

PHYSICOCHEMICAL, MICROBIOLOGICAL AND SENSORY CHANGES OCCURRING IN TVAROG WRAPPED IN ECO LEAN AND VACUUM PACKED DURING REFRIGERATED STORAGE

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The objective of the research was to assess the changes in the quality and shelf life of tvarog wrapped in Eco Lean and packed either under vacuum or atmospheric air, during its storage in refrigerated conditions. The performed investigations comprised the following analyses: physicochemical (whey drainage, water and fat content, pH, titrable acidity, content of free fatty acids, colour indices), microbiological (number of acid forming bacteria and total number of microorganisms, numbers of moulds and yeasts, presence of coliform bacteria) and organoleptic (evaluation of such parameters as: consistency, colour, taste, smell and the overall acceptability). Tvarog was analysed directly after its manufacture and after every two consecutive days of its refrigerated storage during the period of 24 days (vacuum packed tvarog) and 10 days (air packed tvarog). On the basis of the results obtained, it was found that the wrapping of fresh white cheese in Eco Lean and vacuum packing, when compared with air packing, results in a considerable reduction of unfavourable physicochemical changes and sensory properties in the course of its refrigerated storage. The number of yeasts exceeded the level of 10^3 cfu/g after 24 days of the vacuum packed and after 10 days of the air packed tvarog storage. In comparison with air packing, vacuum packing allows extending by 2.7 times the shelf life of fresh white cheese stored at a temperature of $5 \pm 1^\circ\text{C}$. The vacuum packed tvarog exhibited good quality over the period of its 3-week storage in refrigerated conditions.

INTRODUCTION

Until recently, the majority of fresh white cheeses produced in the country were packed traditionally. The packaging materials employed in the traditional system included parchment paper as well as parchment laminate with aluminium foil. In recent years, beside the above-mentioned materials, a new packaging material, called Eco Lean, appeared in Poland showing interesting physical properties. The pro-ecological character of this material resulting from its composition, possibilities of reducing packaging costs as well as relatively high mechanical strength and permeability in relation to water vapour, fat and UV radiation [Czerniawski, 2003] all caused that also tvarog manufacturers became interested in this material. Regardless of the type of the above-mentioned packing materials, the shelf life of traditionally packed fresh white cheeses during refrigerated storage is relatively short. In the light of the existing regulations expected from modern packaging materials, this system of tvarog packing is insufficient from the point of view of the product protection and, in addition, it fails to meet the requirements of modern distribution [Panfil-Kuncewicz & Kuncewicz, 1996].

Vacuum packing is the earliest and simplest form of atmosphere modification on an industrial scale and has, for some time, been used to pack food articles, including such milk products as cheeses [Pikul, 2003]. The application of a packaging material characterised by appropriate perme-

ability for water vapour and gases (carbon dioxide, oxygen and nitrogen) such as, for example, PA/PE foil allows maintaining vacuum throughout the shelf life of the packed product [Humphreys, 1996]. In conditions of tight packaging, the oxygen content in the closed space drops to the level below 1%. In this method, the main factors affecting the lengthening of the shelf life of the packed product are: lack of oxygen access and low storage temperature. It is believed that vacuum packing allows maintaining the high quality of the product in its natural shelf life rather than extending this period [Subramaniam, 1998]. This is associated, primarily, with the impossibility of the total removal of oxygen from the package, even in the situation when the deep vacuum is applied. Vacuum packing with a simultaneous storage in refrigerated conditions, first and foremost, inhibits the growth of such aerobic microorganisms as: yeasts, moulds, staphylococci and streptococci from the *Enterococcus* genus. Vacuum packing inhibits the development of the aerobic and facultative microflora which causes food deterioration. However, even at reduced oxygen content, some psychrotrophic or anaerobic microorganisms can still develop. Most frequently, they are *Micrococcus* bacteria or lactic acid bacteria whose development does not result in such unfavourable sensory changes which can be observed in the case of regeneration of typical aerobic microflora causing food deterioration. Hence, vacuum packaging extends the shelf life of food products [Blakistone, 1998].

There is little information in literature on the subject concerning the evaluation of the quality and shelf life of fresh white cheese packed under vacuum [Steinka & Stankiewicz, 2001; Panfil-Kuncewicz & Kuncewicz, 2000] or packed traditionally using Eco Lean [Karczewska *et al.*, 2005]. Moreover, no data is available discussing the effect of the simultaneous packing of tvarog in Eco Lean and vacuum packing in PA/PE foils on its quality and shelf life.

