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Influences of Whey protein concentrate based-antimicrobial edible film coatings on soft cheese properties

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Abstract

Whey protein concentrate (WPC) edible coating films contain sorbate, nisin, sorbate & nisin or marjoram oil as preservative systems were successfully used in soft cheese manufacture. Chemical composition, texture profile analyses and sensory properties were determined in addition to microbiological examination for fresh treatments and during storage at (5±2°C). Coated cheese treatments exhibited that fresh treatments have no significant differences in moisture and ash contents. Coated soft cheese with marjoram oil WPCM has the lowest pH value meanwhile, the control treatment has the highest. Coated soft cheese treatments have less weight loss compared to the control treatment. The WPCM cheese has the lowest hardness value on contrast to the control was the highest. Control treatment with no coating has the highest TBC comparing by coated treatments. There were no detected coliforms counts in all samples when fresh as well as during storage. Addition of WPC resulted to improve the preference of coated soft cheese especially WPCSN and WPCM treatments.

***Keywords*:** Soft cheese; Whey protein concentrate (WPC); Edible film; Coatings.

1. Introduction

Soft cheese is one of the most important types of cheeses for the consumers especially in Egypt. Soft cheese is susceptible to the contamination by the different microorganisms as bacteria, yeasts and moulds. Packaging food such as soft cheese by edible coatings and films have an effect to protect from spoilage, reduce weight loss through the control of oxygen and carbon dioxide exchange rate and act as a carrier of antimicrobial compounds that can improve cheese quality especially during storage **[1].** The utilization of protein and polysaccharide-based biopolymer hybrid nanostructure materials for the food coatings has been increased due to their non-toxicity, biodegradability, ability to form gels and provides compliant protection for products due to its antioxidant and antibacterial properties **[2].** Whey protein as a by-product of cheese-making process is useful in producing transparent, odorless, and flexible edible films and coatings **[3].** Whey protein edible films can enhance the texture and quality of the cheese. Whey proteins edible films containing sorbic, benzoic and lactic acid or bacteriocins as nisin and natamycin can inhibit the growth of bacteria, yeast and mould, **[4]**. In addition, there has been an increase in the use of packing films enriched with essential oils as a new approach **[5].** Essential oils is a well-known source of natural antimicrobial agent against bacterial strains and antiviral moreover, fungi and yeast **[6].** Essential oils extract from marjoram (Origanum majorana) contains antimicrobial compounds of terpinen-4-ol (30.41%), γ-terpinene (13.94%), cis-sabinene hydrate (9.64%), α-terpinene (7.70%), **[7].** The hydroxyl groups in these phenolic compounds can interact with the cell membrane to disrupt membrane structures and cause leakage of cellular components, **[8].**

Therefore, this study was achieved to investigate the manufacture and characterization of soft cheese coated with whey protein concentrate (WPC) edible films containing antimicrobial agents as sorbate, nisin and sorbate & nisin, also containing natural essential oils of marjoram as preservative systems.

2. Material and methods

2.1. Materials:

Fresh buffalo's milk was obtained from Faculty of Agriculture, Cairo University. Whey Protein Concentrate (WPC) contains (80% protein, 4% fat, 2.0% ash and 5.0% moisture) was purchased from Master trade Co., Giza, Egypt. Commercial fine grade sodium chloride (NaCl) was obtained from El-Nasr salins Co., Alexandria, Egypt. Calf rennet powder was procured from Chr. Hansen's Laboratories, Denmark. Nisin as a preservative

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produced by Zhejiang silver elephant Bio–Engineering Co., China, was obtained from Amson international trading Co, Giza – Egypt. Potassium sorbate used as a preservative was purchased from EL-Nasr for Chemicals and Pharmaceutical Industries, Cairo, Egypt. Essential oil of marjoram was obtained from National Research Center (NRC), Giza, Egypt.

2.2. Methods:

2.2.1. Methods of manufacture:

2.2.1.1. Preparation of whey protein concentrate based-antimicrobial edible film coatings:

Whey protein Concentrate (WPC) coating films containing preservative systems were prepared as described by [9] with some modifications as follows:

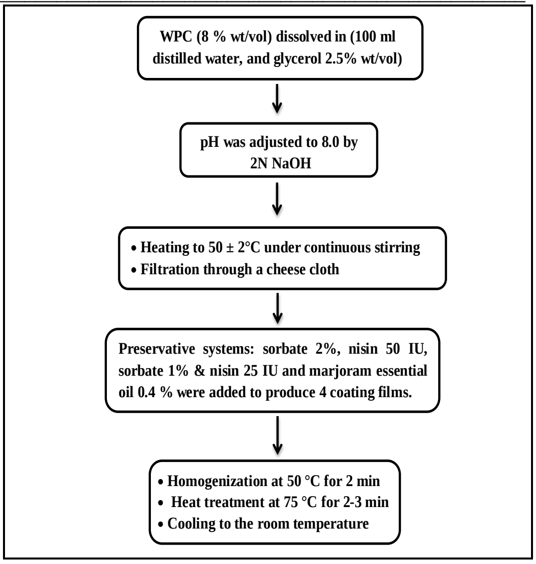

Fig. (1): Preparation of whey protein concentrate (WPC)-based antimicrobial edible film coatings.

