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Effect of whey protein isolate on keeping quality and shelf life of minced meat

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ABSTRACT

Minced meat is a widely consumed and popular meat product, known for its high nutritional value. However, it is prone to contamination by a multitude of microorganisms. Therefore, the aim of this work was to improve the keeping quality and safety of chilled minced meat by using whey protein isolate (WPI) at different concentrations 1, 3, and 5% to assess their effect on the sensory attributes, chemical parameters (pH, total volatile nitrogen, thiobarbituric acid and amino acid content), and bacteriological status including total bacterial count, *Enterobacteriaceae* count, coliform count, and staphylococcal count in minced meat stored at 4°C for 12 days, in addition, detection of some staphylococcal enterotoxins. The results showed that adding different concentrations of WPI improved the treated minced meat samples sensory qualities when compared to the control samples. WPI also improved the chilled minced meat's chemical properties by lowering pH, total volatile nitrogen, and thiobarbituric acid values while raising the amount of essential amino acids when compared to the control group. Furthermore, WPI significantly reduced the bacterial counts. Among the different concentrations tested, 5% of WPI exhibited the most significant improvement in terms of sensory, chemical, and bacterial quality, surpassing the effects observed with 1% and 3% concentrations. Incidence of *Staph. aureus* in the control group and the minced meat treated with 1% and 3% WPI was 60, 20, and 20% respectively. However, no *Staph. aureus* was isolated from the treated samples with 5% WPI. In control samples, enterotoxins A, A+C, and A+D were detected in 20% of each of the examined samples, respectively; enterotoxin D was detected in 20% of sample treated with 3% WPI; enterotoxin A+C was detected in 20% of sample treated with 1% WPI. The study concluded that utilizing WPI as a natural antioxidant and antibacterial preservative for refrigerated minced meat can extend its shelf life up to 12 days, in contrast to the control group, which spoiled completely within 6 days of chilled storage.

Introduction

Minced meat is commonly sold chilled at temperatures between 2-5°C, raising concerns among retailers, consumers, and public health officials regarding its microbiological quality and safety. To ensure longer shelf life and consumer safety, it is crucial to minimize contamination and prevent the growth of spoilage and pathogenic microorganisms. However, refrigerated minced meat within this temperature range can lead to undesirable changes in the product due to microbial growth, resulting in a decline in quality, meat spoilage, and economic losses Elabbasy *et al*. (2014).

According to Bisholo *et al*. (2018), the consumption of contaminated meat and meat products leads to numerous illnesses and a significant number of deaths worldwide. To address this issue, scientists have explored various methods, such as radiation and inorganic chemicals, to reduce contamination in meat products. However, it has been found that chemical preservatives can have harmful effects, including being carcinogenic, teratogenic, and having residual toxicity Costa *et al*. (2019). As a result, there is currently a growing trend towards the use of natural additives, such as whey protein isolate, as a safer alternative to chemical additives.

Whey proteins are byproducts of the cheese-making industry and have traditionally been disposed of as animal feed or used in infant formulas and sports food. However, there are now ongoing efforts to find new uses for whey proteins. Whey protein is composed of several individual protein components, with the two most abundant proteins being β-Lactoglobulin at 50-55% and α-Lactalbumin at 20-25%. These proteins play important roles in various food applications and contribute to the functional properties of whey protein Van-Vliet *et al*. (2004).

Whey protein hydrolysate (WPH) has made a breakthrough in inhibiting oxidative deterioration and improving the quality of meat products during storage Yu *et al*. (2023). Thus, whey acts as preservatives increases the shelf life of food at the same time retaining its quality Regalado *et al*. (2006)

Whey components like bioactive peptides, lactoferrin and lactoperoxidase have been researched for their potential as antimicrobial agents in food preservation. Lactoferrin, an iron binding glycoprotein, damages the membranes of bacteria and fungi by binding to them and causing a loss of cytoplasmic fluids. Lactoperoxidase, another protein, acts as a strong oxidizing agent and causes damage to bacteria and their cell membranes. In addition, bioactive peptides of whey protein have been shown to possess antioxidant and antimicrobials (Fitzgerald and Murray, 2006; Brandelli *et al*., 2015; Nongonierma and FitzGerald, 2016; Ali and LaPointe, 2022). So, this work was planned to detect the effect of whey protein isolate (WPI) on the quality and safety of chilled minced meat by sensory evaluation, chemical indices (pH, TBA, and TVN), and bacteriological examination including total bacterial count, *Enterobacteriaceae*, coliform count, and staphylococcal count during storage at 4°C throughout 12 days of chilled storage, in addition determination the incidence of *staph. aureus* and their enterotoxins in treated minced meat with different concentrations of WPI at day six of chilled storage.

Materials and methods

Preparation of whey protein isolate (WPI) concentration

Whey protein isolate (WPI) (NZWPI 895, Caldic, Fonterra, USA). 1, 3 and 5 grams of WPI were added in containers and then 99, 97 and 95 ml of sterilized distal water were added gradually, respectively., the solution was shaken until dissolving to produce WPI at concentration of 1, 3 and

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5%, respectively, then filtrated and exposed to ultraviolet radiation to ensure it was free from microorganisms according to Reed (2010).

