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EXTENDING THE SHELF LIFE OF LOW SALT WHITE SOFT CHEESE BY MODIFIED ATMOSPHERE PACKAGING AND ULTRAVIOLET USAGE.

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ABSTRACT

Low salt white soft cheese was manufactured by ultrafilteration – yeast suspension was added to the resultant retentate to get a rate of contamination equal to 500 cell/gm. Cheese was packed in polyethylene bags and air was substituted by gas nitrogen in some of these bags, and in some other the polyethelyne bags, were exposed to ultraviolet lamp after substitution of air for 30 seconds. Cheese samples were divided into two groups where one of these group was stored at room temperature and the other, at refrigeration temp. During storage cheese samples were periodically analysed for yeast count and organoleptically assessed. The obtained results revealed that the modification of the atmosphere packaging by nitrogen gas led to slowing or delaying in yeast growth on cheese surface as the yeast count obviously decreased when compared with untreated samples. Also by delaying the detection of the off - or yeasty flavour in treated cheese. No positive effect could be detected due to the ultraviolet treatment. In general one could conclude that modified atmosphere packaging by nitrogen gas didn't prevent microbiological spoilage but it led to a relative delaying to it.

INTRODUCTION

Many foods spoil rapidly in air due to moisture loss or uptake, reaction with oxygen and the growth of aerobic microorganisms i.e., bacteria, yeast and moulds. Microbial growth results in changes in texture, colour, flavour and nutritional value of the food. Storage of foods in a modified gaseous atmosphere can maintain quality and extend product shelf life by slowing chemical and biochemical deteriorative reaction and by slowing or in some instances preventing the growth of spoilage microorganisms. Modified atmosphere packaging (MAP) is defined as the packaging of a perishable product in an atmosphere which has been modified so that its composition is other than that air (Hintlian and Hotchkis, 1986). There are three main gases used in modified atmosphere packaging namely O_2 Co_2 and N_2 in addition to Co and noble gases (He, Ar and Ne) which are applied in a very narrow limit.

MAP has the potential to increase the shelf life of a number of dairy products. These include fat-filled milk powders, cheeses and fat spreads. In general these products spoil due to the development of oxidative rancidity in the case of powders and or the growth of micro-organisms particularly yeast and moulds in the case of cheese.

White soft cheese is an ideal substrate for film yeasts to develop. This slime film may be thin and watery or quite thick and even ropy. It varies in colour from white to yellow to brown or fluorescent brown and it may have a rancid, putrid or fruity odour.

Nowadays, yeast growth in the form of slime film appears on the cheese surface represent a big problem to low salt white soft cheese

manufacturer due to the huge amount return to the plant. Therefore dealing with this problem in a trail to solve it is considered a very important point from the economical point of view.

MATERIAL AND METHODS

Materials:

Cow's milk for white soft cheese manufacturing was obtained from Dina Farm.

- **Rennet:** Maxiren 1800 mg 100% chymosin purified from cluyveromyces lactis, made in France.
- **$Cacl_2$:** Case, 77-80% cash, made in Italy, was purchased from private company in El-Gish Street, Cairo-Egypt.
- **Salt:** Sodium chloride according to the Egyptian specification No. 2732-1/2005 concerned with food salt, was obtained from the Egyptian Company for salts and minerals.
- Skimmilk powder Sweden was purchased from Knofa Company, Nasr City, Cairo, Egypt.
- **Nitrogen gas:** a nitrogen cylinder was obtained from Emylcogen Company, Zagazig Governorate.

EXPERIMENTAL PROCEDURE

1. Isolation and identification of yeast from spoiled cheeses

A) Isolation

Plates of wort agar medium (Lodder, 1952), containing (%, w/v): Malt extract, 1.5; peptone, 0.075; maltose, 1.575; dextrin, 0.275 glycerol, 0.236; diopotassium phosphate, 0.1; ammonium chloride, 0.1; agar agar, 1.5; were streaked using suitable dilutions of spoiled cheese and incubated at 30°c for 48 hrs. Colonies of yeasts were confirmed by Gram reaction and morphology. Well-separated yeast colonies were picked on nutrient glucose agar and incubated at 30°C for 48 hr where purity checks were carried out using Gram reaction.

B)Identification

Isolated yeasts were identified microscopically using reference images from Barnet et al. (2000) along with their ability to produce hyphae, ascospores, buds, lactose fermentation and urea hydrolysis. It was found that 60% of the isolates were related to the genus Kluyveromyces and the other 40% were belonging to Saccharomyces.

2.Yeast inoculum

The yeast inoculum prepared for cheese contamination was prepared in nutrient glucose broth, incubated for 24 hrs and standardized microscopically to a density of 10^5 yeast cell/ml in a 5% peptone water solution.