Table (1): Thickness, Water Solubility (WS), Water Vapour Transmission (WVT), Water Vapour Permeability (WVP), Transparency (T) at 550 WL, Tensile strength (TeS) and Elongation (E) of Whey protein concentrate edible coating films.

Edible coating films*	Thickness (mm)	WS (%)	WVT (g/s.m^2)	WVP (g.mm/m^2-mmHg)	T (%)	TeS (N/m^2)	E (%)
WPCS	0.435^{A}	25.51^{ABC}	8.30^{F}	0.036^{C}	43.13^{A}	4.215^{D}	19.48^{F}
WPCN	0.278^{AB}	34.02^{A}	18.69^{BCD}	0.052^{B}	35.07^{BC}	4.104^{D}	19.19^{F}
WPCSN	0.243^{B}	28.30^{ABC}	17.30^{DE}	0.042^{B}	41.24^{AB}	4.376^{D}	19.69^{F}
WPCM	0.248^{AB}	26.38^{ABC}	18.00^{CDE}	0.050^{B}	36.17^{ABC}	12.874^{B}	44.23^{C}

WPCS*: Whey protein concentrate + sorbate; WPCN: Whey protein concentrate + nisin
WPCSN: Whey protein concentrate + sorbate & nisin; WPCM: Whey protein concentrate + marjoram oil 0.4%.
ABC: Means with the same letter among treatments are not significantly different.

2.2.1.2. Soft Cheese Manufacture:

Soft cheese were manufactured according to **[10]** with some modifications as illustrates in Fig. (2):

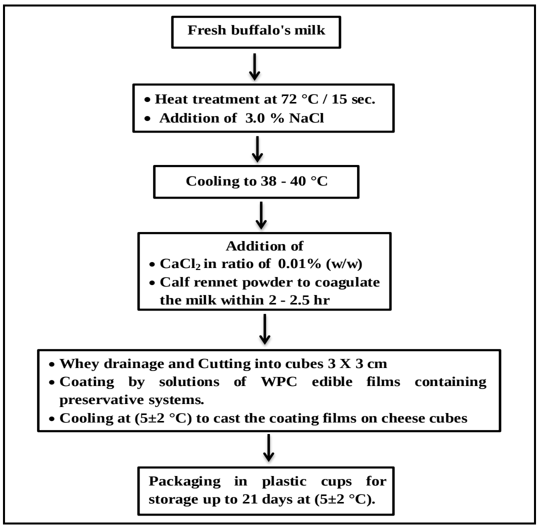

Fig. (2): Manufacture of soft cheese coated by WPC antimicrobial edible films containing preservative systems.

2.2.2. Methods of analysis:

2.2.2.1. Chemical composition and Weight loss (%):

Moisture, fat, total nitrogen (TN), ash, water soluble nitrogen (SN) contents and weight loss as (%) of soft cheese samples were determined according to **[11].** Weight loss was determined using the following equation:

$$\textbf{Weight loss (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

Where: W_1 is the sample weight when fresh and W_2 is the sample weight at storage intervals.

pH values:

pH values of soft cheese samples were measured using a digital laboratory Jenway 3510 pH meter, UK. Bibby Scientific LTD. Stone, Stafford shire, ST 15 OSA.

2.2.2.2. Texture profile analyses:

Texture profile measurements of coated soft cheese were carried out using testing model (FTC, Food Technology Corporation TMS-Pro, strling, Virginia, USA).

2.2.2.3. Microbial enumerations:

Viable total bacterial count (TBC):

Viable total bacterial counts (TBC) as (log cfu/ml) were enumerated using plate count agar medium according to the method described by **[12].** The plates were incubated aerobically at 37 °C for 48 h.

Aerobic spore-formers bacteria (SFB):

Aerobic spore-formers bacterial counts (log cfu/ml) were detected using tryptone glucose yeast extract agar. The samples were heated to 80 °C for 10 min and cold immediately to 10 °C before numeration. The plates were incubated aerobically at 37°C for 24 – 48 h as the method described by **[13].**

Yeast and mould:

Yeast and mould counts (log cfu/ml) were enumerated using potato dextrose agar and incubated at 25±2°C for 3-5 days according to **[14].**

Coliform bacterial count:

Coliform bacterial counts (log cfu/ml) were detected according to the method described by **[15].** using Maconkey agar. Plates were incubated at 37°C for 24 - 48 h.