Collection, preparation, and treatment of minced meat samples with WPI according to Barbosa et al. (2009)

A total of 14 kilograms of minced meat were purchased from the butcher shops in Damanhour city, El-Behaira governorate, Egypt. The collected samples were kept in sterile polyethylene bag, preserved in an ice box and transported to the postgraduate laboratory, Food Hygiene Department, Animal Health Research Institute, El- Behira government, Egypt. The minced meat samples were divided into 4 groups (about 3.5 Kg for each group) and each group was represented by 35 samples (100g for each). The first group was prepared as control (untreated group) and the other 3 groups were treated with 1, 3, and 5% WPI, respectively by mixing with samples. The previously treated minced meat samples were labeled and each single sample was separately packaged in polyethylene bags. The experiment was conducted for 12 days of chilling storage at 4°C. Each group was subjected to sensory, chemical, and bacteriological assessment at zero time (within 2 hours after treatment) then periodically every 2 days until the appearance of decomposition signs in each group (zero, 2, 4, 6, 8, 10 and 12 days). The scheme was replicated 5 times.

Sensory evaluation of treated minced meat samples with different concentrations of WPI according to Lawless and Heymann (2010)

Twenty adults who were untrained and unaware of the experimental approach were given 100±10 grams of minced meat for each concentration. The samples were coded with a specific number and the panelists were asked to rate the overall acceptance (color, odor, and texture) while the samples were fresh (uncooked). After that, the samples were cooked without any additives and presented to the panelists to evaluate their sensory qualities. The panelists drank warm water between each sample and used a ten-point descriptive scale; 4.5-5 "Very good"; 4-4.5 "Good" ; 3-4 "Acceptable"; 2-3" Unacceptable"; 1-2: "Bad" ; S: "Spoiled". This was used for assessing appearance, smell, texture, taste, and overall acceptability. The sensory evaluation was repeated after different period.

Chemical indices of treated minced meat samples

Potential of hydrogen ion concentration (pH) measurement according to EOS, 63-11/(2020)

Ten ml of neutralized distilled water and 10 grams of minced beef samples were blended. After 10 minutes of constant shaking at room temperature, the mixture was set aside. A pH electrical meter was used to calculate the pH value (Bye model 6020, USA). The pH meter was calibrated using two buffer solutions with precisely defined pH values (alkaline pH 7.01, acidic pH 4.01). To clean the pH electrode, neutralized water was used, and it was introduced to the homogenizer after the temperature correction system was adjusted.

Determination of total volatile nitrogen "TVN" according to EOS: 63-9/(2006)

In a clean distillation flask, 300 ml of distilled water and 10 grams of minced meat samples were combined and carefully mixed. The prior mixture was supplemented with two grams of magnesium oxide and an anti-foaming agent. 25 ml of 2% boric acid and a few drops of indicator were put to a 500 ml receiving flask. The receiver tube of the receiving flask was positioned so that it dropped below the boric acid solution. Within 10 minutes, the distillation flask reached boiling temperature. Distillation followed for another 25 minutes. Then titration of TVN was done against H_2SO_4 M 0.1 was performed until pink color was appeared, TVN was calculated according to the following formula:
TVN/l00g= (mls H_2So_4 n 0.1 for sample – ml H_2So_4 n 0.1 for Blank)x14

Determination of thiobarbituric acid number "TBA" according to EOS: 63-10/(2006)

The test depends on determination of malonaldehyde (MDA) as a product of lipid peroxidation. Briefly, 50 ml of distilled water were mixed with ten grams of prepared minced meat samples and transferred to a distillation flask, then antifoaming agent and 50 ml of diluted hydrochloric acid were added to flask. The distillation flask was heated for distillation of 50 ml of diluted hydrochloric within 10 minutes from the beginning of boiling .Accordingly, 5 ml of a distilled solution was put in a tube with cover, then 5 ml of prepared thiobarbituric acid, the tube was covered and put on water bath and boiled for 35 minutes, then cooled by water for 10 minutes. By using Spectrophotometer (UNICAM969AA Spectronic, USA), the absorbance of sample was measured under wavelength 538 n m^3.
TBA value= absorbance of sample x 7.8 (malonaldehyde (mg) /Kg).

Fractionation of amino acids

The technique recommended by Mabbott (1990) for fractionation of amino acids was applied by Gas Liquid Chromatography (GLC).

