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AMELIORATION OF PHYSICO-CHEMICAL FEATURES AND ANTIBACTERIAL ACTIVITY OF BALADY YOGHURT USING PECTIN

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ABSTRACT

This study was designed to evaluate the antibacterial effect of pectin against Enteropathogenic *Echerichia coli* (EPEC) and *Listeria monocytogenes (L. monocytogenes)* in Balady yoghurt as a food model. Sensory properties including taste, odor, color and texture were evaluated. Syneresis, viscosity, adhesiveness and cohesiveness were applied as rheological evaluation. In addition, physico-chemical properties such as pH value, titratable acidity, moisture and total solids were measured. Pectin incorporated in yoghurt significantly reduced EPEC and *L. monocytogenes* populations. Pectin at the concentration of 0.6% and 1.2% was accompanied by a significant decrease in the average count of EPEC with a reduction in percentage varied from 94.2 to 97.7% and 97.3 to 99.3%, respectively, during the last four days of the experiment. Against *L. monocytogenes*, pectin was bactericidal at a concentration of 1.2% on the 4th day of the experiment. Moreover, pectin at the rate of 0.6% and 1.2% significantly improved color, odor and texture of the yoghurt, while the taste was significantly improved at the concentration 0.6%. The best overall acceptability (OAA) of yoghurt was obtained at 0.6% pectin, according to the panelist review. Pectin was found to a significant decrease in syneresis; and increase viscosity, adhesiveness and cohesiveness of the yoghurt. On the physico-chemical properties, pectin did not affect pH and titratable acidity; while it decreased the moisture and increased total solids of the yoghurt. In conclusion, our present study shows that pectin has the potential to control EPEC and *L. monocytogenes* in Balady yoghurt with beneficial effects on its sensorial, rheological and physico-chemical properties.

Keywords: Balady yoghurt, pectin, physico-chemical, reheological, antibacterial activity, Enteropathogenic *Echerichia coli*, *Listeria monocytogenes*.

INTRODUCTION

Food is a vital resource for human growth and health since it provides the body with the nutrients it needs (Ali *et al.,* 2023). To preserve the highest possible level of food quality, the food industry has strictly embraced a number of new and standard strategies (Monteiro *et al.,* 2018). Dairy products have special chemical characteristics among a variety of food products. The texture, flavor, odor and nutritional value are all significantly influenced by these characteristics (deMan *et al.,* 2018).

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Yoghurt is a popular dairy product because of its delicious flavor and texture, as well as its nutritional value and bioactive ingredients (Tiwari *et al.,* 2021). In terms of manufacturing and consumption, yoghurt, which is recognized as a functional food, has attracted a lot of attention worldwide. Probiotics and functional foods have been increasingly combined in the dairy business in recent years, particularly with the creation of probiotic functional yoghurts (Munteanu-Ichim *et al.,* 2024). The main component of yoghurt, milk, which is derived from animals like cows, goats, and sheep, has a significant impact on the product's inherent quality. Therefore, maintaining yoghurt's microbiological safety is crucial to its quality, since it includes controlling spoiling to preserve freshness, as well as avoiding the presence of dangerous organisms (Wang *et al.,* 2025).

During the incubation and packaging of yoghurt, there are risks from airborne pathogens that cannot be easily managed at this point. Additionally, the materials used for packaging, along with workers and tools may also introduce microbial contam-ination. Pathogenic *Escherichia coli* (*E. coli*) that belongs to a group of facultative anaerobic bacteria may lead to illness in healthy people due to specific harmful factors, such as adhesins, invasins, toxins and capsules (Sora et al., 2021). Enterohemorrhagic *E. coli* (EHEC) is particularly resilient against acid and can thrive in yoghurt's low acidity (Yun *et al.,* 2021). *E. coli* can endure the fermentation process and remains present for a long time during yoghurt storage (Curtrim *et al.,* 2016). *Listeria monocytogenes* (*L. monocytogenes*) is an opportunistic pathogen that grows with or without oxygen and thrives at temperatures between 0 and 45 °C. It can survive in environments with a pH range of 4 to 9.6 (Ryser, 2011) and can remain alive in yoghurt kept at cold storage temperature (Yang and Yoon, 2022). Notably, *L. monocytogenes* poses a serious risk to vulnerable groups, such as immunocompromised people, infants, elderly individuals and pregnant women, as it is able to cross the blood brain and the fetoplacental barriers (Radoshevich and Cossart, 2018).

Today, buyers are increasingly focused on the quality of the items they purchase. To meet this demand, producers are continually enhancing their product lines by adding various ingredients to yoghurts such as pieces of fruits and vegetables, nuts, dried fruits, herbs and grains (Farag *et al.,* 2021). As the addition of dietary fiber to food products could yield nutritional advantages and improve their functional properties, the dairy industry is increasingly emphasizing the use of diverse additives known for their health benefits (Kaczmarczyk *et al.,* 2012).