That is why the main purpose of the performed investigations was to assess the quality and shelf life of fresh white cheese wrapped in the Eco Lean and packed under vacuum or air, during its storage in refrigerated conditions.

MATERIALS AND METHODS

The experimental material was acid tvarog manufactured from the extra class milk by the traditional method in a dairy plant in the region of Wielkopolska. Milk was inoculated by cultures of freeze-dried mesophilic lactic acid bacteria type XT 302 of Chr. Hansen Company containing *Lactococcus lactis ssp. lactis*, *Leuconostocs mesenteroides ssp. cremoris* and *Lactococcus lactis ssp. cremoris*. After cooling down to a temperature of $5 \pm 1^\circ\text{C}$, a block of tvarog weighing 306 ± 25 g was prepared, wrapped by hand in Eco Lean and then placed in bags of PA/PE barrier foil. One part of the samples prepared in this way was vacuum packed at 200–300 mbar vacuum, while the remaining part was packed in the air atmosphere. Tvarog packed in the way described above was stored at a temperature of $5 \pm 1^\circ\text{C}$. Experimental tvarog was analysed directly after its manufacture and then, consecutively, after every two days of storage for 24 days (vacuum packed tvarog) and for 10 days

(tvarog packed in air atmosphere). Physicochemical and microbiological measurements and assays as well as organoleptic assessment were carried out.

Using standard methods, the quantity of whey drainage, water, protein and fat content, active (pH) and titratable acidity were determined. The content of free fatty acids was determined by the method Deeth and Fitz-Gerald [1975]. Measurements of the $L^* a^* b^*$ colour indices were conducted using the Hitachi H-3000 apparatus and the white index was calculated from the data obtained. The total number of microorganisms as well as the numbers of acid forming bacteria were determined in accordance with appropriate regulations [Polish Standard, 1993a; 1977]. Total numbers of yeasts and moulds as well as presence of the coliform bacteria were all carried out according to standard methods [Polish Standard, 1993b,c]. The organoleptic assessment was carried out by a 6-person team of trained panellists. The team estimated such attributes as: consistency, colour, flavour, taste and the overall acceptability. A 10-point evaluation scale was employed by the panellists [Matuszewska *et al.*, 1998].

The Excel calculation sheet as well as the SPSS/PC+ statistical program were used to carry out a statistical analysis of the results obtained, whereas the Duncan test was employed to establish differences between mean values.

RESULTS AND DISCUSSION

The manufactured tvarog contained 72.80% water, 19.36% protein and 16.40% fat as required by appropriate standard [Polish Standard, 1991]. The highest increment of the whey drainage occurred after two days of storage of the

TABLE 1. The influence of packaging methods and refrigerated storage time on the changes of selected physicochemical properties of tvarog.

Time of storage (days)	Packaging methods	Whey drainage (%)	Water content (%)	Active acidity (pH)	Titratable acidity ($^\circ\text{SH}$)	Free fatty acids ($\mu\text{Eq/g}$)	White colour index (%)
0	vacuum	0	72.80 Aa	4.46 Aa	64.6 Aa	0.50 Aa	95.69 Aa
	air	0	72.80 Aa	4.46 Aa	64.6 Aa	0.50 Aa	95.69 Aa
2	vacuum	1.81 Ab	70.96 Ab	4.46 Aa	65.5 Aab	0.53 Aab	95.68 Aa
	air	1.36 Bb	71.41 Bb	4.46 Aa	66.2 Ab	0.60 Ab	95.65 Aab
4	vacuum	2.10 Ac	70.68 Ac	4.45 Ab	66.8 Abc	0.60 Abc	95.62 Aab
	air	1.50 Bb	71.28 Bc	4.45 Ab	68.4 Ac	0.75 Ac	95.58 Ab
6	vacuum	2.20 Ad	70.60 Ac	4.45 Ab	68.0 Acd	0.68 Acd	95.56 Abc
	air	1.68 Bc	71.09 Bd	4.43 Bc	72.4 Bd	0.96 Bd	55.39 Bc
8	vacuum	2.39 Ae	70.39 Ad	4.45 Ab	69.5 Ade	0.76 Ad	95.52 Acd
	air	1.83 Bd	70.96 Be	4.41 Bd	82.4 Be	1.38 Be	95.25 Bd
10	vacuum	2.94 Af	69.90 Ac	4.44 Ac	70.7 Aef	0.88 Ae	95.45 Ade
	air	2.07 Be	70.74 Bf	4.39 Bc	90.5 Bf	1.56 Bf	95.12 Be
12	vacuum	3.06 g	69.75 f	4.44 c	72.1 f	0.96 ef	95.40 ef
14	vacuum	3.17 h	69.65 f	4.43 d	74.6 g	1.03 f	95.36 fg
16	vacuum	3.31 i	69.49 g	4.43 d	76.8 h	1.23 g	95.29 g
18	vacuum	3.31 i	69.43 g	4.42 e	78.8 i	1.40 h	95.19 h
20	vacuum	3.49 j	69.29 h	4.42 e	82.4 j	1.57 i	95.13 hi
22	vacuum	3.68 k	68.97 i	4.41 f	84.5 k	1.70 j	95.11 hi
24	vacuum	4.04 l	68.84 j	4.41 f	85.6 l	1.77 j	95.09 i