Bacteriological examination of minced meat treated with different concentrations of WPI

Twenty-five grams of minced beef samples were weighed under aseptic condition and homogenized for 1 min in a laboratory blender containing 225 ml of 0.1 % a sterile peptone water (Oxide CM0009) for preparation of an original dilution of 1: 10. Ten-fold serial dilutions up to 106 were prepared to cover the expected range of samples contamination. using plate count agar for estimation of the total aerobic bacterial count, plates were incubated at 37°C for 24 hours according to ISO 21528-2 (2004) *Enterobacteriaceae* were quantified using violet red bile glucose agar medium and plates were incubated at 37°C for 24 hours. The coliform count was calculated using violet red bile agar medium (VRB), and plates were incubated at 37°C for 48 hours according to ISO 7218 (2007). According to FDA (2001) the staphylococcal count was performed using the Baird Parker agar medium supplemented with egg yolk tellurite emulsion and incubated at 37°C for 48 hours. Identification of *staph. aureus* occurred according to MacFaddin, (2000). Finally, detection of enterotoxins A, C, and D which is produced by *staph. aureus* was performed according to Shingaki *et al*. (1981) using Passive Latex Agglutination technique "RPLA".

Statistical Analysis

The statistical analysis system (SAS, 2014), Cary, USA, Version 9.3) software was used to statistically analyze the data. The mean and standard deviation "SD" of the organoleptic, chemical, and bacteriological parameters were displayed. Tukey's Studentized Range (HSD) post-hoc test (p0.05) and a nested procedural model ($p<0.05$) were used to compare significant means. Reduction percent calculated as follow:
Reduction (R) %= Initial load-New count× 100/Initial load

Results and Discussion

Overall acceptability of minced meat treated with different concentrations of WPI

The control minced meat sample was spoiled after the sixth day of storage at 4°C. The addition of WPI at 1, 3, and 5% significantly improved

the overall acceptability for the sensory properties of minced meat. Samples treated with 1% WPI maintained their overall acceptability until the tenth day of cold storage, while samples treated with 3% and 5% WPI maintained their overall acceptability until the 12th day. Moreover, samples containing 5% WPI had the highest acceptability, while those with 1% WPI had the least enhancement as shown in Table 1. Adding whey protein extract to minced meat can enhance various sensory attributes, such as taste, texture, and juiciness, leading to a more favorable eating experience. These findings were supported by El-Magoli *et al.* (1996) who reported that the addition of whey protein concentrate can positively impact the overall acceptability of meat products, including minced meat-based products like low fat ground beef.

Table 1. Overall acceptance grades of treated minced meat with different concentrations of WPI during refrigeration at 4°C.

Storage period	Control	WPI		
		1%	3%	5%
Zero day	4.9	4.9	4.9	4.9
2nd day	3.2	4.3	4.5	4.6
4th day	1.5	4	4.3	4.5
6th day	1.3	3.5	4.1	4.3
8th day	spoiled	3.3	4	4.1
10th day	spoiled	3	3.5	3.8
12th day	spoiled	2.2	3.1	3.4

4.5- 5: Very good; 4-4.5: Good; 3-4: Acceptable; 2-3: Unacceptable; 1-2: Bad.

Chemical analysis of minced meat treated with different concentrations of WPI

Effect of different concentrations of WPI on pH values of treated minced meat

The recorded data in Table 2, indicated that minced meat samples treated with different levels of whey protein isolate (WPI) 1, 3, and 5% had lower pH values compared to the control samples. The effect of WPI on lowering the pH values of minced meat was most pronounced after 12 days of storage, especially when the concentration of WPI increased to 5%. The pH increases in control minced meat indicate meat spoilage due to protein breakdown into free amino acids, which results in the generation of NH3 and amines by chemical alkaline reaction Karabagias *et al.* (2011). The addition of whey protein (WPI) at concentrations of 3% and 5% to minced meat has been found to result in a lower pH and increased shelf life till the end of day 12 of experiment during chilling storage. According to EOS 1694, (2005) state that the pH of meat should not exceed 6.5, therefore WPI 5% gives best results on day 12 of storage.

The addition of whey protein (WPI) at concentrations of 3% and 5% to minced meat has been found to result in a lower pH of the minced meat during chilling storage, this might be due to when whey protein is added to minced meat, it can bind water and increase the water-holding capacity (WHC) of the meat. This can lead to a decrease in the pH of the meat during chilling storage. The increased WHC can result in the retention of more water in the meat, leading to a higher concentration of acids and a lower pH (Ha *et al*., (2019).

Effect of different concentrations of WPI on total volatile nitrogen (TVN) content of minced meat

The amount of nitrogen that is released because of protein decomposition caused by microorganisms and/or tissue proteolytic enzymes during storage can be measured by the Total Volatile Nitrogen (TVN) content Gibriel *et al.* (2007). TVB-N is frequently used to estimate the rate of deterioration and shelf life of different types of meat Morshdy et al. (2021). TVN mean values of control minced samples were increased as shown in Table 2, and were exceed the permissible limits established by EOS-1694 (2005) (TVN should not exceed 20 mg/100 grams) by the sixth day, this might be due to quick growth of spoilage bacteria which caused protein breakdown and the production of free amines such trimethylamine and dimethylamine as well as ammonia Rukchon *et al.* (2011). Treated minced meat with different concentration of WPI showed significant decrease in TVN values as compared with control samples. Additionally, compared to lower concentrations of WPI (1 and 3%), the highest concentration of WPI (5%) was more successful in lowering TVN value, especially on day 12 of chilled storage. Based on the EOS-1694 (2005) guidelines, it is recommended that the total volatile nitrogen (TVN) in minced meat should not exceed 20 mg/100 grams. The addition of whey protein (WPI) at a concentration of 5% produced the most favorable outcome in terms of reducing TVN, as it did not exceed the permissible limit until the 12th day of the experiment.