Numerous researchers have sought to discover plant-based additives that positively impact human health. These additives frequently have a considerable influence on the physical, chemical and microbiological traits, as well as the texture and sensory qualities of yoghurt. The reviewed studies indicate that yoghurts enhanced with additives hold greater value, particularly regarding their health-promoting components, such as fiber, phenolic compounds, vitamins, fatty acids and minerals (Wajs *et al.,* 2023).

Pectin, a natural substance found in fruits and vegetables, is recognized as a safe food additive (E440) without a specified maximum daily intake (Khedmat *et al.,* 2020). Additionally, pectin has recently been referred to as "a universal medicine" due to its wide-ranging physiological effects (Zaitseva *et al.,* 2020). Research has also highlighted the antibacterial properties of new citrus pectins (Ciriminna *et al.,* 2021). Of particular note, adding pectin to a semi-solid food like yoghurt causes changes in rheological, sensory and tribological characteristics that depend on the size and concentration of the fiber

(Kieserling *et al.,* 2019). Thus, the current research focused on examining the impact of pectin against EPEC and *L. monocytogenes*, and also evaluating how pectin affects the sensory, rheological and physico-chemical properties of Balady yoghurt.

MATERIALS AND METHODS

1- Strains used

Enteropathogenic *E. coli* (ATCC®25922) and *L. monocytogenes* (ATCC®7644) reference strains were obtained from Animal Health Research Institute, Cairo, Egypt.

2- Yoghurt manufacture

Raw milk was homogenized and boiled for 15 min. Then, it suddenly cooled to 45°C and incubated with 2% yoghurt culture (Sfakianakis and Tzia, 2014).

The prepared boiled milk was subdivided into 9 parts (before the incubation) for the following additions:

1st part (without additives) as a negative control.

2nd part for addition of EPEC (10^6/ml) as a positive control.

3rd part for addition of *L. monocytogenes* (10^6/ml) as a positive control.

4th part for addition of pectin (0.6%) plus EPEC (10^6/ml).

5th part for addition of pectin (1.2%) plus EPEC (10^6/ml).

6th part for addition of pectin (0.6%) plus *L. monocytogenes* (10^6/ml).

7th part for addition of pectin (1.2%) plus *L. monocytogenes* (10^6/ml).

8th part for addition of pectin (0.6%) to study sensory, rheological and physicochemical features.

9th part for addition of pectin (1.2%) to study sensory, rheological and physicochemical features.

All groups were incubated at 37°C until curdling (3-4 h) and then cooled and preserved at 4 °C. Sensory, rheological, physicochemical features and microbiological analysis were carried out daily for 7 days. Each experimental parameter was represented in triplicate.

3- Microbiological analysis

EMB agar media (OXOID) was used for enumeration of EPEC and Oxford agar media (Hi media) for *L. monocytogenes.*

Microbiological count data are expressed as colony forming units (cfu)/g of yoghurt.

4- Sensory evaluation

Samples were scored by a regular score panel, depending on a 9-point's hedonic scale (from 1 as dislike extremely to 9 as like extremely). The sensory properties were evaluated depending on 4 main attributes, such as color, taste, odor and texture. Furthermore, the results of the four main attributes were expressed in the overall acceptability (OAA). Sensory evaluation was performed by thirty researchers at Assiut Animal Health Research Institute.

5- Rheological examination

5.1. Syneresis

Whey separation of yoghurt samples was estimated using the centrifugal method described by Parvarei *et al.* (2021), where 10 g of yoghurt sample was centrifuged at 260× g for 10 min at 4°C. The quantity of whey separated at the top of the coagulum inside centrifuge tubes was recorded. The weight of separated whey was measured and the percentage of syneresis determined following the formula: Syneresis (%) = (whey separated/10) × 100.

5.2. Viscosity, cohesiveness and adhesiveness

They were measured by 9-points hedonic scale (from 1 as dislike extremely to 9 as like extremely) with the help of thirty researchers from Animal Health Research

Institute, Assiut branch. They assessed viscosity by stirring the yoghurt with a spoon to gauge resistance and evaluating the thickness perceived during tasting.

Panelists assessed yoghurt cohesiveness by focusing on texture and mouth feel. Cohesiveness refers to the degree to which the yoghurt holds together during consumption. Yoghurt adhesiveness was measured by using a spoon to scoop the yoghurt noting the force required to separate the yoghurt from the spoon. More adhesive yoghurt will cling to the spoon, requiring greater effort to detach. During tasting, panelists assessed how the yoghurt adheres to oral surfaces, such as tongue and palate.

6- Physical examination
pH value
pH value was examined according to AOAC (2005), using a pH meter (AD11, Adwa, waterproof pH-Temp pocket tester with replaceable probe, Romania).