2.2.2.4. Sensory evaluation:

Sensory evaluation of soft cheese coated with edible films were carried out by the staff members at Food Technology Research Institute, Giza. Egypt. Cheese samples were sensory evaluated according to **[16]** when fresh and during storage at (5±2°C). The Sensory evaluation were attributed for: Appearance & color (10 point), Body & texture (40 point), and flavour (50 point).

2.2.2.5. Statistical analyses:

One-way analysis of variance (ANOVA) was performed using SAS software v. 9.2 with PROC GLM procedure **[17].** Two-way with interaction analysis of variance were carried out according to (MSTAT-C) 2.10 computer software package as described by **[18].** The statistical analyses were carried out at $p \leq 0.05$ among means of three replicates for each treatment when fresh and during storage for 21 days at (5±2°C).

3. Results and Discussion

3.1. Chemical composition:

Chemical changes of soft cheese coated with edible films containing sorbate (WPCS), nisin (WPCN), sorbate & nisin mixture (WPCSN), and marjoram oil (WPCM) when fresh and during storage at (5±2°C) are represented in Table (2):

Moisture content data cleared that in fresh treatments there were no significant differences in moisture content for all coated cheese. The control treatment has moisture content slightly lower than coated cheese treatments.

After 7 days of storage moisture content decreased significantly in all treatments, the control treatment being the lowest. Among cheeses with edible films it was noticed that, treatments of WPCS & WPCM were higher than that of WPCN & WPCSN. These could be due to differences of edible coating properties as water vapour transmission (WVT) and water vapour permeability (WVP). By extending the storage intervals, there were a more reduction in moisture content of all treatments coated by edible films. The results are agreed with **[19].**

Table (2): Chemical composition (%) of coated soft cheese when fresh and during storage at (5±2°C).

Treatments*	Storage periods (days)			
	Fresh	7	14	21
		Moisture		
Control	63.31^{A}	60.45^{H}	ND	ND
WPCS	63.32^{A}	62.35^{C}	59.60^{J}	57.98^{I}
WPCN	63.32^{A}	61.53^{D}	60.10^{I}	59.96^{I}
WPCSN	63.32^{A}	61.53^{D}	60.10^{I}	59.96^{I}
WPCM	63.32^{A}	62.85^{B}	61.04^{I}	60.78^{G}
		Fat		
Control	17.00^{G}	17.35^{EF}	ND	ND
WPCS	17.01^{G}	17.28^{F}	17.43^{D-F}	17.64^{D}
WPCN	17.03^{G}	17.26^{F}	17.40^{D-F}	17.62^{D}
WPCSN	17.05^{G}	17.23^{FG}	17.37^{EF}	17.56^{DE}
WPCM	18.00^{C}	18.21^{C}	18.54^{B}	18.92^{A}
		TN		
Control	2.06^{AB}	1.72^{E}	ND	ND
WPCS	2.25^{A}	1.93^{B-D}	1.81^{C-E}	1.76^{DE}
WPCN	2.25^{A}	1.86^{C-E}	1.77^{DE}	1.72^{E}
WPCSN	2.24^{A}	1.97^{B}	1.94^{B-D}	1.81^{C-E}
WPCM	2.25^{A}	2.08^{AB}	1.99^{BC}	1.89^{B-E}
		Ash		
Control	3.772^{A}	3.827^{A}	ND	ND
WPCS	3.771^{A}	3.790^{A}	3.795^{A}	3.801^{A}
WPCN	3.772^{A}	3.778^{A}	3.782^{A}	3.795^{A}
WPCSN	3.772^{A}	3.780^{A}	3.786^{A}	3.792^{A}
WPCM	3.770^{A}	3.779^{A}	3.785^{A}	3.796^{A}

*** : The same as Table (1)**
ABC: Means with the same letter among treatments are not significantly different.

Fat content of soft coated cheese treatments showed that, fat content in fresh soft cheese ranged from 17 to 18 %. Treatment of WPCM has fat content higher than other treatments including the control.

Furthermore, fat content for all treatments were increased by extending the storage period. The increase in fat content during storage resulted to the decrease in moisture content i.e., increase in total solids (TS) content. These results are in agreement with **[20].**

Total nitrogen (TN) content of soft coated cheese treatments when fresh were 2.06, 2.25, 2.25, 2.24 and 2.25 for control, WPCS, WPCN, WPCSN and WPCM treatments respectively. The data revealed that the control cheese has TN content lower than other treatments. There were no significant differences among coated soft cheese treatments.

By increasing the storage there were a decrease in TN content of all treatments, the highest content was being 1.89 % for WPCM sample meanwhile, the lowest was being 1.72 % for WPCN sample. The reduction in TN content of soft cheese could be due to the water loss during storage and hence the release of whey proteins and protein hydrolysis, **[21].**

For the determined ash content when fresh, no significant differences reported among all cheese treatments.