Vavrusova *et al.* (2015) explored the potential of whey protein as an antioxidant in preventing oxidative deterioration of protein during the storage of meat products. Therefore, our findings suggest that whey protein can delay or retard protein oxidation in the treated samples. In addition, Peng *et al.* (2023) discovered that whey protein demonstrated excellent antioxidant activity, leading to effective inhibition of oxidation and enhancement of the gel quality of ground meat.

Table 2. Effect of different concentrations of WPI on chemical parameters (pH, TVN and TBA) of treated minced meat during refrigeration at 4°C.

Storage periods	Control	pH value		
		WPI 1%	WPI 3%	WPI 5%
Zero day	5.69±0.01 a	5.65±0.01 a	5.64±0.01 a	5.62±0.01 a
2nd day	6.12±0.01 a	5.76±0.01 b	5.71±0.01 b	5.68±0.01 b
4th day	6.47±0.02 a	5.90±0.02 b	5.82±0.01 b	5.77±0.01 b
6th day	6.93±0.01 a	6.07±0.03 a	5.95±0.02 b	5.84±0.02 b
8th day	spoiled	6.26±0.03 a	6.11±0.03 b	6.02±0.03 b
10th day	spoiled	6.53±0.04 a	6.30±0.03 b	6.16±0.03 b
12th day	spoiled	spoiled	6.59±0.04 a	6.34±0.04 b
		TVN		
Zero day	2.18±0.13 a	2.15±0.13 a	2.12±0.12 a	2.07±0.12 a
2nd day	9.93±0.52 a	5.09±0.27 b	4.65±0.01 c	4.11±0.01 d
4th day	17.05±0.76 a	8.81±0.02 b	7.08±0.01 c	5.56±0.01 d
6th day	28.41±1.35 a	13.65±0.51 b	11.69±0.02 c	8.89±0.02 d
8th day	spoiled	17.87±0.69 a	15.33±0.03 b	12.02±0.02 c
10th day	spoiled	24.73±1.07 a	18.14±0.02 b	15.65±0.02 c
12th day	spoiled	spoiled	22.86±0.04 a	19.74±0.04 b
		TBA		
Zero day	0.04±0.01 a	0.04±0.01 a	0.04±0.01 a	0.04±0.01 a
2nd day	0.38±0.02 a	0.17±0.01 b	0.11±0.01 b	0.08±0.01 b
4th day	0.79±0.05 a	0.41±0.03 b	0.25±0.02 c	0.18±0.01 c
6th day	1.13±0.06 a	0.66±0.03 b	0.42±0.03 c	0.29±0.02 c
8th day	spoiled	0.82±0.03 a	0.59±0.03 b	0.44±0.02 c
10th day	spoiled	1.05±0.07 a	0.76±0.02 b	0.62±0.03 c
12th day	spoiled	spoiled	0.97±0.04 a	0.81±0.03 b

Mean values with different superscripts in the same rows are significantly different at ($P<0.05$).

Effect of different concentrations of WPI on thiobarbituric Acid (TBA) content of minced meat

TBA is a lipid oxidation indicator Abd El-Khalek and Zahran, (2013). TBA values are used to measure the concentration of secondary lipid oxidation products, such as aldehydes and carbonyls of hydrocarbons, which can cause off-aromas in meat Safa *et al*., (2015). In our study, TBA values of control minced meat exceeded the permissible limits established by

EOS-1694 (2005) (not more 0.9 mg MDA/kg) by day 6 during storage but treated minced meat with different concentration of WPI does not exceed the same permissible limit during different periods of storage. Treated minced meat samples with WPI specifically 3 and 5% continued to have normal flavour until the end of storage at 12 days without any rancidity while, rancid flavor in the control samples began to develop on the sixth day of storage as shown in Table 2. Based on the EOS-1694 (2005) guidelines, it is recommended that the thiobarbituric Acid in minced meat should not exceed 0.9 mg/ MDA/kg. The addition of whey protein (WPI) at a concentration of 5% produced the most favorable outcome in terms of reducing TBA, as it did not exceed the permissible limit until the 12th day of the experiment. This could be attributed to the presence of bioactive peptides in whey protein isolate that possess antioxidant properties. These peptides are known to inhibit lipid oxidation, which is a significant contributor to TBA formation in meat products. This finding is supported by Prabhu (2006), who observed the antioxidant activity of whey protein in pork and other lipid-rich products. Gomide *et al.* (2022) reported that antioxidant activity observed in ground meat treated with WPI may be due to the presence of amino acids which have antioxidant activity such as tryptophan, proline, histidine, and tyrosine.