7- Chemical examination
7.1. Titratable acidity (TA) (AOAC, 2005)
A yoghurt sample (10 g) was added to 20 ml distilled water and mixed thoroughly, and then 1 ml of phenolphthalein (1% alcoholic solution) indicator was added to the mixture. The content was titrated against standard sodium hydroxide solution N/10 until faint pink color persisted for about 10-15 sec for complete neutralization. The total acidity was expressed as lactic acid% (normal value 0.7-0.8%).

Acidity %= 0.009 × volume of N/10 NaOH (ml) × 100/ Weight of sample (g), where 0.009 is equivalent to lactic acid normality.

7.2. Moisture content (AOAC, 2000)
Moisture % was estimated by drying in an oven at 100° C until constant weight was recorded. Moisture%= (W2 - W3)/(W2 - W1) X 100, Where: W1 = weight of empty dish, W2 = weight of dish with the sample before drying, and W3 = weight of dish with the sample after drying.

7.3. Total solids%
Total solids = 100 – moisture %.

Statistical analysis
Data were obtained from three replicates and expressed as mean±SEM. They were statistically analyzed by one way ANOVA followed by Tukey post-hoc test for multiple comparisons. A SPSS program (version 16.0) was used for analysis. $P<0.05$ was considered significant.

RESULTS

Table 1: Effect of pectin on the survival rate of EPEC in the examined samples of yoghurt (count by $\log_{10}$ cfu/g).

Day	Control	Pectin 0.6%	Pectin 1.2%
1	5.8±4.6	5.7±4	5.7±4
2	5.8±4.5	4.8±3.3	4.4±3.4
3	5.7±4.5	4.5±3.3	4.2±2.7
4	5.7±4.4	4.3±2.7	3.9±2.2
5	4.9±3.7	3.4±1.7	3.0±1.7
6	4.8±3.2	3.3±1.8	2.8±1.3
7	4.6±3.2	3.0±1.4	2.4±1.6

Table 2: Effect of pectin on the survival of *L. monocytogense* in the examined samples of yoghurt (count by $\log_{10}$ cfu/g).

Day	Control	Pectin 0.6%	Pectin 1.2%
1	6.8±5.0	5.7±4.0	5.4±4.4
2	6.7±5.0	3.6±2.7	2.8±1.2
3	5.5±3.7	3.5±2.4	2.4±1.7
4	5.4±4.0	3.3±2.0	<2
5	4.9±4.0	2.6±1.4	<2
6	4.6±4.0	2.4±1.7	<2
7	4.4±4.0	2.0±1.7	<2

Table 3: Reduction % of EPEC and L. monocytogenes count in Balady yoghurt samples after treatment with 0.6% and 1.2% pectin.

Day	EPEC		L. monocytogenes	
	0.6 %	1.2%	0.6 %	1.2%
1	20.2	23.1	91.6	96
2	89.2	95.3	98.8	99.9
3	94.2	97.3	99	100
4	96.3	98.3	99.3	100
5	96.5	98.6	99.4	100
6	97.2	98.6	99.4	100
7	97.7	99.3	99.6	100

Table 4: Effect of pectin on sensory properties of yoghurt.

Day	**Color**			**Taste**			**Odor**			**texture**		
	Plain	**Pectin 0.6%**	**Pectin 1.2%**	**Plain**	**Pectin 0.6%**	**Pectin 1.2%**	**Plain**	**Pectin 0.6%**	**Pectin 1.2%**	**Plain**	**Pectin 0.6%**	**Pectin 1.2%**
1	7.5	8	7	7.5	8	7	7	8	8	6	7	7
2	7.5	8	7	8	8.5	7	7	8	8	6	7	7.5
3	8	8.5	7	8	8.5	7.5	7	8	8.5	6.5	7	8
4	8	8.5	7	8	8.5	7	8	8	8.5	6.5	8	8.5
5	8	8.5	7.5	7	8.5	6	7	8	8.5	6	8	8.5
6	8	8.5	7.5	7	8	6	7	8	8	6	8	8
7	8	8.5	7	7	8	6	6	7	8	5	7	7.5