3.Total viable yeast count in cheese

Ten gram of cheese were aseptically homogenized in 100 ml of 1% peptone water solution from which suitable dilutions were prepared for inoculating wort agar plates. Yeast colony forming units (cfu) were counted

after incubation at 30°c for 48 hrs. The suspected yeast colonies were confirmed by Gram staining and morphology.

4.Manufacture of cheese

White soft cheese was manufactured from cow's milk with the following chemical properties: Fat% : 3.5, SNF: 8.5, protein: 2-95, Sp. gr: 1.030, TA: 0.17 according to the method applied on a large scale in some dairy factories. The method could be summarized as follows:

Milk was pasteurized at 80°c/15 sec. and homogenized at 110 bar. During pasteurization, milk fat was standardized to 5% fat with polyhydrogenated palm kernel oil (Tekerline) at 1.5% by injection in pasteurization line before homogenization step. Pasteurized milk was warmed up to 48°C, then was ultrafiltered using a spiral wound continuous apparatus. By the end of ultrafiltration step skim milk powder was added to the resultant retentate at 3.3%, then after retentate was pasteurized at 75°c/15 sec. and homogenized at 100 bar. Salting was applied at 3% salt, then 0.1% $Cacl_2$ and 0.005% rennet powder was added. Retentate was packed in stainless steal plates and incubated for 2 hr at 40°C, then after cheese plates were transferred into refrigerator for at least 6 hr. Cheese is then cut into small cubes and packed in polyethylene bags.

5.Cheese Judging.

Cheese was organoleptically assessed by a group of panelists from the staff member of the dairy department of faculty of agriculture, Cairo University

6.Statistical Analysis

The obtained data was statistically analyzed according to Fogiel, (1988).

7.Experimental treatments

Thirty two kg retentate was taken after salting and before renneting and was divided into two parts, the first part is 12.8 kg which was divided into two division each was 6.4 kg. The first division was renneted and left for coagulation, then was cut into 16 cubes and packed in polyethylene bags. Eight cubes were stored at room temperature and the rest were stored at refrigeration temp. (T_1).

The second division was treated as the first one except that the cheese in the polyethylene bags were flushed with nitrogen gas until the bags were full of nitrogen then some gas was pushed out and the bags were tightly closed before storage (T_2). The second part approx. 19.2 kg was inoculated with 200 ml of yeast suspension to get a contamination rate equal to 5×10^2 cell/gm. After inoculation, rennet was added and retentate was left for coagulation as previously mentioned. The obtained cheese was divided into small cubes and the cubes were divided into two groups each was 24 pieces, one group was stored at room temp. and the other at refrigeration temperature. Each group was subdivided into three treatments as follows:

1. The first treatment consisted of 8 cubes which were packed in polyethylene bags with no other treatment (T_3).
2. The second treatment (T_4) was packed and flushed with nitrogen gas as in T_2.

3. The third one (T_5) was completely similar to the second one (T_4) except that cheese bags were exposed to ultraviolet lamp for 30 second.

All samples were stored as previously mentioned at room and refrigeration temp. until spoilage which was terminated by judging, and samples were taken for judging and yeast analysis every five days at room temp. and every 10 days at refrigeration temp.

RESULTS AND DISCUSSION

Data presented in tables 1 & 2 and Figures 1 & 2 represent the changes in yeast count in low salt white soft cheese stored at both room and refrigerator temperature as affected by packaging in modified atmosphere and the exposure to ultra violet lamp. It is clear from table (1) that yeast count increased in all treatments throughout the storage period but in different ratios.

In treatment one (T_1) which represents the control sample, yeast count increased from 10×10^1 to 5×10^6 after 20 days storage with the development of off flavour in cheese after 15 days and the rate of increment was significant all over the storage period..

In treatment two (T_2) which represents the same control cheese packed in modified atmosphere, the data indicate that the rate of increase in yeast count was very slow and decreased too much when compared to T_1 as the total yeast count reached 20×10^2 after 20 days with no off flavour, which represent 0.04% of the count in its corresponding untreated control sample, then the count highly increased to reach 25×10^5 after 25 days with obvious off flavour. Statistically the rate of increase was not significant until the 20^{th} days. Concerning the inoculated cheese samples in T_3, T_4 and T_5. The obtained data clearly show that yeast count in T_3 increased rapidly from 5×10^2 to be 13×10^6 after 5 days, then increased with some fluctuation to reach 80×10^6 after 15 days/ and 65×10^5 after 20 days with obvious yeasty flavour in the cheese. On the other hand cheese packed in modified atmosphere in treatment four (T_4) showed a very low increment rate as the yeast count increased to reach 44×10^3 after 15 days which represent 0.055% of its corresponding sample without nitrogen gas (T_3). Statistically there was an obvious significant increase in yeast count in (T_3) from the start point, while in (T_4) and (T_5), the significant increase appeared only after 15 days of storage.