Effect of different concentrations of WPI on amino acid composition in treated minced meat sample

The chemical and nutritional composition of each meat product is greatly varied from one product to another as it contains different kinds of tissues and sometimes a mixture of meat of various organs Lawrie (2022). According to the findings in Table 3, it was observed that the concentrations of essential amino acids generally increase with higher concentrations of whey protein, this is because meat inherently contains a good amount of essential amino acids, as documented by Alina and Ovidiu (2007). Furthermore, whey protein is considered a valuable source of essential amino acids, as highlighted by Hoffman and Falvo (2004). Hence, combining meat with whey protein can enhance the overall content of essential amino acids in the treated meat.

On the other hand, the decrease in non-essential amino acid content in the treated minced meat with whey protein isolate may be attributed to enzymatic reactions and microbial activities of the remaining microbial count in treated samples during refrigeration, which can lead to chemical spoilage, oxidation, and the conversion of amino acids into organic acids and ammonia, as mentioned by Amit *et al.* (2017).

Effect of different concentrations of WPI on bacteriological quality of minced meat

Effect of different concentrations of WPI on aerobic bacterial count of minced meat

The Aerobic Plate Count (APC) is a measure of the bacterial population in meat products; a higher APC usually indicates lower quality and shorter shelf-life Kim and Yim, (2016).

Results observed in Table 4, explain the effect of whey protein on minced meat aerobic bacterial count, the obtained results cleared that, the aerobic bacterial count in minced meat differ significantly ($P<0.05$) among different concentrations of whey protein at different period of experiment. The values of aerobic bacterial count of control minced meat samples were higher than treated minced meat with different concentration of WPI till sixth day of storage. Mean values of aerobic bacterial count in treated minced meat with WPI at concentration 5% were lower than treated meat with WPI at 1 and 3%. Treated minced meat with WPI 1% started to decompose after day10 of storage, while treated minced meat with WPI 3 and 5% still fit for consumption till the end of experiment. The higher concentrations of WPI (3 and 5%) were more effective in decreasing bacterial count than lower concentration (1%).

The highest reduction percent in aerobic plate count observed by using of WPI at concentration 5% which was 99.6% while using of WPI at concentration 1 and 3% gave reduction percent of 98.5 and 99.2%, respectively on the 6th day of storage (Table 4).

According to our findings, whey protein isolate exhibits antimicrobial properties. This observation is consistent with the findings of (Fitzgerald and Murray (2006); Korhonen and Pihlanto (2006); Brandelli *et al.* (2015); Nongonierma and FitzGerald (2016) and Ali and LaPointe (2022), who reported that whey protein contains varying levels of antimicrobial components such as lactoperoxidase, lactoferrin, lysozyme, immunoglobulins,

Table 3. Fractionation of amino acid composition (%) in the examined samples of control and whey protein treated minced meat.

	Amino acids	Control	WPI 1%	WPI 3%	WPI 5%
Essential amino acid	Arginine	5.4	5.9	6.2	6.3
	Cystine	1.1	1.4	1.9	2.1
	Histidine	3.2	3	3.3	3.6
	Isoleucine	4.5	4.7	5.2	5.4
	Leucine	10.3	9.9	10.4	10.5
	Lysine	6	6.3	6.1	6.4
	Methionine	3.4	3.5	3.7	3.8
	Phenylalanine	4.9	5.1	5.4	5.4
	Threonine	3.3	3.7	4	4.1
	Tryptophan	2.2	2.4	2.5	2.6
	Valine	4.3	4.8	4.4	4.7
	Total	48.6	50.7	53.2	54.9
Non-Essential amino acid	Alanine	6.1	5.3	4.8	4.7
	Aspartic acid	10.2	9.5	9.2	8.8
	Glutamic acid	14.5	14.1	13.6	13.3
	Glycine	7.3	7.6	6.9	6.8
	Proline	5	4.7	4.3	4.1
	Serine	3.4	3.2	2.9	2.6
	Tyrosine	2.8	2.5	2.4	2.3
	Total	49.3	46.9	44.1	42.6

and bioactive peptides that have been found to possess antimicrobial activities.

Effect of different concentrations of WPI on *Enterobacteriaceae* count of treated minced meat

Results observed in table (4) show that *Enterobacteriaceae* count in control minced meat samples was higher than treated minced meat with different concentration of WPI till sixth day of chilled storage and control samples started to decompose after six days of storage. Mean values of *Enterobacteriaceae* count in minced meat treated with WPI at concentration 5% were lower than minced meat treated with WPI at 1 and 3%, respectively. Treated minced meat with WPI 1 % started to decompose after tenth day of storage while treated with 3 and 5% WPI still fit for consumption till end of experiment. This indicates that when whey protein concentration increases, the count of *Enterobacteriaceae* decreases. Moreover, the treated samples with the highest concentration of whey protein contain lower *Enterobacteriaceae* count than the control samples or samples with lower concentration of whey protein. The highest reduction percent in *Enterobacteriaceae* count observed by using of WPI at concentration 5% which was 97.1% while using of WPI at concentration 1 and 3% gave reduction percent of 94.8 and 95.9%, respectively, on the 6th day of storage.