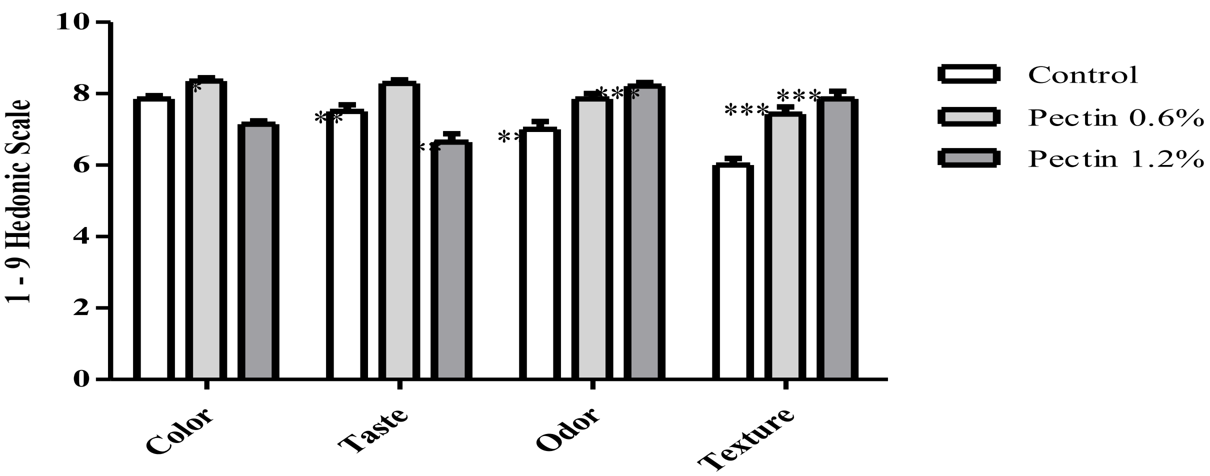

Figure 1: Sensory properties of Balady yoghurt inoculated with 0.6% and 1.2% pectin during 7 days of cold storage at 4 °C. Sensorial attributes scored by 9-point hedonic scale resulting from tasting panelists. Addition of pectin 0.6% has a significant effect on taste, odor and texture of Balady yoghurt with no significance on color. Pectin 1.2% has a significant effect on taste, odor and texture.

Table 5: The overall acceptability scores of yoghurt during 7 days of cold storage by 9-point hedonic scale resulting from tasting panelists.

Day	Plain	Pectin 0.6%	Pectin 1.2%
1	7	8	7.5
2	7	8	7.5
3	7	8	8
4	7.5	8.5	8
5	7	8.5	8
6	7	8	7
7	6.5	7.5	7

Table 6: Effect of pectin on syneresis of yoghurt (values are mean ±SE).

Day	**Plain yoghurt samples**	**Pectin 0.6%**	**Pectin 1.2%**
1	35±1.1	20±0.5	9±0.2
2	36±0.5	20±0.5	9±0.5
3	36±0.5	23±1.0	9±0.5
4	43±1.1	25±0.5	10±0.2
5	43±0.5	25±0.5	10±0.5
6	45±1.0	25±0.5	10±0.2
7	45±1.0	26±0.5	10±0.2

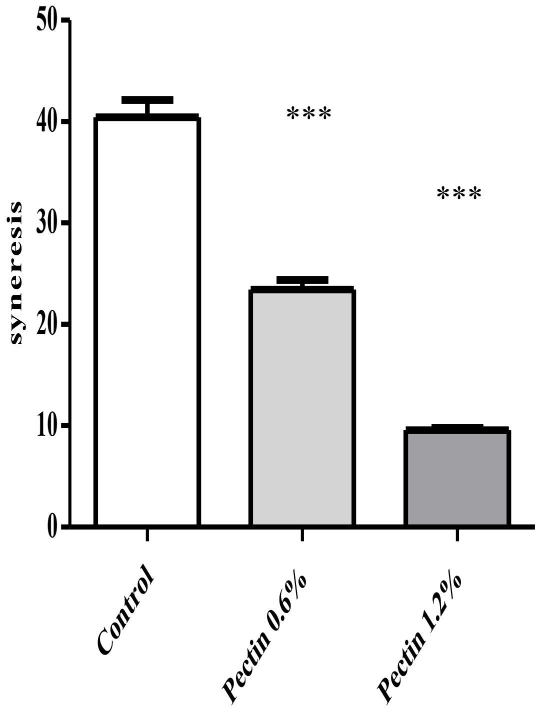

Figure 2: Effect of 0.6% and 1.2% pectin on syneresis of Balady yoghurt during 7 days of cold storage. Addition of pectin with both 0.6% and 1.2% has a significant effect on yoghurt syneresis with P value <0.001.

Table 7: Effect of pectin on apparent viscosity, cohesiveness and adhesiveness.

Day	**Viscosity**			**Cohesiveness**			**Adhesiveness**		
	Plain	**Pectin 0.6%**	**Pectin 1.2%**	**Plain**	**Pectin 0.6%**	**Pectin 1.2%**	**Plain**	**Pectin 0.6%**	**Pectin 1.2%**
1	6	7	6	6	6	5	6	7.5	7.5
2	6	7	6	5	6	5	6	7.5	7.5
3	7	7	6	6	6	5	6	7.5	7.5
4	8	8	7	6	7	6	7	8	8
5	7	8.5	8	6	7.5	7	7.5	8	8
6	7	8.5	8	6	7.5	7	7	8	8
7	7	8.5	8	7	7.5	7	7	8	8

