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PRODUCTION AND PROLONG SHELF LIFE OF KARISH (WHITE EGYPTIAN CHEESE) USING EDIBLE COATING FILMS

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Abstract

The present study aimed assessing the microbiological quality of Karish cheeses and examining the microbiological quality of coated and uncoated karish cheese samples with edible whey protein films, also studying the inhibitory effect of varied concentration of Rosemary essential oil as antimicrobial agent after incorporated it with the tested film samples. The study also investigated the rheological and organoleptic properties of karish samples before and after coating and during storage periods at 4^0C and 25^0C.

Key words: karish cheese, edible coating, rosemary, whey protein, storage periods.

Introduction

Cheese making began about 8000 years ago and now there are excess of 1000 cheese varieties worldwide. Cheese is the generic name for group of fermented milk based food product's, produced in a great range of flavours and forms through the world (Tom *et al.,* 2001). Soft cheese may be considered as unstable product with irresponsible shelf life. Most traditional cheese is usually produced under poor hygienic conditions with different manufacturing technologies that are dependent on the geographical location (Freitas and Malcata1999).

The recent microbiological guidelines for various ready to eat foods according to HPA and NSW (New South Wales Food Authority 2009) classified food that contains uncooked fermented ingredients such as cheese in the fifth category of the standard limit of microbiological quality (cfu per gram).

In the last years, there has been a growing interest in the use of edible materials in food packaging. The cheese industry is clearly one of sectors many materials have good opportunity for application, as shown by the recent developments on edible coatings and films for cheese. Edible coatings and films, besides its edibility, can be used to reduce weight loss and prevent the microbiological spoilage through the control of oxygen and carbon dioxide exchange rate and as a carrier of antimicrobial compounds (Maria *et al.,* 2018).

Edible films and coatings are thin layers of edible materials applied on food products that play an important role on their conservation, distribution and marketing. Some of their functions are to protect the product from mechanical damage, physical, chemical and microbiological activities. Their use in food applications and especially highly perishable products such as cheeses is based on some particular properties such as cost, availability, functional attributes, mechanical properties (flexibility, tension), optical properties (brightness and opacity), the barrier effect against gases flow, structural resistance to water and microorganisms and sensory acceptability (Falguera *et al.,* 2011).

Whey proteins are obtained from cheese and casein manufacture and contain diverse amount of proteins with distinctive properties. It is available as whey protein concentrate (WPC) or whey protein isolate (WPI) according to the protein content, 20–80% and > 90%, respectively, they have good film forming capacity and present as main advantages the low barries properties, namely to oxygen, volatile aromas and lipids (Maria *et al.,* 2018).

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Whey proteins dispersed in aqueous solutions can form transparent, flexible and tasteless films and have water insolubility due to the network stabilization by disulfide covalent bonds. The unique characteristic of milk whey proteins make them excellent candidates for incorporation into edible film and coatings to control mass transfer in food systems (Bourtoom 2008).

Evaluation of antimicrobial effects of Rosemary (*R. officinalis* L.) essential oils against *Staphylococcus* spp. Rosemary (*Rosmarinus officinalis* L.), originally grow in southern Europ. Its harp and oil are commonly used as spice and flavoring agents in food processing for its desirable flavor, high antioxidant activity and lately as antimicrobial agent (Quattara *et al.,* 1997, Lo *et al.,* 2002). Moreno *et al.,* (2006) reported that rosemary plants are rich sources of phenolic compounds with high antimicrobial activity against both Gram-positive and Gram-negative bacteria. High percent of the antimicrobial activity they attributed to carnosic acid and carnosol.It is clear that rosemary extracts have bioactive properties, but their antimicrobial activities have not beendeeply characterized. Antimicrobial activities of plant essential oils have been known for centuries, but their strong flavour limited their use in food (Del-Cammpo *et al.,* 2000).

Materials and Methods

Materials

Fresh skimmed milk

Was obtained from Faculty of Agriculture Cairo University

Yoghurt starter

Culture for direct vat set (ycx11, thermophilic culture yo-flex) which is available in local market was used in the manufacture.

Chemicals

Candelilla wax was obtained from Stahl bash Inc., New York, N.Y., U.S.A., glycerol was purchased from El-Gomhoria Company, rosemary oil was purchased from local market and stored at 5ºC±1ºC and the culture media; Plate count agar, Brilliant green bile a gar, spore forming media, Nutrient Agar, MacConkey agar, Potato dextrose agar and Barid Parker agar were obtained from Hansen Laboratories A/S, Copenhagen, Denmark. Whey protein Concentrate from localCompany.