Our findings were confirmed by Molayi *et al*. (2018) who reported that the whey protein–alginate coating incorporated with the lactoperoxidase system in different levels could significantly control *Enterobacteriaceae* content in chicken meat.

Effect of different concentrations of WPI on coliform count of treated minced meat

The total aerobic and coliform count are important indicators of general microbial contamination, and the presence of these bacteria in high numbers indicates a higher potential for spoilage and the presence of pathogens in meat (De Oliveira *et al*., 2021).

Results observed in Table 4 demonstrate that coliform mean values of control minced meat samples were higher than treated minced meat with different concentrations of WPI till the sixth day of storage. Mean values of coliform count in treated minced meat with 5% of WPI were lower than treated minced meat with WPI at 1 and 3%. Treated minced meat with 1 % started to decompose after tenth day of storage, while treated with 3 and 5 % still fit till the end of experiment. This indicates that higher concentration of WPI (3 and 5%) is more successful in decreasing the count of coliform than lower ones (1%).The highest reduction percent

Table 4. Effect of different concentrations of WPI on different bacterial counts of chilled minced meat samples (n=5).

Treatment	Control	Aerobic bacterial count					
		WPI 1%		WPI 3%		WPI 5%	
Storage time		Count	R %	Count	R %	Count	R %
Zero day	$6.9\times10^{3}\pm0.4\times10^{3\,a}$	$6.9\times10^{3}\pm0.4\times10^{3\,a}$	------	$6.9\times10^{3}\pm0.4\times10^{3\,a}$	------	$6.9\times10^{3}\pm0.4\times10^{3\,a}$	------
2nd day	$1.8\times10^{5}\pm0.1\times10^{5\,a}$	$5.2\times10^{4}\pm0.3\times10^{4\,b}$	71.1	$3.9\times10^{4}\pm0.2\times10^{4\,c}$	78.3	$3.0\times10^{4}\pm0.1\times10^{4\,d}$	83.4
4th day	$1.3\times10^{6}\pm0.1\times10^{6\,a}$	$9.4\times10^{4}\pm0.8\times10^{4\,b}$	92.8	$6.6\times10^{4}\pm0.3\times10^{4\,c}$	94.9	$5.2\times10^{4}\pm0.3\times10^{4\,d}$	96
6th day	$2.4\times10^{7}\pm0.3\times10^{7\,a}$	$3.5\times10^{5}\pm0.2\times10^{5\,b}$	98.5	$1.7\times10^{5}\pm0.1\times10^{5\,c}$	99.2	$8.5\times10^{4}\pm0.7\times10^{4\,d}$	99.6
8th day	spoiled	$7.9\times10^{5}\pm0.8\times10^{5\,a}$	------	$3.6\times10^{5}\pm0.4\times10^{5\,b}$	------	$1.0\times10^{5}\pm0.1\times10^{5\,c}$	------
10th day	spoiled	$1.2\times10^{6}\pm0.1\times10^{6\,a}$	------	$5.7\times10^{5}\pm0.5\times10^{5\,b}$	------	$1.2\times10^{5}\pm0.1\times10^{5\,c}$	------
12th day	spoiled	spoiled	------	$1.6\times10^{6}\pm0.1\times10^{6\,a}$	------	$4.5\times10^{5}\pm0.3\times10^{5\,b}$	------
				Enterobacteriaceae count			
Zero day	$4.1\times10^{3}\pm0.2\times10^{3\,a}$	$4.1\times10^{3}\pm0.2\times10^{3\,a}$	------	$4.1\times10^{3}\pm0.2\times10^{3\,a}$	------	$4.0\times10^{3}\pm0.2\times10^{3\,a}$	------
2nd day	$9.7\times10^{4}\pm1.1\times10^{4\,a}$	$3.2\times10^{4}\pm0.1\times10^{4\,b}$	67	$2.0\times10^{4}\pm0.1\times10^{4\,c}$	79.4	$1.4\times10^{4}\pm0.1\times10^{4\,d}$	85.6
4th day	$5.9\times10^{5}\pm0.3\times10^{5\,a}$	$4.8\times10^{4}\pm0.4\times10^{4\,b}$	91.9	$3.9\times10^{4}\pm0.2\times10^{4\,c}$	93.3	$2.1\times10^{4}\pm0.1\times10^{4\,d}$	96.4