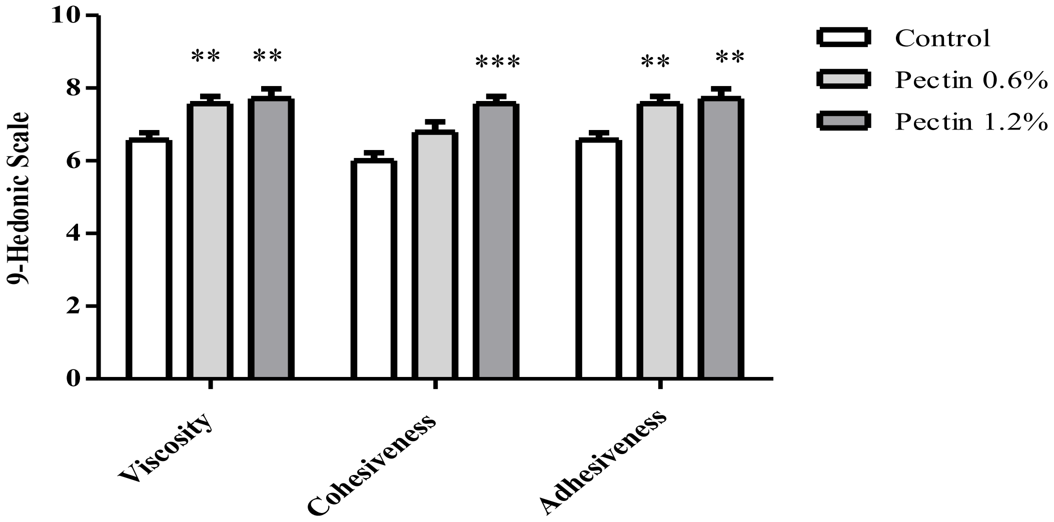

Figure 3: Effect of 0.6% and 1.2% pectin on apparent viscosity, cohesiveness and adhesiveness during 7 days of cold storage of Balady yoghurt. Addition of 0.6% pectin has a significant effect on viscosity and adhesiveness with P value < 0.001. Addition of 1.2% pectin has a significant effect on the three parameters (P<0.001).

Table 8: Effect of pectin on yoghurt pH and titratable acidity % (values are mean ±SE).

Day	pH			TA		
	Plain	Pectin 0.6%	Pectin 1.2%	Plain	Pectin 0.6%	Pectin 1.2%
1	4.7±0.12	4.7±0.21	5±0.17	0.75±0.02	0.76±0.02	0.78±0.01
2	4.9±0.06	4.7±0.15	5±0.15	0.77±0.02	0.76±0.02	0.78±0.01
3	4.8±0.12	4.8±0.13	5±0.12	0.79±0.03	0.75±0.02	0.85±0.03
4	4.6±0.06	4.6±0.10	4.7±0.12	0.85±0.02	0.93±0.07	0.97±0.07
5	4.3±0.06	4.4±0.06	4.5±0.12	1.14±0.09	1.17±0.06	1.19±0.02
6	4.4±0.06	4.5±0.06	4.5±0.17	1.17±0.04	1.19±0.07	1.19±0.02
7	4.2±0.06	4.4±0.12	4.5±0.15	1.17±0.06	1.19±0.06	1.19±0.04

Table 9: Effect of pectin on moisture content and total solids% (values are mean ±SE).

Day	Moisture %			Total solids %		
	Plain	Pectin 0.6%	Pectin 1.2%	Plain	Pectin 0.6%	Pectin 1.2%
1	85±0.4	84±0.4	82.5±0.2	15±0.2	16±0.1	17.5±0.2
2	84.5±0.6	83.8±0.3	82.3±0.9	15.5±0.2	16.2±0.2	17.7±0.1
3	84.1±0.8	83.5±1	82.3±0.1	15.9±0.3	16.5±0.2	17.7±0.1
4	84±0.4	83±0.9	82±0.2	16±0.4	17±0.4	18±0.2
5	83.6±0.7	82.7±0.3	82±0.7	16.4±0.4	17.3±0.4	18±0.2
6	83.5±1	82.5±0.8	82±0.6	16.5±0.4	17.5±0.2	18±0.1
7	83.4±0.9	82.3±0.7	82±0.8	16.6±0.2	17.7±0.2	18±0.1

DISCUSSION

In order to produce yoghurt with improved quality attributes, pectin was applied at a concentration of 0.6% based on a previous study (Arioui *et al.*, 2016), and the concentration was subsequently doubled to evaluate its impact on yoghurt quality.