Microorganism

Pathogenic strains including

Bacteria

The bacterial strains used in this investigation (Gram positive and Gram-negative) were kindly supplied by the Microbiology Department, Faculty of Agriculture, Cairo University. These strains were *Staphylooccus aureus*, ATTCC 25923; *Escherichia coli*, ATCC 25922; *Bacillus cereus*, ATCC 33018; *Salmonella typhimurium,* ATCC 20231. Cultures were maintained on nutrient agar slants 4°C and sub cultured at 37°C in nutrient broth for 24 hours prior to incubation.

Fungi

Isolates of fungi, *Aspergillus niger* obtained from Microbiology center CATM, Ain-Shams University, Cairo, Egypt.

Yeast

Candida albicans, CAIM-22 was obtained from Microbiological Center CAIM, Ain–Shams University, Cairo, Egypt. The cultures were maintained on slants of appropriate medium, where the bacteria maintained on nutrient agar, the yeast-like fungus was kept on sabourad's dextrose agar medium. All microorganisms were kept on slants of their appropriate medium at 4°C until used.

Methods

Whey protein based film preparation

The WPC (Whey Protein Concentrate) films were formed with the modification of the method described by Kim *et al.,* (2013). Whey protein concentrate (5% wt/vol) was dissolved in distilled water and glycerol (5% wt/vol) was added. The pH was adjusted to 8.0 with 2 N NaOH. Then, solutions were heated to 90±2°C while being stirred continuously. Candelilla wax (0.8% wt/vol) was added during heating per film. After homogenizing for 2 min using Stuart SHm2 homogenizer, the film solutions were filtered through a layer of cheese cloth. Rosemary oils in (0.1, 0.2, 0.3 and 0.4%) were added to the film solutions as essential oil concentrations per film.

Manufacture of Karish cheese

Buffalo´s skim milk was heated to 85ºC for 15sec and cooled to 38-40ºC. Active starters of *Streptococcus thermophilus* and *Lactobacillus bulgarics* (2%w/w). Incubation at45°C for 2-3hrcoating added onto surface. Whey draining Coating (oil + whey protein) the end Storage at (4°C and 25°C) for 25 days and analyzed at zero time, 5, 10, 15, 20 and 25th day.

Cheese coating

The antimicrobial edible coating solution, as well as the commercial nonedible coating (positive controll), was adjusted to pH 7.0 (using 1mol/LNaOH) to guarantee that the coating were devoid of any significant antimicrobial activity associated with pH itself. The coating was applied directly on the surface of cheese after

manufacture (in the absence of any of any other type of protective coating added onto surface). Coating were applied by dib-ping karish cheese for 2 min until all surface were covered, with the residual coating being allowed to drip off. The cheese was left until the coating was essentially dry. The cheese were packed in plastic cans and stored at (4°C & 25°C) according to Ramos *et al.,* (2012).

Chemical analysis

Determination of Moisture, ash, fat, protein and pH Were determined according to the standard method described by AOAC (2000).

Weight loss

Karish cheese samples were individually weighted in zero time after the storage period. The mass loss (w) was determined with the following equation:

$$W\% = (mi - mt) / mt \times 100$$

Where mi is the initial weight and mt is the weight at time t

Microbiological analysis

Total count bacteria were determined on plate count agar according to APHA (1978).

Total coliform and *E.coli* were determined on Maconkey agar according to Greenberg *et al*. (1998). *Staphylococcus aureus* were determined were Bairid Parker agar according to Baird-Parker (1962).

Salmonella were determined on Brilliant Green Agar (CM263, Oxide, 1998).

Spore-forming bacteria count were determined on (Tryptone glucose yeast extract agar) according to (Difco manual 1984).

Yeast and mold were enumerated using Potato dextrose agar according to Duncan (2011).

Statistical Analysis

Procedure of the Statistical Analysis Systems (SAS2004) was used to analyse the and the differences between means were detected by Duncan's Multiple Rang Test Duncan (1955).

Table 1: Changes in the chemical composition of Karish cheese during storage periods at 4°C and 25°C for un-coating and coating cheese.