6th day	$1.4\times10^{6}\pm0.1\times10^{6\,a}$	$7.2\times10^{4}\pm0.5\times10^{4\,b}$	94.8	$5.8\times10^{4}\pm0.3\times10^{4\,c}$	95.9	$4.0\times10^{4}\pm0.3\times10^{4\,d}$	97.1
8th day	spoiled	$1.7\times10^{5}\pm0.1\times10^{5\,a}$	------	$8.1\times10^{4}\pm0.7\times10^{4\,b}$	------	$6.2\times10^{4}\pm0.4\times10^{4\,c}$	------
10th day	spoiled	$4.9\times10^{5}\pm0.3\times10^{5\,a}$	------	$1.4\times10^{5}\pm0.1\times10^{5\,b}$	------	$9.1\times10^{4}\pm0.6\times10^{4\,c}$	------
12th day	spoiled	spoiled	------	$5.3\times10^{5}\pm0.4\times10^{5\,a}$	------	$2.2\times10^{5}\pm0.1\times10^{5\,b}$	------
				Coliforms count			
Zero day	$2.7\times10^{3}\pm0.1\times10^{3\,a}$	$2.7\times10^{3}\pm0.1\times10^{3\,a}$	------	$2.7\times10^{3}\pm0.1\times10^{3\,a}$	------	$2.7\times10^{3}\pm0.1\times10^{3\,a}$	------
2nd day	$5.1\times10^{4}\pm0.4\times10^{4\,a}$	$1.0\times10^{4}\pm0.1\times10^{4\,b}$	80.4	$8.7\times10^{3}\pm0.9\times10^{3\,c}$	82.9	$6.5\times10^{3}\pm0.1\times10^{3\,d}$	87.2
4th day	$2.3\times10^{5}\pm0.2\times10^{5\,a}$	$3.1\times10^{4}\pm0.2\times10^{4\,b}$	86.5	$1.8\times10^{4}\pm0.1\times10^{4\,c}$	92.1	$1.0\times10^{4}\pm0.1\times10^{4\,d}$	95.6
6th day	$4.9\times10^{5}\pm0.1\times10^{5\,a}$	$5.0\times10^{4}\pm0.4\times10^{4\,b}$	89.7	$3.2\times10^{4}\pm0.4\times10^{4\,c}$	93.5	$1.9\times10^{4}\pm0.2\times10^{4\,d}$	96.1
8th day	spoiled	$8.9\times10^{4}\pm1.0\times10^{4\,a}$	------	$5.5\times10^{4}\pm0.7\times10^{4\,b}$	------	$4.7\times10^{4}\pm0.5\times10^{4\,c}$	------
10th day	spoiled	$2.1\times10^{5}\pm0.2\times10^{5\,a}$	------	$9.3\times10^{4}\pm1.1\times10^{4\,b}$	------	$7.0\times10^{4}\pm0.5\times10^{4\,c}$	------
12th day	spoiled	spoiled	------	$3.1\times10^{5}\pm0.2\times10^{5\,a}$	------	$1.4\times10^{5}\pm0.1\times10^{5\,b}$	------
				Staphylococcal count			
Zero day	$1.5\times10^{3}\pm0.1\times10^{3\,a}$	$1.5\times10^{3}\pm0.1\times10^{3\,a}$	------	$1.5\times10^{3}\pm0.1\times10^{3\,a}$	------	$1.5\times10^{3}\pm0.1\times10^{3\,a}$	------
2nd day	$2.9\times10^{4}\pm0.3\times10^{4\,a}$	$7.7\times10^{3}\pm0.5\times10^{4\,b}$	73.5	$5.3\times10^{3}\pm0.4\times10^{3\,c}$	81.7	$4.2\times10^{3}\pm0.3\times10^{3\,d}$	85.1
4th day	$7.5\times10^{4}\pm0.6\times10^{4\,a}$	$1.9\times10^{4}\pm0.2\times10^{4\,b}$	74.7	$8.2\times10^{3}\pm0.6\times10^{3\,c}$	89	$6.9\times10^{3}\pm0.5\times10^{3\,d}$	90.8
6th day	$2.0\times10^{5}\pm0.1\times10^{5\,a}$	$3.3\times10^{4}\pm0.2\times10^{4\,b}$	83.5	$1.7\times10^{4}\pm0.1\times10^{4\,c}$	91.5	$9.4\times10^{3}\pm0.8\times10^{3\,d}$	95.3
8th day	spoiled	$5.6\times10^{4}\pm0.4\times10^{4\,a}$	------	$2.9\times10^{4}\pm0.3\times10^{4\,b}$	------	$1.9\times10^{4}\pm0.1\times10^{4\,c}$	------
10th day	spoiled	$9.7\times10^{4}\pm1.0\times10^{4\,a}$	------	$6.0\times10^{4}\pm0.4\times10^{4\,b}$	------	$4.2\times10^{4}\pm0.3\times10^{4\,c}$	------
12th day	spoiled	spoiled	------	$9.9\times10^{5}\pm1.1\times10^{5\,a}$	------	$6.5\times10^{5}\pm0.7\times10^{5\,b}$	------

Mean values with different superscripts in the same rows are significantly different at ($P<0.05$).

