sodium Nitrate 0.005). Treatment T4 (Liquid Whey 5 ml) exhibited the highest counts of lactic acid bacteria over the 7, 14, and 21-day periods, achieving values of 100.00, 155.00, and 233.33 (cfu/g), respectively. In contrast, treatment T5 (Liquid Whey 5 ml + Ascorbic acid 5%) recorded values of 84.66, 141.93, and 214.99 (cfu/g) for the same intervals. Treatment T1 (Sodium Nitrate 0.005%) yielded 62.83, 75.55, and 101.66 (cfu/g) for the durations of 7, 14, and 21 days, respectively. The elevated concentration of lactic acid bacteria in therapies utilizing liquid whey is attributable to the high prevalence of these bacteria, particularly *Lactobacillus*, inside the liquid whey itself. This strain is crucial in suppressing the proliferation of other harmful organisms found in the meat (Mazorra-Manzano et al. 2020). The statistical analysis results indicated a considerable rise in the quantity of lactic acid bacteria with prolonged storage periods. The fermentation occurring in the sausage components during storage produces lactic acid bacteria, with whey serving as a source of these bacteria (Mazorra-Manzano *et al.*, 2020).

Table (5) : Lactic acid bacteria count in sausage treatments during a 21-day storage period at 4°C

Treatments	Day				Mean
	1	7	14	21	
T1	0.00	61.83	75.33	101.66	59.70
T2	0.00	82.03	140.33	210.33	108.17
T3	0.00	84.66	162.92	200.00	111.90
T4	0.00	100.00	155.00	223.33	119.58
T5	0.00	84.66	141.93	214.99	110.40
Mean	0.00	82.64	135.10	190.06	
L.S.D.	0.466				0.521

T1- Sodium Nitrate 0.005% ; T2- Sodium Nitrate 0.005%+Liquid Whey 5ml ; T3- Sodium Nitrate 0.002%+Liquid Whey 5ml , T4- Liquid Whey 5ml ; T5- Liquid Whey 5ml+ Ascorbic acid 5%. LSD value at ($p \leq 0.01$).

E. coli : Table (6) presents the *E. coli* bacterial count in refrigerated sausages across the durations of 1, 7, 14, and 21 days of refrigeration. The table indicates a substantial reduction ($p<0.01$) in the E. coli count (cfu/g) across all treatments and storage durations of 1, 7, 14, and 21 days. The findings demonstrate a notable reduction in E. coli count for treatment T2 (Sodium Nitrate 0.005% + Liquid Whey 5 ml) over the 21-day storage period, culminating at 30.00 (cfu/g). Treatment T5 (Liquid Whey 5 ml + Ascorbic Acid 5%) exhibited E. coli count of 32.02 (cfu/g) during the same duration, while treatment T1 (Sodium Nitrate 0.005%) recorded 159.66 (cfu/g) in that period. The reduction in E. coli count observed with treatments involving liquid whey or liquid whey combined with ascorbic acid can be ascribed to the unfavorable conditions for bacterial proliferation created by whey in sausage, particularly due to the presence of lactic acid bacteria that suppress the growth of pathogenic bacteria typically found in meat products, such as *Listeria* and *Clostridium* *botulinum* (Kononiuk and Karwowska, 2020). Statistical analysis indicated a substantial rise in E. coli count during the 21-day storage period for all treatments when compared to the 1, 7, and 21-day storage periods.

Table (6) : E. coli bacterial count in sausage treatments during a 21-day storage period at 4°C

Treatments	Day				Mean
	1	7	14	21	
T1	0.00	0.00	0.00	159.66	39.92
T2	0.00	0.00	0.00	30.00	7.50
T3	0.00	0.00	0.00	112.66	28.16
T4	0.00	0.00	0.00	45.03	11.26
T5	0.00	0.00	0.00	32.03	8.01
Mean	0.00	0.00	0.00	75.88	
L.S.D.	0.361				0.403

T1- Sodium Nitrate 0.005% ; T2- Sodium Nitrate 0.005%+Liquid Whey 5ml ; T3- Sodium Nitrate 0.002%+Liquid Whey 5ml , T4- Liquid Whey 5ml ; T5- Liquid Whey 5ml+ Ascorbic acid 5%. LSD value at ($p \leq 0.01$)

Conclusions

The use of whey in sausage production is an innovative method for manufacturing nitrite-free meat products. The results obtained indicate a good effect of adding acidic whey with ascorbic acid on the quality of the resulting sausages. Meat products subjected to this addition exhibited a reduction in secondary oxidation markers and a decrease in pH relative to the control group. Furthermore, the results demonstrated that the use of whey retained the microbiological quality of the sausages . Consequently, the incorporation of acidic whey with ascorbic acid in processed meat products is advocated as a substitute for nitrates.

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