There was an increase of ash content in soft cheese during storage due to the decrease in moisture content. Ash content of soft cheese treatments at the end of storage were being 3.801, 3.795, 3.792 and 3.796 % for WPCS, WPCN, WPCSN and WPCM treatments respectively. These results are in agreement with **[20].**

3.2. pH values:

The pH values for coated soft cheese treatments with different edible films are illustrates in Fig (3). Soft cheese has pH values ranged from 6.47 to 6.50 for fresh treatments. The WPCM treatment has the lowest meanwhile; the control treatment has the highest pH value comparing with the other treatments.

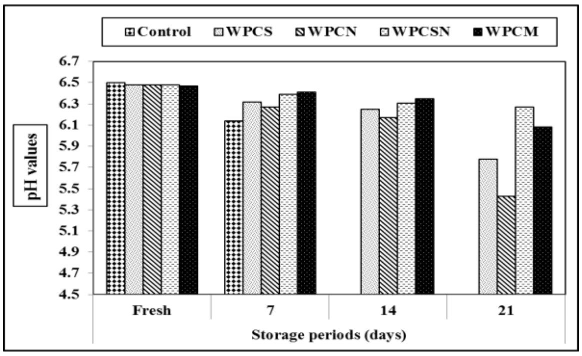

Fig. (3): pH values of coated soft cheese when fresh and during storage at (5 ± 2°C).

During storage at (5±2°C) pH values of all treatments decreased as the storage increased. This could be due to the hydrolysis of residual lactose into cheese curd to lactic acid and free fatty acids developed by storage as the manipulation of bacterial spp. **[22, 23].** Furthermore, the reduction in pH also could be due to the enzymatic activity of heat resistant proteinases present in cheese curd. pH values ranged from 6.27 – 5.43 at the day 21^{th} of storage. From the data, it can be found that the lower pH value was for the WPCN treatment that has higher TBC comparing by the other treatments.

3.3. Soluble nitrogen (SN):

Soluble nitrogen values as (%) of coated soft cheese samples are illustrated in Fig. (4). Soluble nitrogen values for fresh samples were 0.495, 0.540, 0.540, 0.630 and 0.675 % for the control, WPCS, WPCN, WPCSN and WPCM respectively. This could be due to the difference in activity of TBC during curding time. Moreover, primary proteolysis could be due to residual coagulant or milk plasmin, **[22].**

Gradual increase in SN content of cheese samples were determined during storage. The highest SN content was for WPCN sample meanwhile, the lowest was for that of WPCM. This could be due to differences of TBC resulted to differences of water vapour transmission (WVT) and water vapour permeability (WVP) of edible films.

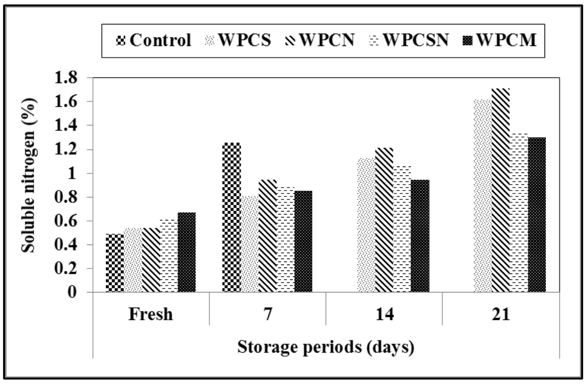

Fig. (4): Soluble nitrogen of coated soft cheese when fresh and during storage at (5 ± 2°C).

3.4. Weight loss of coated soft cheese:

Differences in weight loss as (%) of coated soft cheese and the control treatments at (5±2°C) are illustrated in Fig (5). The weight loss of cheese is considered as a result of whey loss. As a result of the cheese coating after 7 days of storage, coated cheese treatments have lower weight loss performance when compared to the control treatment, **[24].** The WPCM and WPCSN treatments have weight losses lower than that of WPCS and WPCN. The weight losses of coated cheese were influenced by the coating film properties that can control the whey release of cheese curd, **[25].**

All coating films have reduced the weight loss of soft cheese during storage. The weight loss was the higher for the control treatment and the lower for that of WPCM. In another meaning, edible film contain marjoram oil was the most effective for coating that when compared by the other treatments. The increased barrier quality of coating films to water vapour can be due to less weight loss, **[20].** Differences in weight loss among all treatments were significant. At the end of storage, the results indicated an increase in weight loss of coated treatments. This could be due changes in pH values affected the protein network cohesion. The highest value was for WPCS treatment, while the lowest was for that of WPCSN.