Concerning the last treatment T_5 in which the cheese packed under modified atmosphere was exposed to ultraviolet from ultraviolet lamps, it could be concluded that no positive effect could be detected and attributed to the ultra violet rays as there was no big difference between the yeast count in T_5 when compared with T_4 and the differences were not significant. It is worthy to note here that cheese samples in T_2 doesn't spoil until the end of the 20^{th} day and the yeasty flavour was observed after 25 days.

The same previous trend was also observed with cheese stored at refrigeration temperature. It was obvious from the presented data in table (2), that the yeast count increased in all treatments but in a lower rates when compared with that stored at room temperature. It was also clear that the cheese packed in modified atmosphere in T_2 and T_4 recorded the lowest

count when compared with its corresponding values in T_1 and T_3 respectively. As the yeast count reached 16 x 10^3 in T_2 and 30 x 10^4 in T_4 after 40 days which represent 0.16 and 1.66% from their corresponding value in T_1 and T_2 respectively. No improvement could be detected due to the ultraviolet treatment in (T_5) as the rate of increase was slightly higher in the first twenty days, then obviously increased during the last 20 days when compared with (T_4). Statistically there was significant increase in yeast count after 20, 40, and 30 days in (T_1), (T_2) and (T_4) respectively, and after 10 and 20 days in (T_3) and (T_5) respectively.

Also it is worthy to note that the shelf life of cheese in T_2 extended 10 days more than cheese in T_1 and also the shelf life for cheese in T_4 and T_5 extended 10 days more than cheese in T_3.

In conclusion, it is possible that N_2 concentration could affect ascosporogenous and basidosporgenous yeast. In fact no investigations on specific yeast species behaviour in modified atmosphere containing different concentrations of N_2 are reported in the literature and are strongly required. While it was reported that 100% N_2 at 4°C was able to completely inhibit growth of staphylococci, lactobacilli and bacillus for 2 days in whey cheese (Pintado & Halcata, 2000).

Table (1): Yeast counts in cheese samples as affected by modified atmosphere packaging during the storage at room temperature

Storage Period (days)	T_1 Control	T_2 Content cheese + N	T_3	T_4	T_5
Fresh	10 x 10^1	10 x 10^1	5 x 10^2	5 x 10^2	5 x 10^2
5	88 x 10^2	12 x 10^1	13 x 10^6	11 x 10^3	8 x 10^3
10	10 x 10^3	20 x 10^1	10 x 10^6	20 x 10^2	17 x 10^3
15	*10 x 10^5	42 x 10^1	80 x 10^6	44 x 10^3	26 x 10^3
20	5 x 10^6	20 x 10^2	65 x 10^5	*50 x 10^4	*30 x 10^4
25		35 x 10^5			

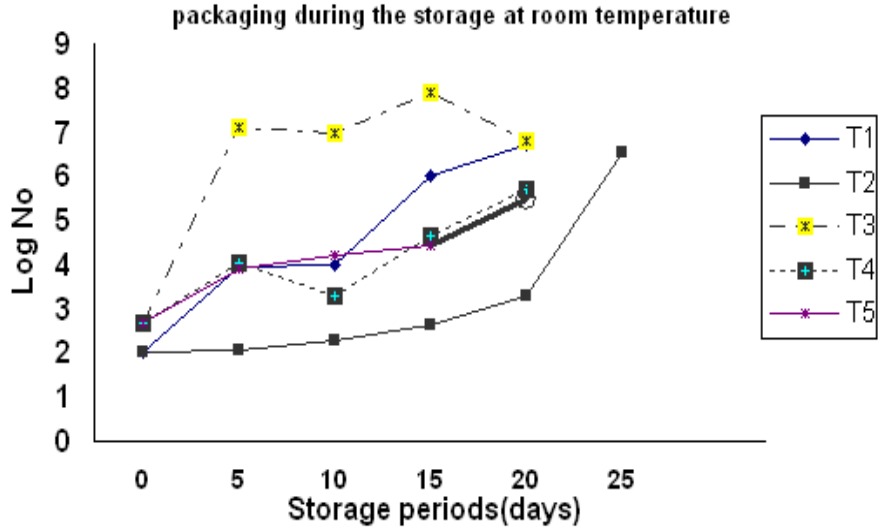
*** off flavour was easily detected by panelists.**
LSD for log No. was 1.1357

Table (2): Yeast counts in cheese samples as affected by modified atmosphere packaging during the storage at refrigeration temperature