Antibacterial activity

In the current study, pectin was examined as an edible supplement in Balady yoghurt. In relation to the microbiological analysis, Tables 1, 2 and 3 gave a picture of the pectin's impact on EPEC and *L. monocytogenes*. The data in Table 3 showed that adding pectin at a dose of 0.6% to Balady yoghurt containing EPEC significantly decreased (P<0.001) the bacterial count by 20.20% compared to control at the first day of storage. While, at the dose of 1.2%, the reduction increased to 23.10% at the same day. During refrigeration storage for 48 h, the effect of pectin (0.6%) showed a raise in the activity to 89.20% (Table 3). The increased antibacterial activity was observed by the end of 72 h reaching 96.30% and still significant. Then, the reduction increased to reach 97.70% by the end of the seventh day. On the other hand, at 1.2% pectin, the effect was significant and nearly steady, where the reduction was within the range of 95.30-99.30% (Table 3). Nearly similar results were obtained by Presentato *et al.* (2020) as they reported a powerful *in vitro* activity of lemon pectin against *E. coli*. According to Cirimnna *et al.* (2024), lemon pectin may have an antibacterial impact on *E. coli* because it induces an oxidative stress inside the bacterium by producing reactive oxygen species (ROS) at the bacterial surface, which damages and kills the bacteria. In contrast, Boudouaia *et al.* (2023) found a different finding, demonstrating that pectin had no antibacterial impact on *E. coli*. This discrepancy may result from a variety of reasons, including the molecular weight, ambient conditions and the degree of esterification of the pectin.

Against *L. monocytogenes*, pectin also emerged immediate action after addition (zero h). The bacterial count significantly decreased at a concentration of 0.6%, reaching 91.60%, then grew to 96% at a doubling concentration (Table 3). During refrigeration storage after 24 h, the reduction increased to 98.80% for 0.6% concentration and 99.90% by double concentration. At the end of 96 h of storage, the achieved reductions were 99.40 and 100% for 0.6% and 1.2% doses, respectively. In the same context, Huang *et al.* (2021) found that pectin added to packing film helped eradicate *L. monocytogenes* from fresh food. In a related study, Presentato *et al.* (2020) found that pectin extracted from lemon peel exhibits strong antibacterial activity against *Staphylococcus aureus*, a Gram-positive bacterium that readily contaminates food. According to Gao *et al.* (2023), the pectin oligosaccharide in seed melon had a strong antibacterial action toward *Staphylococcus aureus* and *E. coli*. Of note, pectin's inhibitory effect was primarily caused by the contraction or rupture of cell membranes, which resulted in bacterial cell necrosis or apoptosis.

Sensory analysis

This study also addressed the possible changes in physico-chemical properties and storage stability when yoghurt is enriched by pectin beyond the basic elements. The four main parameters of sensory evaluation (color, taste, odor and texture) were studied in this investigation. As shown in Table (4), the plain and pectin enriched yoghurt samples showed good scores for sensory attributes, especially in 0.6% pectin enriched yoghurt samples. In the present study, sensory panelists revealed significant differences in eating quality between pectin-treated and untreated yoghurt (Figure 1). In this context, Arioui *et al.* (2016) found that a higher rate of pectin incorporation greatly enhanced yoghurt's sensory quality. The improvement of yoghurt taste when adding pectin may be due to the interaction between milk proteins and pectin, that leads to unfolding of protein, which makes hydrophobic groups accessible. According to Mao *et al.* (2014), these groups offer more locations for attaching volatile chemicals, which can reduce their release into the gas phase and enhance olfactory perception. Julmohammad *et al.* (2024) examined the effect of two types of pectin which are low-methoxyl pectin (LMP) and high-methoxyl pectin (HMP) on low fat yoghurt. They concluded that 1% LMP gave the highest sensory evaluation by all panelists. Regarding OAA and according to data summarized in Table (5), yoghurt enriched with 0.6% pectin has the highest score of acceptance according to panelists review. Strikingly, yoghurt samples containing 0.6% pectin were rated significantly higher in OAA, than the plain yoghurt samples with p value < 0.001. Same results were obtained by Arioui *et al.* (2016), as they found that the best acceptance was obtained by yoghurt samples incorporated with 0.6% pectin.

In the same context, Julmohammad *et al.* (2024) reported that the highest OAA of yoghurt samples were recorded by 1.0% pectin. While, different results were obtained by Tobil *et al.* (2020), who observed that the OAA of yoghurt containing 0.6% pectin does not have the lowest rating of neither like nor dislike, while 0.2% pectin yoghurt samples recorded the highest acceptance. The high OAA of yoghurt samples could be due to better texture, enhancement of viscosity, reduction of syneresis, enhancement of taste and better visual appeal. Based on the results obtained, the utilization of pectin in dairy industries would lead to a better fermented milk products, that satisfies consumers and provide health benefits due to its great nutritional contents.