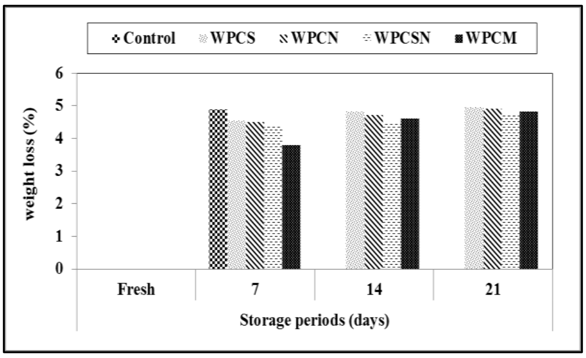

Fig (5): Weight loss as (%) of coated soft cheese when fresh and during storage at (5 ± 2°C).

3.5. Texture profile analyses of coated soft cheese:

The Texture profile analysis: hardness, adhesiveness, cohesiveness, springiness, gumminess and chewiness of coated soft cheese samples are represented in Table (3).

Hardness:

Table (3) mentioned the hardness values of coated soft cheese samples. The fresh samples showed that, the control cheese has the highest hardness value that when compared by coated cheese samples. This could be due to the lower moisture content. **[26]** stated that the hardness of fresh cheese increased in relation to the moisture level all over the time. Among coated cheese samples, WPCS sample has the highest value meanwhile that of WPCM has the lowest. This could be due to the fat content that resulted to more softness of protein network.

Hardness of coated soft cheese samples increased during storage up to 21^{th} days. The hardness of all coated soft cheese samples were increased in relation to the weight loss due to the decrease in moisture content during storage **[21].** The highest value of hardness was observed for the WPCS sample. This might be due to the lower moisture content. The cheese sample coated with marjoram oil (WPCM) reported the lowest hardness value. **[27]** mentioned that decrease in the water activity (aw) of the curd during storage due to changes in water binding by the new carboxylic acid and amino groups formed by hydrolysis. Moreover, changes in pH could cause a migration and precipitation of calcium phosphate influencing the solubility of caseins, high pH cheeses are softer than more acid cheeses.

Adhesiveness:

As shown in Table (3), among coated samples the lowest value of adhesiveness was being 0.229 mj for the sample of marjoram oil (WPCM) and the highest was for that of WPCS being 0.284 mj. The control sample exhibited the highest value comparing by the other samples. This could be due to the lower moisture content.

By extending the storage adhesiveness values of all coated samples increased. The sample of WPCS was the highest followed by that of WPCN while that of WPCM was the lowest. The increase of adhesiveness values during storage could be due to the differences in moisture and fat contents of the sample.

Cohesiveness:

Table (3) shows the mean levels of cohesiveness for coated soft cheese when fresh and during storage comparing by the control sample. In fresh samples the reported cohesiveness values were; 0.94, 0.74, 0.73, 0.72, 0.72 for control, WPCS, WPCN, WPCSN and WPCM respectively. The control sample has the highest value for cohesiveness in contrary to that of WPCM has the lowest. This could be as a result to the more fat content of WPCM sample, **[21]**.

After storage for 21 days the results indicated that, the highest value of cohesiveness being 1.11 for the coated sample of WPCN and the lowest cohesiveness value being 0.95 for that of WPCS. This could be due to the decrease in pH values.

Springiness:

Table (3) represents the springiness values of the control and coated soft cheese samples. For fresh samples the reported springiness values were 2.97, 2.83, 2.80, 2.71 and 2.66 mm for control, WPCS, WPCN, WPCSN and WPCM respectively. The control sample has higher springiness value than that of other coated samples. Among coated samples the WPCS sample has the higher and that of WPCM has the lower. Cheese springiness could be more dependent on moisture and fat contents, **[21].**

At the end of the storage the reported springiness values were increased to 4.63, 4.85, 4.22 and 4.74 mm for WPCS, WPCN, WPCSN and WPCM samples consequently. The highest value of springiness was for the sample of WPCN on contrast to that of WPCSN was the lowest.

This could be related to the weight loss of samples, in addition to the pH value that affected the protein network during storage. So, changes in cheese springiness during storage could be a function of cheese composition, **[21]**.

Table (3): Texture analysis of coated soft cheese when fresh and during storage at (5 ± 2°C).