Temperature		4ºC			25ºC		
Storage period (days)		Zero	15	25	Zero	15	25
Moisture	Un-coating	70.54^{c}	69.61^{e}	68.60^{g}	70.54^{c}	68.20^{h}	67.41^{i}
	Coating	70.80^{b}	70.89^{a}	69.84^{d}	70.79^{b}	69.40^{f}	68.61^{g}
Ash	Un-coating	1.17^{a}	1.18^{a}	1.19^{a}	1.18^{a}	1.19^{a}	1.20^{a}
	Coating	1.16^{a}	1.17^{a}	1.18^{a}	1.17^{a}	1.18^{a}	1.19^{a}
Fat	Un-coating	0.17^{a}	0.18^{a}	0.19^{a}	0.18^{a}	0.19^{a}	0.20^{a}
	Coating	0.15^{a}	0.16^{a}	0.17^{a}	0.16^{a}	0.16^{a}	0.18^{a}
Protein	Un-coating	15.43^{ef}	15.56^{d}	15.89^{b}	15.48^{e}	15.83^{d}	16.12^{a}
	Coating	15.31^{g}	15.42^{f}	15.75^{c}	15.35^{g}	15.55^{d}	15.79^{c}
pH	Un-coating	4.28^{bc}	4.23^{cd}	$4.18d^{e}$	4.24^{c}	4.12^{ed}	4.10^{de}
	Coating	4.37^{a}	4.25^{c}	4.15^{e}	4.31^{b}	4.16^{e}	4.14^{e}

Mean values followed by different superscripts in the same column row are significantly different at $p \leq 0.05$

Table 2: % of weight loss (WL) of un-coated and coated Karish cheese at 4ºC and 25ºC during different periods of storage (W= initial weight) 25ºC.

State	Un-coated				Coated			
Temp.	4ºC		25ºC		4ºC		25ºC	
Weight	W	WL	W	WL	W	WL	W	WL
Zero time	44.762	0	48.922	0	38.773	0	48.023	0
5 days	44.762	0	48.922	0	38.773	0	48.023	0
10 days	43.905	1.915	47.612	2.686	38.694	0.204	47.523	0.040
15 days	43.457	2.920	47.420	3.087	38.243	1.367	47.172	1.772
20 days	43.016	3.901	46.613	4.720	38.002	1.989	46.510	3.136
25 days	42.910	4.140	46.318	5.393	37.611	2.997	46.012	4.188

Results and Discussion

table 1 shows results of some physical and chemical characteristics of un-coated and coated Karish cheese. It is noticed that there is significant decrease in moisture in the un-coated, for different treated Karish cheese during different storage periods compared with coated samples that scared a high degree of moisture loss. That's to say coating affected and monitored the evaporation of water in Karish of cheese during storage at 4°C and 25°C. Furthermore, the cheese treatments affected by storage temperature, that mean at high temperature (25ºC), the moisture evaporation has been higher than the low degree (4ºC). Moisture contents of Karish cheese was affected by coating temperature and progression of storage. Our results are in agreement with those of Cerqueria *et al*., (2009). They concluded that cheese with coating led to lower values and with chitosan and oil promoted a decrease of water vapour permeability. The decrease in the moisture content was detected in the uncoated cheese. This result is in harmony with those of (Youssef *et al*., 2018).

Chemical changes in the Karish cheese were found to be statistically show significant difference ($p \leq 0.05$) until the end of 25 days; ripening period. Moisture, Protein of cheese sample significantly decreased all types un-coating compered coated. The result also showed that the coating process with (whey protein+rosemaryoil) film. The packging material has significant influence on

outage formed by the loss moisture in cheese. pH values are significantly affect by coating in karish cheese from table 1 and storage processes affected pH significantly ($p \leq 0.05$). The pH level of Karish cheese during ripening is shown table 1 and decreased linear during ripening (Farbod *et al.,* 2015) reported significantly decreased in pH level. Changein Fat and Ash were not significantly differ with coating and un-coating during storage period of Karish cheese.

Results shown in table 2 indicated that in all uncoated and coated samples showed decrease in weights after 5 days of storage period at both 4°C and 25°C of storage can cause both an expulsion of serum from cheese and decrease in cheese moisture content. Changes in weight during storage temperatures revealed the effect of temperature on such type of cheese. The obtained results show a moderate decrease in weight for uncoated samples specially at 4°C, while at 25°C high loss was detected. On the other hand, coated samples have less weight loss at 4°C and 25°C than uncoated cheese samples, as the period of storage progressed. Also, weight loss is higher in un-coated than coated samples (Karish cheese).

These results are agree with those obtained by Kavas *et al*., (2015). The concluded that coated cheese samples with film decreased weight losses of Karish cheese and positive effect in terms of quality prevented economic losses by the improved properties of used coated due to the water vapour because the film is good water barrier in many studies, coating with different films prevented the passage of water vapour and thus decreased the economic loses our results is in harmony with those of Youssef *et al*., (2019).