A–B, a–l; different capital letters at average values for packaging methods and different small letters for time of refrigerated storage represent statistically significant differences at the level of $p=0.05$

tvarog. It was found that with the lengthening of the storage time, the whey drainage also increased. The increase of the whey drainage was statistically significantly higher in the vacuum packed tvarog than in the tvarog packed in the air atmosphere (Table 1). A similar increase in the amount of whey drainage from vacuum-packed tvarog during storage was also reported by [Ziółkowski *et al.*, 2003]. Changes in the amount of whey drainage depending on the way of tvarog packing exerted a decisive influence on the decrease of water content during its storage. The vacuum packed tvarog was characterised by a lower water content than the tvarog packed in the air atmosphere (Table 1). Steinka & Stankiewicz [1999] revealed that the dynamics of changes in the water content during tvarog storage was different in vacuum and non-vacuum packages. From the experiments carried out by Panfil-Kuncewicz *et al.* [2001] it is evident that the content of water in the vacuum-packed tvarog stored for 3 weeks increased.

The packaging system had a significant influence on the tvarog pH value after 6-day storage in refrigerated conditions. A considerable stabilisation of active acidity was found throughout the storage period in the vacuum-packed tvarog. In the case of tvarogs packed in the air atmosphere, pH values decreased considerably from the 4th day of storage (Table 1). It is evident from investigations carried out by other researchers that vacuum-packed fresh white cheeses did not show significant pH changes during storage in refrigerated conditions [Steinka & Stankiewicz, 1999; Ziółkowski *et al.*, 2003]. It was demonstrated in our investigations that from the 6th day on, the stored vacuum-packed tvarog was characterised by lower titrable acidity than the tvarog packed in the air atmosphere. After 24 days of storage, the vacuum-packed tvarog showed titrable acidity of

85.6°SH, while the tvarog packed in the air atmosphere reached titrable acidity of 90.5°SH after 10 days. Changes in the titrable acidity of tvarogs were also investigated by Steinka & Stankiewicz [2001]. The authors reported increases in tvarog titrable acidity from 15 to 20°SH during storage. [Panfil-Kuncewicz & Kuncewicz, 2000] reported considerably smaller increments of titrable acidities of vacuum-packed tvarog stored in refrigerated conditions.

The mean content of free fatty acids in the examined tvarogs directly after their manufacture was 0.50 $\mu\text{Eq/g}$ of the product. A statistically significantly lower content of free fatty acids was found from the 6th day of storage at 4–6°C onwards in the vacuum-packed tvarogs in comparison with samples packed in the air atmosphere (Table 1). After 24 days of storage, the amount of free fatty acids in the vacuum-packed tvarog increased by 3.5 times [Panfil-Kuncewicz *et al.*, 2001], when analysing vacuum-packed tvarog, reported a similar initial content of free fatty acids and their almost 2-fold increase during cheese storage.

Regardless of the tvarog packing method, a statistically significant decrease of the colour white index value was observed with the lengthening of the storage time. From the 6th day of storage onwards, the vacuum-packed tvarog was characterised by higher values of the colour white index than the tvarog packed in the air atmosphere (Table 1). The closer are the values of the colour white index to 100%, the closer is the tvarog colour to that of the ideal white.

Directly after the manufacture of the tvarog and its packing, the acid forming bacteria count amounted to 8.9×10^8 cfu/g, whereas the total bacteria count – 9.8×10^8 cfu/g tvarog (Table 2). These results confirm earlier observations that lactic acid bacteria introduced with the dairy starter are the dominating microorganisms in tvarog

TABLE 2. The influence of packaging methods and refrigerated storage time on the numbers of the examined microorganisms of tvarog.