Treatments*	Storage periods (days)			
	Fresh	7	14	21
	Hardness (N)			
Control	11.60^{CD}	13.42^{B}	ND	ND
WPCS	9.75^{FG}	10.24^{FG}	11.26^{D}	14.32^{A}
WPCN	9.35^{G}	10.35^{EF}	11.29^{D}	13.95^{AB}
WPCSN	7.65^{H}	7.73^{H}	10.09^{FG}	12.35^{C}
WPCM	6.40^{I}	7.23^{HI}	9.56^{FG}	11.15^{DE}
	Adhesivenes (mj)			
Control	0.577^{A}	0.589^{A}	ND	ND
WPCS	0.284^{B}	0.295^{B}	0.345^{B}	0.353^{A}
WPCN	0.276^{B}	0.294^{B}	0.339^{B}	0.350^{A}
WPCSN	0.267^{B}	0.271^{B}	0.276^{B}	0.324^{A}
WPCM	0.229^{B}	0.251^{B}	0.293^{B}	0.321^{B}
	Cohesiveness			
Control	0.94^{A}	1.01^{A}	ND	ND
WPCS	0.74^{A}	0.82^{A}	0.91^{A}	0.95^{A}
WPCN	0.73^{A}	0.80^{A}	0.90^{A}	1.11^{A}
WPCSN	0.72^{A}	0.75^{A}	0.87^{A}	1.05^{A}
WPCM	0.72^{A}	0.74^{A}	0.82^{A}	1.03^{A}
	Springiness (mm)			
Control	2.97^{EF}	3.96^{A-D}	ND	ND
WPCS	2.83^{F}	3.45^{C-F}	3.91^{A-E}	4.63^{AB}
WPCN	2.80^{F}	3.37^{C-F}	3.90^{B-E}	4.85^{A}
WPCSN	2.71^{F}	2.98^{EF}	3.16^{D-F}	4.22^{A-C}
WPCM	2.66^{F}	2.91^{F}	3.33^{C-F}	4.74^{AB}
	Gumminess (N)			
Control	9.70^{E}	10.30^{E}	ND	ND
WPCS	7.15^{GH}	8.13^{F}	14.55^{C}	18.01^{B}
WPCN	6.50^{HI}	7.94^{FG}	11.51^{D}	19.30^{A}
WPCSN	5.65^{I}	8.73^{F}	10.44^{E}	18.73^{AB}
WPCM	4.60^{J}	7.89^{FG}	12.21^{D}	19.05^{A}
	Chewiness (mj)			
Control	29.06^{C}	32.54^{B}	ND	ND
WPCS	19.40^{H}	21.51^{FG}	25.23^{E}	34.71^{A}
WPCN	18.38^{I}	21.06^{G}	24.93^{E}	32.64^{B}
WPCSN	17.48^{I}	19.43^{H}	22.14^{F}	29.35^{C}
WPCM	11.81^{K}	15.33^{J}	21.67^{FG}	27.89^{D}

*** : The same as Table (1)**
ABC : Means with the same letter among treatments are not significantly different.

Gumminess:

Gumminess of coated soft cheese presented in Table (3) were showed significant differences among cheese treatments. The gumminess values when fresh were being 9.70, 7.15, 6.50, 5.65 and 4.60 N for control, WPCS, WPCN, WPCSN and WPCM samples respectively. All coated samples exhibited lower values than the control that being the highest. The sample of WPCS has the higher meanwhile, WPCM has the lower, that when compared by the other coated samples. This could be due to the moisture and SN content.

Gumminess values were gradually increased significantly by extending the storage for all coated samples. At the end of storage the reported gumminess values were 18.01, 19.30, 18.73, 19.05 N for WPCS, WPCN, WPCSN and WPCM samples respectively.

Chewiness:

Chewiness of coated soft cheese samples when fresh and during storage are reported in Table (3). According to the results, chewiness values were 29.06, 19.40, 18.38, 17.48 and 11.81 mj for control, WPCS, WPCN, WPCSN and WPCM samples respectively. The control sample has higher chewiness value than that of coated soft cheese different samples that being lower.

The data of the 14^{th} day of storage indicated that the chewiness increased with the increase of storage for soft cheese samples. At the end of storage the highest value of chewiness being 34.71 mj for WPCS sample and the lowest being 27.89 mj for WPCM sample. The increase of chewiness values during storage was significant The more loss of moisture contents the higher hardness and chewiness values. The results are agreed with **[28, 29].**

3.6. Microbial numerations of coated soft cheese:

Total bacterial counts (TBC), Aerobic spore-formers bacteria, yeast & mould and coliforms numerations as log cfu/ml for soft cheese coated by WPC edible films contains different preservative systems are illustrated in Fig. (6).

Total bacterial count (TBC):

Fig. (6) illustrates the total bacterial count (TBC) numerations of coated soft cheese treatments. The data cleared that, the control treatment with no coating has the highest TBC comparing by the other coated treatments. Moreover, among treatments coated by edible films with added different preservative systems, the highest TBC was for WPCN treatment that detected 4.14 log cfu/ml, meanwhile, the lowest was for the WPCM treatment that detected 3.22 log cfu/ml.