Bacterial contamination might be due to poor sanitary conditions and may also due to the improper pasteurization as was reflected by its survival in the milk after its pasteurization however, percentage of moisture content of cheese product will encourage the growth of these microorganisms, therefore Karish cheese which contain less and less amount of moisture than white cheese and white cheese with vegetable oil, had the least of bacterial counts as shown in tables 3 and 4.

Table 3: Some of microorganisms count in the tested Karish cheese Uncoated during storage at 4°C and 25°C.

Storage period in days	Storage temp °C	Uncoated Samples						
		Counts of hygienic indicators			Counts of public health significance			
		Total bacterial counts cfu× 10^3	Coliforms cfu × 10	*E. coli*cfu × 10	*S.aureus*-cfu × 10	*Salmonella* spp.cfu /25g	Spore forming bacteria cfu × 10^2	Yeasts and Molds cfu × 10
0	25	14.0	28.0	6.4	3.4	nd	15.0	94.0
5	4	17.1	35.5	7.9	4.4	nd	20.2	125.5
	25	21.5	39.5	10.2	6.4	nd	23.5	154.3
10	4	22.5	44.2	9.5	5.7	nd	28.5	165.0
	25	30.2	57.9	14.9	9.3	nd	33.3	237.2
15	4	27.9	55.6	11.3	7.1	nd	36.9	218.5
	25	41.3	82.4	20.0	12.4	nd	46.4	323.0
20	4	33.2	69.7	14.8	8.7	nd	46.0	279.9
	25	55.9	114.9	27.9	16.0	nd	64.6	445.9
25	4	39.6	81.2	19.8	10.9	nd	57.0	366.6
	25	74.5	156.8	37.1	20.2	nd	85.5	597.0

nd: not detected

Table 4: Some of microorganisms count in the tested Karish cheese Coated during storage at 4°C and 25°C.

Storage period in days	Storage temp °C	Coated Samples						
		Counts of hygienic indicators			Counts of public health significance			
		Total bacterial counts cfu× 10^3	Coliforms cfu × 10	*E. coli*cfu × 10	*S.aureus*-cfu × 10	*Salmonella* spp.cfu /25g	Spore forming bacteria cfu × 10^2	Yeasts and Molds cfu × 10
0	25	14.0	28.0	6.4	3.4	nd	15.0	94.0
5	4	14.4	29.9	6.6	3.5	nd	15.4	112.5
	25	19.4	31.7	6.8	4.0	nd	18.2	120.0
10	4	14.8	31.2	6.9	3.8	nd	16.0	116.4
	25	22.8	41.4	7.3	5.0	nd	18.5	155.7
15	4	15.4	33.8	7.3	4.2	nd	16.7	124.2
	25	25.7	54.9	7.9	6.3	nd	22.6	184.4
20	4	16.0	37.0	7.8	4.6	nd	17.6	135.0
	25	29.6	68.3	8.7	7.9	nd	27.9	238.3
25	4	16.8	42.0	8.3	5.1	nd	19.5	150.4
	25	36.4	84.0	9.8	9.5	nd	42.0	282.0

nd: not detected

Total bacterial count

The mean count of total bacteria was 14×103 cfu/g immediately after manufacture and increased to 39.6×103 cfu/g in uncoated samples stored at 4°C (about 2.8 fold) and 74.5×103 cfu/g in uncoated samples stored at 25°C for 25 days (about 5.3 fold), whereas, in coated samples, the count increased to16.8×103

cfu/g (about 1.2 fold) and 36.4×103 only (about 2.6 fold) and after 25 days of storage at 4°C and 25°C, respectively, tables 3 and 4.

Coliforms count

In the same study, higher incidence of coliforms in Karish cheese samples was recorded with mean value of 28.0×10 cfu/g immediately after processing tables 3 and 4. It increased to 81.2×10 cfu/g (about 2.9 fold) and 156.8×10 cfu/g (about 5.6 fold) in uncoated Karish cheese samples stored at 4°C and 25°C, respectively, after 25 days of storage. Coated samples, the count reached to 42.0×10 cfu/g (about 1.5 fold) and 84.0×10 cfu/g (about three fold) at 4°C and 25°C for 25 days of storage respectively. It is evident that there is a clear effect of whey protein edible coating to maintain the shelf life of cheeses during storage.