Time of storage (days)	Packaging methods	Number of acid forming bacteria (cfu/g)	Number of total microorganisms (cfu/g)	Number of yeasts (cfu/g)
0	vacuum	8.90 E+08 Aa	9.80 E+08 Aa	9 Aa
	air	8.90 E+08 Aa	9.80 E+08 Aa	9 Aa
2	vacuum	7.80 E+08 Ab	8.80 E+08 Aa	31 Aa
	air	8.40 E+08 Aa	9.50 E+08 Aa	32 Aa
4	vacuum	4.20 E+08 Ac	5.30 E+08 Ac	57 Ab
	air	6.50 E+08 Bd	7.70 E+08 Bd	122 Ab
6	vacuum	9.50 E+07 Ad	1.20 E+08 Ad	131 Ac
	air	1.60 E+08 Bc	2.50 E+08 Bc	277 Bc
8	vacuum	2.60 E+07 Ac	3.40 E+07 Ae	224 Ad
	air	5.60 E+07 Bd	6.60 E+07 Bd	602 Bd
10	vacuum	8.70 E+06 Af	9.30 E+06 Af	290 Ac
	air	1.70 E+07 Be	2.40 E+07 Be	1333 Be
12	vacuum	3.40 E+06 g	4.30 E+06 g	357 f
14	vacuum	1.60 E+06 g	2.20 E+06 g	441 g
16	vacuum	8.70 E+05 g	9.80 E+05 g	558 h
18	vacuum	6.60 E+05 g	7.60 E+05 g	617 i
20	vacuum	4.30 E+05 g	5.40 E+05 g	678 j
22	vacuum	2.70 E+05 g	4.50 E+05 g	797 k
24	vacuum	1.80 E+05 g	2.90 E+05 g	1167 l

A-B, a-l; different capital letters at average values for packaging methods and different small letters for time of refrigerated storage represent statistically significant differences at the level of $p=0.05$

and their numbers in the finished product reach hundreds of millions in 1 g [Molska, 1988]. It was demonstrated that, with the lengthening of the storage time, both numbers of acid forming bacteria as well as the total bacteria count decreased. Moreover, it was found that from the 4th day of storage onwards, numbers of bacteria decreased faster in the vacuum-packed tvarog than in the cheese packed in the air atmosphere. This can probably be attributed to the smaller amount of oxygen found in tvarogs packed in vacuum than in air. The observed reduction in the numbers of acid forming bacteria could have been caused by the accumulation of metabolites which were toxic for the acid forming bacteria and by the low temperature of storage [Molska, 1988].

The initial number of yeasts in the examined tvarog did not exceed 10³ cfu/g of the product (Table 2). It was demonstrated that in comparison with air packing, the vacuum packing lowered statistically significantly the number of yeasts from the 6th day of the tvarog storage in refrigerated conditions. The results obtained confirmed the fact that yeasts can disqualify tvarog for consumption when their numbers exceed 10³ cfu/g of the product [Polish Standard, 1997]. This level was exceeded on the 24th day of storage of the vacuum-packed tvarog and on the 10th day of storage of samples packed in the air atmosphere.

The coliform bacteria were absent in 0.001 g and moulds in 1 g of tvarog. Panfil-Kuncewicz & Kuncewicz [2000], analysing the shelf life of tvarogs packed either under vacuum or CO₂ enriched atmosphere, demonstrated that both packaging systems extended the shelf life of tvarogs by inhibiting the development of moulds, yeasts and coliform bacteria. Pintado & Malcata [2000] found that vacuum packing of cheeses inhibited the development mainly of yeasts and moulds.

The results of the organoleptic evaluation showed, from the 6th day of storage onwards, a statistically significantly better consistency of the vacuum-packed than the air-packed tvarog (Table 3). With the increase of the storage time, the tvarog consistency became slightly crumbly. Similar relationships, from the 8th day of storage onwards, were found in the case of changes of colour and smell of the tvarog. The observed colour changes resulted from the loss of its uniformity on the tvarog surface. The unfavourable smell changes appeared as unclean smell. From the 6th day of storage, the taste of the vacuum-packed tvarog was better than the taste of tvarog packed in the air atmosphere. The deterioration of this parameter was associated with the development of an excessively sour taste. With the lengthening of the storage time of tvarog, the overall acceptability of the product decreased, which was associated mainly with the unfavourable flavour. The Eco Lean wrapping, irrespective of the applied system of packing, allows avoiding visible whey drainage inside the package. In addition, it makes it possible to re-wrap the tvarog again once it has been taken out of the PA/PE foil and used partially. It also protects the cheese from drying during storage in refrigerated conditions.