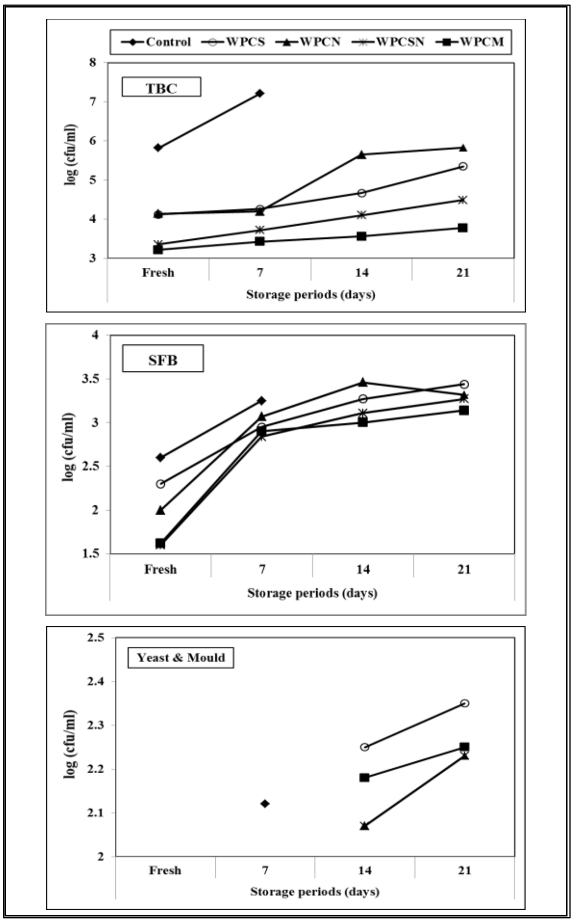

Fig. (6): Microbial numerations as (log cfu/ml) of coated soft cheese when fresh and during storage at (5±2°C).

After storage for 7 days, coated soft cheese treatments with added preservatives had lower TBC comparing by the control treatment that has a more obvious increased numeration resulted to exclude the control treatment. At the end of storage 21th day, the coated soft cheese treatment WPCN has the highest TBC count that detected 5.83 log cfu/ml. This could be due to the water solubility (WS), water vapour transmission (WVT) and Water vapour permeability (WVP) affected the manipulation of TBC. In contrast coated soft cheese WPCM treatment has the lowest TBC that detected 3.78 log cfu/ml. These could be due to the effective of marjoram oil to preserve cheese and prevent microbial manipulation to increase even under storage conditions. Antimicrobial compounds penetration into the food product is affected by storage conditions such as: pH value and water activity, as well as the concentration, **[30].**

Aerobic spore-formers bacteria (SFB):

Aerobic spore-formers bacteria (SFB) counts of coated soft cheese treatments are shown in Fig. (6). The results when fresh showed that the numeration of spore-forming bacteria were 2.60, 2.30, 2.00, 1.60, 162 log cfu/ml for control, WPCS, WPCN, WPCSN and WPCM treatments respectively. The control treatment with no coating has the highest count of SFB meanwhile that of WPCSN has the lowest.

Spore forming bacterial count increased to 3.25, 2.95, 3.07, 2.84, 2.90 log cfu/ml for control, WPCS, WPCN, WPCSN and WPCM treatments at the day 7th of storage, respectively. The control sample was excluded by extending the storage period. At the end of storage, spore-forming bacteria subsequently increased by a rate of 0.49, 0.25, 0.43 and 0.24 log cfu/ml for WPCS, WPCN, WPCSN and WPCM treatments consequently, that when compared by the day 7th of storage. It is clear that, the minimum increasing rate was for the treatments of WPCN and WPCM. These could be due to the effectiveness of nisin and marjoram oil to inhibit SFB and preserve soft cheese.

Yeast and Mould:

Fig. (6) shows yeast and mould count of coated soft cheese treatments. The data cleared that fresh treatments of all coated soft cheese and the control have no yeast and mould numerations.

After 7th days of storage, the control treatment has yeast and mould count of 2.12 log cfu/ml and coated soft cheese have no detected yeast and mould. These could be due to the effect of edible film coatings contain preservatives. Moreover, difference of yeast and mould growth among coated cheese and the control sample could be attributed to the reduction of available oxygen in coated samples, **[21].** Mould and yeast counts at the end of storage was the highest for the treatment of WPCS while was the lowest for that of WPCN and WPCSN. This could be due to the WPC coatings were effective in restricting the growth of yeast and mould, **[31].** In addition, adding nisin & sorbate as preservative system being more effective to inhibit the growth of yeast and mould.

Identification of Coliforms:

There were no detected coliforms counts in all samples when fresh as well as during storage. This could be due to the heat treatment of milk during cheese manufacture. Consequently, these indicate that, soft cheese samples were manufactured under good sanitation and hygienic conditions, so it could not be to detect any contamination by coliform bacteria, **[32].**

3.7. Sensory evaluation:

The sensory properties of coated soft cheese treatments with different preservatives were evaluated to: colour & appearance, body & texture and flavour when fresh and during storage at (5±2°C) and reported in Table (4) as follows:

Colour & appearance (C&A):

Table (4) mentioned the differences of colour and appearance (C&A) scores among control treatment and coated soft cheese treatments when fresh and during storage. Fresh control, WPCS and WPCN treatments scored 8 slightly less than that of WPCM treatment that scored 9. Differences among coated cheese could be due to the differences among coating films in thickness that affected the appearance of coated cheese.