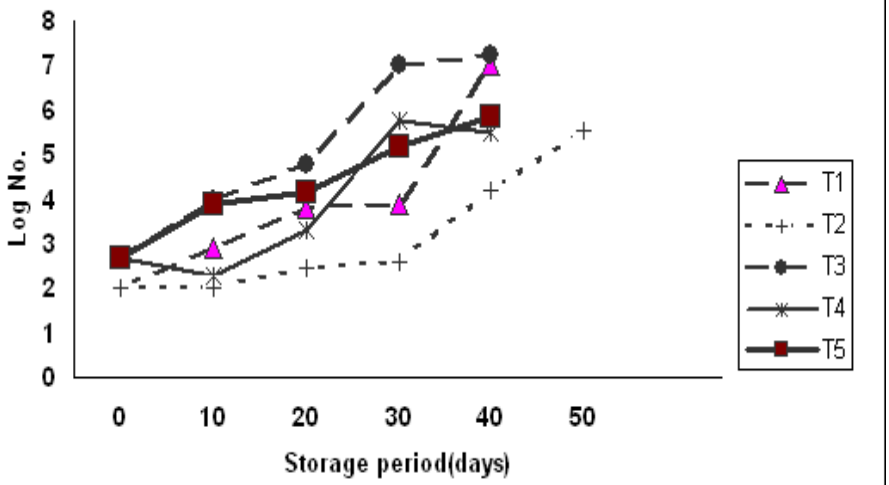
Storage period (days)	T_1	T_2	T_3	T_4	T_5
Fresh	10 x 10^1	10 x 10^1	5 x 10^2	5 x 10^2	5 x 10^2
10	8 x 10^2	10 x 10^1	10 x 10^3	2 x 10^2	8 x 10^3
20	6 x 10^3	28 x 10^1	6 x 10^4	2 x 10^3	15 x 10^3
30	8 x 10^3	40 x 10^1	*10 x 10^6	60 x 10^4	15 x 10^4
40	*10 x 10^6	16 x 10^3	18 x 10^6	30 x 10^4	*7 x 10^5
50 days		*36 x 10^4			

*** off flavour was easily detected by panelists.**
LSD for log No. was 1.1567.
T_1 : control cheese without yeast inoculation or nitrogen gas exposure.
T_2: control cheese without yeast inoculation but with gas exposure.
T_3: Cheese samples with added yeast inoculum.
T_4: Cheese samples with added yeast inoculum + nitrogen gas exposure.
T_5: Cheese sample with added yeast inoculum + nitrogen gas exposure and ultraviolet treatment.

Fig(1): yeast count in Cheese samples as affected by modified atmosphere packaging during the storage at room temperature



Fig(2): yeast count in Cheese samples as affected by modified atmosphere packaging during the storage at refrigerator temperatures



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إطالة مدة حفظ الجبن الأبيض الطري المنخفض الملح بالتعبئة تحت ظروف معدلة

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يهدف هذا البحث إلى دراسة تأثير الاستبدال الجزئي للهواء بغاز النيتروجين في عبوات تخزين الجبن الأبيض المنخفض الملح على مدة حفظ هذا الجبن وقدرة الخمائر على النمو تحت هذه الظروف. فقد تم تصنيع جبن أبيض بطريقة الترشيح الفوقي Ultra filtration، وتم إضافة معلق الخميرة أثناء التصنيع إلى المركز الناتج من الترشيح للوصول بعدد الخلايا إلى ٥٠٠ خلية/جم، وتم تعبئة الجبن في أكياس بلاستيك مع عمل استبدال جزئي للهواء بغاز النيتروجين داخل بعض الأكياس. وفي بعض العينات المعاملة بالنيتروجين تم إمرار الأكياس البلاستيك تحت لمبة أشعة فوق بنفسجية لمدة ٣٠ ثانية من على بعد ١٥ سم. وتم تقسيم العينات إلى مجموعتين، حيث خزنت إحداها على درجة حرارة الغرفة مع متابعتها بالتحكيم الحسي وعد خلايا الخميرة كل ٥ أيام، وتم تخزين الأخرى على درجة حرارة الثلاجة، وتم متابعتها كل ١٠ أيام. وقد أظهرت النتائج المتحصل عليها أن إحلال غاز النيتروجين محل الهواء في أكياس تعبئة الجبن قد أدى إلى تأخير نمو الخميرة متمثلاً في الأعداد المنخفضة بالمقارنة بعينات الكنترول التي لم يستبدل فيها الهواء بالنيتروجين وكذلك تأخر ظهور عيب الرائحة، مما أدى إلى زيادة مدة حفظ الجبن سواء على حرارة الغرفة أو حرارة الثلاجة، ولم يكن هناك تأثير إيجابي لمعاملة أكياس الجبن بالأشعة فوق البنفسجية حيث لم يحدث أي تحسن مقارنة بالعينات المعاملة باستبدال الغاز فقط. وعليه، يمكن القول أن المعاملة بالنيتروجين ولو أنها لم تمنع الفساد الميكروبيولوجي إلا أنها أدت إلى تأخيره نسبياً.