Syneresis

Table (6) showed the syneresis values, in which, the average syneresis value of the plain yoghurt samples was higher than those of pectin enriched yoghurt samples. The syneresis values along days of examination were of a minimum value of 35±1.1% and a maximum value of 45±1.0% for plain yoghurt, while the minimum value was 20±0.5% and the maximum value was 26±0.5% for 0.6% pectin enriched yoghurt samples. On the other hand, yoghurt enriched with 1.2% pectin showed lower values of syneresis, with a minimum of 9±0.2% and a maximum of 10±0.2%. Interestingly, syneresis was significantly reduced due to the addition of pectin (Figure 2). Similar results were reported by Arioui *et al.* (2016) who found that the rise in pectin rates in yoghurt is inversely proportional to the whey exudation (P< 0.01). This could be due to pectin's absorption on the surface of milk casein micelles that boosts whey exudation when added to yoghurt, increasing the fermented milk's capacity to hold water (Everett *et al.,* 2005). Related results were obtained by Julmohammad *et al.* (2024), as they reported that syneresis values decrease with increasing pectin content. The additional flavoring ingredients that raised the total solids and revealed inadequate whey drainage may be the cause of the variation in syneresis readings. In the same context, the increase in protein content and fiber amount may have caused the decrease in syneresis and increase in water holding capacity (WHC) in yoghurt with pectin added; that is, water separation in yoghurt decreased as a result of protein interactions and fiber (Marand *et al*., 2020). Pectin from okra improved water-holding capacity and decreased whey exudation when added to yoghurt, which improved customer acceptability (Tobil *et al.,* 2020).

Viscosity evaluation

Regarding viscosity (Table 7), 0.6% pectin enriched yoghurt was more viscous than plain and 1.2% pectin enriched yoghurt along all days of the experiment with p value <0.001. In the same context, Arioui *et al.* (2016) reported that the viscosity of yoghurt added with pectin rose during the fermentation and post-acidification phases. The amount of galacturonic acid and the degree of esterification both raise the viscosity of pectin. Additionally, it is impacted by factors, like pH and the kind and concentration of salt (Reichembach and Petkowicz, 2021). Particularly in dairy products and dairy analogs, pectin is known to display stabilizing mechanisms, increasing viscosity, establishing an effective network structure that can sustain suspended particles without being noticeable in the mouth, and concealing potentially interactive elements in dispersed systems are some methods that can be used to attain stabilizing properties (Phillips and Williams, 2020; Guo *et al.,* 2022; Wusigale *et al.,* 2020). Bio macromolecules called stabilizing agents, or stabilizers, can reduce the separation of dispersed or heterogeneous media or stop sedimentation (Guo *et al.,* 2021; Rubinstein and Colby, 2003; Wusigale *et al.,* 2020). Pectin has been shown to increase food's viscosity, which is helpful for producing a thick texture (Natalia and Mikhailo, 2023).

Cohesiveness and adhesiveness

During the post acidification period, the panelists qualified the experimental yoghurt added, with 0.6% of pectin having better values of cohesiveness (Table 7). During the storage period, adhesiveness also increased when yoghurt supplemented with pectin. By concentration 1.2%, pectin had a significant effect on cohesiveness and adhesiveness with P value <0.001. Arioui *et al.* (2016) reported similar outcomes, that adding pectin improved the experimental yoghurt's cohesiveness and adhesiveness.

pH and acidity

The two used concentrations of pectin caused a slightly alkalizing effect to the examined yoghurt compared to control. That effect was non-significant, and the pH values were still within normal values (Table 8). The pH values of the three groups of the experiment were nearly similar (Table 8). During the storage time, there was a slight reduction in the pH in the plain, 0.6% and 1.2% pectin enriched yoghurt samples. The reduction in pH during storage can be attributed to the utilization of residual carbohydrate by viable microorganisms. Moreover, the decline in pH values may also be due to increasing the fermentation time and the contribution of acidity of the added stabilizer (Marete *et al.,* 2024). Of note, there was an insignificant increase in pH with increasing the pectin concentration. Tobil *et al.* (2020) reported nearly identical results, as they showed an insignificant rise in pH over the course of 14 days of cold storage for yoghurt samples enhanced with two forms of pectin at varying amounts (0.2, 0.4, and 0.6%). While slightly different results were obtained by Arioui *et al.* (2016), who found the decrease in pH was slow and progressive mean values of 4.13, 4.12 and 4.05 at 7th, 14th and 21st days of storage at 4°C, respectively.

Titratable acidity

Regarding titratable acidity that affects the product's flavor and measures the total amount of acid present, (Table 8) showed the average values of titratable acidity for the plain, 0.6% and 1.2% pectin enriched yoghurt. For plain yoghurt samples, the lowest value was 0.75±0.02 while the highest value was 1.17±0.06. It was obvious that after adding pectin, there was an insignificant effect on titratable acidity. As it is clear in Table (8), titratable acidity of yoghurt fortified with 0.6% pectin ranged from 0.76±0.02 to 1.19±0.06 during 7 days of cold storage. Nearly similar values were obtained after adding 1.2% pectin, ranged from 0.78±0.01 to 1.19±0.04. In a related study, Arioui *et al.* (2016) recorded that there was a highly significant increase in acidity (P<0.01) on average of 70.29ºD at 2 h and reach 90.5ºD after 4 h. In addition, during this phase, a progressive increase in lactic acid of the experimental yoghurt, as 95.12 ºD on average after 7 days of storage at 4ºC. Additionally, it seemed that the rise in lactic acid throughout the fermentation period was proportionate to the rise in pectin addition to 0.6% (P<0.01). However, the evaluation of lactic acid in yoghurt was not significantly impacted by the addition of pectin during the post-acidification stage. Different results were obtained by