Evaluated C&A were reduced to different scores by increasing the storage up to 21^{th} days, WPCM treatment scored 7 that being higher than other coated treatments. These could be due to the colour tending to be more yellowish as the more water vapour and weight loss as the appearance of coated films changed.

Body and texture (B&T):

Body and texture (B&T) of coated soft cheese treatments as reported in Table (4) exhibited that fresh coated treatments have higher scores comparing by the control. This could be due to the control treatment has a more firm B&T. This could be proved that, the use of edible films as coatings improved the barrier properties against water vapor resulted to reserve the moisture content of coated soft cheese by comparison to the control. The treatment of WPCM has a better body and texture followed by that of WPCSN.

By extending the storage, the body and texture evaluation reduced for all treatments. The highest was for that of WPCM while, the lowest was for that of WPCS.

Flavour:

Flavour scores of coated soft cheese treatments were shown in Table (4). Coated soft cheese treatments have higher flavour scores than the control. Flavour scores ranged from 46 to 49 for coated soft cheese treatments. Addition of WPC resulted to improve the flavour of samples. The treatment of WPCM has the highest score among coated treatments. This could be due to the flavour of marjoram oil has an effect on cheese flavour. **[33]** reported that active films incorporated with plant extracts presented the characteristic of aroma.

During storage, cheese flavour scores decreased for all samples including the control. At the end of storage, soft cheese coated by WPCM film has the higher score and that of WPCS has a less score.

Table (4): Sensory properties of coated soft cheese when fresh and during storage at (5±2°C).

Character Assessed	Treatments*				
	Control	WPCS	WPCN	WPCSN	WPCM
0 days					
C & A (10)	8^{AB}	8^{AB}	8^{AB}	8.5^{AB}	9^{A}
B & T (40)	37^{A-C}	38^{AB}	37^{A-C}	38^{AB}	39^{A}
F (50)	45^{BC}	46^{BC}	47^{AB}	48^{AB}	49^{A}
T (100)	90^{CD}	92^{BC}	92^{BC}	94.5^{B}	97^{A}
7 days					
C & A (10)	5^{D}	8^{AB}	8^{AB}	8^{AB}	9^{A}
B & T (40)	34^{C-F}	37^{A-C}	36^{A-D}	37^{A-C}	38^{AB}
F (50)	37^{A-C}	45^{BC}	46^{BC}	46^{BC}	48^{AB}
T (100)	76^{IJ}	90^{CD}	90^{CD}	91^{C}	95
14 days					
C & A (10)	ND	7^{BC}	7^{BC}	7.5^{B}	8^{AB}
B & T (40)	ND	34^{C-F}	33^{D-G}	35^{B-E}	36^{A-D}
F (50)	ND	42^{DE}	43^{DE}	44^{CD}	45^{BC}
T (100)	ND	83^{FG}	83^{FG}	86.5^{E}	89^{D}
21 days					
C & A (10)	ND	6^{C}	6^{C}	6^{C}	7^{BC}
B & T (40)	ND	31^{F-H}	31^{F-H}	32^{E-H}	33^{D-G}
F (50)	ND	38^{G}	39^{FG}	42^{DE}	43^{DE}
T (100)	ND	75^{J}	76^{IJ}	80^{HI}	83^{FG}

* : The same as Table (1)
C & A : Color & Appearance
F : Flavor
B & T : Body & texture
T : Total score
ABC: Means with the same letter among treatments are not significantly different.

Total scores:

Total scores of soft cheese treatments are stated in Table (4). The total scores of the coated soft cheese treatments indicated that the overall acceptability was affected by all evaluated sensory characters. All coated soft cheese treatments have total scores higher than the control treatment. The treatments of WPCSN and WPCM have the best preference from the other coated treatments. Coating films enhanced the sensory properties of soft cheese, **[23, 34 & 35].**

At the day 7^{th} of storage, the control treatment has lower total scores than that of other treatments. At the end of storage total scores of the coated soft cheese treatments decreased. The differences among treatments could be due to the deterioration of cheese evaluated sensory properties especially B&T and flavour. Moreover, there were significant decreases of total scores by increasing the storage. The treatments of WPCSN and WPCM were the most preferable.

4. Conclusion

Whey protein concentrate (WPC) edible films used in soft cheese coating contain antimicrobial agents or marjoram oil as preservative systems were resulted to an improving for all characters especially the sensory evaluation. The treatments of WPCSN and WPCM were the most preferable.

5. Conflicts of Interest:

Authors have no conflict of interest. We are agreed upon all the Ethic Rules applicable for this journal.

6. Formatting of funding sources

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