E. coli

The results of *E. coli* were classified as acceptable microbiological quality in uncoated Karish samples after storing for 25 days at 4°C and/or at 25°C, which reached 19.8×10 cfu/g at 4°C and 37.1×10 cfu/g at 25°C. The same results revealed that coated Karish samples, *E.coli* were classified as satisfactory microbiological quality storing for 25 days at 25°C and/or at 4°C which reached 8.3×10 cfu/g at 4°C and 9.8×10 cfu/g at 25°C as shown in tables 3 and 4 (HPA, 2009).

Staphylococcus aureus

Enumeration of *S. aureus* is considered a more sensitive measure of food hygiene practices, it is one of the most important food-borne pathogens foods in ready-to-eat products and *S. aureus* intoxication can result in debilitating illness (Gilbert *et al.,* 2000), who found that all-coagulase-positive. *S. aureus* produce entero-toxins and counts of *S. aureus* in food above 100 cfu/g is considered unwholesome, the entero-toxins produced by this bacterium are heat stable and are not affected by processing temperatures. *Staphylococcus* food contamination is usually traced to workers who are carries and/or to contact with inadequately cleaned equipment. After 25 days of storage at 4°C and/or 25°C while in coated Karish cheese samples tables 3 and 4 were classified as satisfactory microbiological quality after the same period of storage, which the number of *S. aureus* was lower by 53% in coated samples than in uncoated samples at the end of storage period at 4°C and/or 25°C. Nearly similar incidence were reported by Varga (2007), who found that the mean count of *S. aureus* for Karish cheese was between 4,7×10 cfu/g after processing and increased to 23.0 to 24.5×102 cfu/g after 3 weeks of storage at room temperature (about three–fold) and the toxin occurs when large number of coagulase positive cells are present under a proper set condition.

***Salmonella* spp.**

Not detected in all uncoated and coated samples. It may be due to the efficiency of milk pasteurization process before manufacture (Leon *et al.,* 2013) which *Salmonella* spp. is sensitive to hasting and non-spore forming. Mainly, the infection of these organisms might occur either in the field or during handling.

Spore-forming bacteria

A marked incidence were recorded in spore-forming bacteria table 3 and 4 with average of 15.0×102 cfu/g immediately after Karish cheese production which milk pasteurization (73°C for 15sec) was not efficient to eliminate all these microbes. Most of these microorganisms are resistance to heat. The count increased from 57.0×102 cfu/g to 85.5×102cfu/g, after 25 days of storage at 4°C and 25°C, respectively in uncoated samples, while it increased from 19.5×102 cfu/g to 42.0×102 after the same period of storage at 4^0C and 25°C, respectively, in coated samples. The count of spore form bacteria as shown in tables 3 and 4 was less by 51% in coated samples than uncoated samples at the end of storage period at 25°C and was less by 66% by the end of storage period at 4°C than uncoated ones, due to the antimicrobial agent which incorporated in used coating films.

Yeasts and Molds

Sources of Yeast and Mold contamination were the air, floors, walls, equipment, hand, aprons and the brine which responsible for the highest yield of yeast contamination (Seiler and Busse 1990). Since some of yeasts are particularly resistant to such adverse conditions as the high salt concentration. Yeasts however, possess the ability to grow under conditions unfavorable to many bacteria and play an important rolls in the spoilage of dairy products.

The mean count of yeasts and molds after manufacture of Karish cheese was 94×10 cfu/g tables 3 and 4 and increased to 366.6×10 cfu/g at 4°C for 25 days (about four-fold), while it increased to 597×10 cfu/g in uncoated samples stored at 25°C for 25 days (about six-fold). Whereas in coated samples the increasing level of yeasts and molds count was lower which reached to150.4×10 cfu/g after 25 days at 4°C (about 1.5 fold) and to 282.0×10 cfu/g after 25 days at 25°C (about three-fold). Also, these numbers will ensure the active effect of antimicrobial edible coating in coated samples to maintain the shelf life of cheese products.

Conclusion

It can be concluded that the hygiene quality of coated cheese was higher than that of uncoated one and the quality of cheese products significantly affected by the edible packaging. The quality of cheese is significantly affected by the addition of essential oils such as rosemary oil which are identified as the major chemicals components responsible for extending antimicrobial activity. This observation encourage to use this oil for the further studies and it will be used in the commercial scale as well as, this oil considered an antifungal edible coatings oil and improve the shelf life of cheese. The food processor must employ methods for both short and long term preservation of methods must be capable or either sup-pressing or killing both pathogenic and spoilage microorganism without the addition of any toxic substances.

Conflict of Interest

The authors declared that present study was performed in absence of any conflict of interest.

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Author Contribution

All authors contributed equally in all parts of this study.

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