Julmohammad *et al.* (2024) who examined the pH and titrable acidity of the low-fat yoghurt fortified with pectin from the 1st day to the 14th day. From the 1st to the 14th day, the titratable acidity gradually increases as the yoghurt's pH gradually drops. 0.67 ± 0.38 was the highest value, and 0.56 ± 0.32 was the lowest.

Moisture

The water-holding capacity of yoghurt can be described as the ability of the food to hold and retain moisture, such as water, during the application of force, pressure, centrifugation or heating (Hamad, 2021). Importantly, moisture content of yoghurt affects texture and thickness.

According to the obtained results in Table (9), the addition of pectin in a ratio of 0.6% has a significant effect on yoghurt moisture with P value of <0.05. While 1.2% pectin showed a highly significant impact on yoghurt moisture (P<0.001). From the obtained data, pectin doesn't raise the moisture level in yoghurt but improves water retention and helps maintain moist texture, which enhances the product quality.

In a related study, Julmohammad *et al.* (2024) indicated that elevating the pectin content enhances water retention capacity. This phenomenon occurs due to the interplay between proteins and water.

Total solids

Data in Table (9) revealed that addition of pectin in concentrations of 0.6% and 1.2% increased the total solids of yoghurt samples, compared to the control. Addition of pectin in a concentration of 1.2% has a highly significant effect on yoghurt's total solids (P<0.001). Similar results were obtained by Khubber *et al.* (2021), who demonstrated that addition of pectin increased soluble solids content in the examined yoghurt samples. The increase in total solids may be due to the nature of pectin, as it is a soluble fiber that contributes directly to the total solids content when added to yoghurt.

CONCLUSION

In conclusion, the current study investigated that the addition of pectin to milk for manufacturing of Balady yoghurt can be used as a preventive measure to inhibit EPEC and *L. monocytogenes*, as well as improving the sensorial, rheological and physico-chemical properties of the obtained product. Additionally, pectin in a ratio of 0.6% has the best evaluation.

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محاولة تحسين الخصائص الفيزيوكيميائية و الجودة الميكروبية بالزبادي البلدي باستخدام البكتين

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صُممت هذه الدراسة لتقييم تأثير البكتين كمادة مضادة لبكتيريا الإشريكية القولونية والليستيريا المستوحدة في الزبادي البلدي كنموذج غذائي. تم تقييم الخصائص الحسية للزبادي المدعم بالبكتين بما في ذلك الطعم والرائحة واللون والملمس. كما تم تقييم كلا من التآزر واللزوجة والالتصاق والتماسك كتقييم ريولوجي. وأيضا تم قياس الخصائص الفيزيوكيميائية مثل الرقم الهيدروجيني والحموضة القابلة للمعايرة والرطوبة والمواد الصلبة الكلية. أدى دمج الزبادي مع البكتين إلى انخفاض كبير في أعداد بكتريا الإشريكية القولونية والليستيريا المستوحدة. إضافة البكتين بنسب 0.6% و1.2% أدى إلى انخفاض كبير في عدد الإشريكية القولونية بنسبة انخفاض تراوحت من ٩٤,٢ إلى ٩٧,٧٪ و٩٧,٣ إلى ٩٩,٣٪, على التوالي. في اليوم الرابع من التجربة، أظهرالبكتين فعاليةً قاتلةً للبكتيريا الليستيريا المستوحدة بتركيز ١,٢٪ . علاوةً على ذلك ، حسّن البكتين طعم ورائحة وملمس الزبادي بشكل ملحوظ بتركيزي ٠,٦٪ و١,٢٪. وكان أفضل قبول عام (OAA) للزبادي عند تركيز ٠,٦٪. كما تم انخفاضٌ ملحوظٌ في التآزر وزيادةٌ في اللزوجة والالتصاق والتماسك. أما من حيث الخصائص الفيزيائية والكيميائية، فلم يؤثر البكتين على الرقم الهيدروجيني أو الحموضة القابلة للمعايرة، بل قلل من الرطوبة وزاد من إجمالي المواد الصلبة في الزبادي. أظهرت الدراسة أن للبكتين تأثير مضاد لبكتريا الإشريكية القولونية والليستيريا المستوحدة في الزبادي البلدي، مع تأثيراتٍ إيجابيةٍ على خصائصه الحسية والريولوجية والفيزيائية والكيميائية .