Table 5. Incidence of *Staph. aureus* and their enterotoxins isolated from the chilled minced meat samples on day 6 of storage.

Treatment	No of examined samples	Positive *Staph. aureus*		Enterotoxins			
		No	%	A	D	A+C	A+D
Control	5	3	60	1 (20%)	-	1(20%)	1(20%)
WPI 1%	5	1	20	-	-	1(20%)	-
WPI 3%	5	1	20	-	1(20%)	-	-
WPI 5%	5	0	0	-	-	-	-

in coliform count observed by using of WPI at concentration 5% which was 96.1%, while using of WPI at concentration 1 and 3% gave reduction percent of 89.7 and 93.5%, respectively, on the 6th day of storage.

According to our findings, whey protein isolate with different concentrations has positive effect in decreasing coliforms count in examined minced meat, and these results were supported by Yamauchi *et al.* (2006) who reported that lactoferrin content in whey protein exhibits bactericidal activity against pathogens such as coliforms.

Effect of different concentrations of WPI on total staphylococcal count of minced meat

Results observed in table (4) demonstrate that Staphylococcal mean values of control minced meat samples were higher than treated minced meat with different concentrations of WPI till the sixth day of storage. Mean values of Staphylococcal count in treated minced meat with 5% of WPI were lower than treated minced meat with WPI at 1 and 3%. Treated minced meat with 1% started to decompose after tenth day of storage, while treated with 3 and 5% still fit till the end of experiment. This indicates that higher concentration of WPI (3 and 5%) is more successful in decreasing the count of Staphylococci than lower ones (1%).

The highest reduction percent in Staphylococcal count observed by using of WPI at concentration 5% which was 95.3%, while using of WPI at concentration 1 and 3% gave reduction percent of 83.5 and 91.5%, respectively, on the 6th day of storage (table 4). This reduction might be due to peptides derived from whey protein isolate such as lactoferrin which may inhibit the growth of *Staph. aureus*. The inhibition could be due to the disruption of the bacterial cell wall or interference with essential cellular functions, these findings were supported by several authors have reported antibacterial activity of lactoferrin in vitro against pathogens including *Staph. aureus* (Murdock and Matthews, 2002; Da Silva *et al*., 2012).

Our results were confirmed by Osman *et al.* (2016) who claimed that hydrolysates goat whey protein showed the highest antibacterial activity against *Staph. aureus*.

Incidence of *staph. aureus* and their enterotoxins in chilled minced meat samples on the sixth day of storage

For the biochemical identification of *Staph. aureus* isolated from both control and treated minced meat using different concentrations of whey protein isolate (WPI), 5 samples were analyzed from each treatment. The results indicated that the incidence of *Staph. aureus* in the control group and the treated minced meat with 1% and 3% WPI was 60%, 20%, and 20% respectively. However, no *Staph. aureus* was isolated from the treated samples with 5% WPI (table 5). These findings suggest a potential dose-dependent response, indicating that higher concentrations of WPI may have the ability to inhibit the growth of *Staph. aureus*.

Staph. aureus is an important foodborne pathogen, most frequently associated with enterotoxins associated with intoxication Akbar *et al.* (2019). Among this group of toxins, enterotoxins A, B, C, and D have frequently been detected in red and poultry meat products Normanno *et al.* (2007). The enterotoxins SEA and SEB produced by the bacterium are heat stable and resist most proteolytic enzymes Rall *et al.* (2010). Enterotoxins coding genes are present on genomic DNA. The release of these enterotoxins in food commodities are responsible for food poisoning Nawrotek *et al.* (2005).

According to the data presented in table (5) the occurrence of enterotoxins secreted by *Staph. aureus* was as follows: in the control samples, enterotoxin A was detected in 20%, enterotoxins A+C was detected in 20%, enterotoxins A+D was detected in 20%. Our findings were supported by Tarabees *et al.* (2016) who could isolate enterotoxins A, B, C, and D from minced meat samples collected from different supermarkets in Menoufia Governorate.

In the samples treated with 1% WPI, enterotoxins A+C was detected in 20%; in the samples treated with 3% WPI, enterotoxin D was detected in 20% of examined samples. Treated minced meat with whey protein isolate showed a decrease in enterotoxin production; this is consistent with Xu *et al.* (2014) who found that some natural additives have been shown to repress enterotoxin production and directly interact with enterotoxins to inhibit their mechanism of action.

Conclusion

It was concluded that whey protein isolate "WPI" improves the sensory, chemical, and bacteriological quality of minced beef in turn whey protein has significant antioxidant, antibacterial properties on the bacteria under study, the results are concentration dependent, with increasing whey protein isolate concentration from 1 to 5 %, the more significant effect on the sensory, chemical, and bacteriological qualities. Treating minced meat with WPI has been shown to result in a reduced occurrence of *Staphylococcus aureus* strains and, therefore, a decrease in the presence of enterotoxins.

Conflict of interest

The authors declare that they have no conflict of interest.

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