CONCLUSIONS

On the basis of the results obtained, it was found that the wrapping of fresh white cheese in Eco Lean and vacuum packing, when compared with packing in the air atmosphere, reduced considerably the unfavourable physico-chemical and sensory changes during its storage in refrigerated conditions. The number of yeasts exceeded the level of 10³ cfu/g of the product after 24 days of storage of the

TABLE 3. The influence of packaging methods and refrigerated storage time on sensoric attributes of tvarog.

Time of storage (days)	Packaging methods	Sensoric attributes (score scale)				
		Consistency	Colour	Smell	Taste	Overall acceptability
0	vacuum	9.1 Aa	8.3 Aa	9.2 Aa	9.0 Aa	9.0 Aa
	air	9.1 Aa	8.3 Aa	9.2 Aa	9.0 Aa	9.0 Aa
2	vacuum	8.1 Ab	8.0 Aab	8.2 Ab	8.6 Ab	7.7 Ab
	air	8.0 Ab	7.9 Aa	8.1 Ab	8.7 Ab	7.6 Ab
4	vacuum	7.7 Ac	7.7 Abc	7.7 Ac	8.1 Ac	7.2 Ac
	air	7.3 Ac	7.4 Ab	7.4 Ac	8.0 Ac	6.8 Ac
6	vacuum	7.4 Ac	7.4 Acd	7.4 Ad	8.0 Ad	6.9 Ad
	air	6.5 Bd	7.0 Ab	7.1 Ad	7.0 Bd	6.1 Bd
8	vacuum	7.1 Ae	7.1 Ade	7.3 Ae	7.4 Ae	6.6 Ac
	air	5.5 Be	5.7 Bc	6.0 Be	5.9 Be	5.1 Be
10	vacuum	6.9 Af	6.8 Ade	7.0 Af	7.0 Af	6.3 Af
	air	5.0 Bf	5.4 Bc	5.2 Bf	5.1 Bf	4.7 Bf
12	vacuum	6.6 g	6.6 ef	6.8 g	6.6 g	6.0 g
14	vacuum	6.3 h	6.2 fg	6.6 h	6.4 h	5.7 h
16	vacuum	5.9 i	6.0 fg	6.3 i	6.2 i	5.5 i
18	vacuum	5.6 j	5.8 gh	6.0 j	5.7 j	5.3 j
20	vacuum	5.3 k	5.7 gh	5.8 k	5.4 k	5.1 k
22	vacuum	5.1 l	5.7 gh	5.6 l	5.2 l	4.9 l
24	vacuum	4.9 m	5.4 h	5.3 m	4.9 m	4.7 m

A–B, a–m; different capital letters at average values for packaging methods and different small letters for time of refrigerated storage represent statistically significant differences at the level of $p=0.05$

tvarog packed in vacuum and after 10 days of storage when the cheeses was packed in the air atmosphere. Vacuum packing, when compared with packing in the air atmosphere, allowed extending by 2.7 times the shelf life of tvarog stored at a temperature of $4\pm 1^{\circ}\text{C}$. Vacuum-packed tvarog can be stored in refrigerated conditions for 3 weeks.

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ZMIANY FIZYKOCHEMICZNE, MIKROBIOLOGICZNE I SENSORYCZNE ZACHODZĄCE W TWAROGU ZAWINIĘTYM W ECO LEAN I ZAPAKOWANYM PRÓŻNIOWO PODCZAS CHŁODNICZEGO PRZECHOWYWANIA

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Celem pracy była ocena zmian wyróżników jakości i trwałości sera twarogowego zawiniętego w Eco Lean i zapakowanego próżniowo oraz w powietrzu podczas jego przechowywania w warunkach chłodniczych. Przeprowadzono pomiary i oznaczenia fizykochemiczne (wycieku serwatki, zawartości wody i tłuszczu, pH, kwasowości miareczkowej, zawartości wolnych kwasów tłuszczowych, wyróżników barwy), mikrobiologiczne (liczby bakterii kwaszących i ogólnej liczby bakterii, liczby pleśni oraz drożdży i obecność bakterii z grupy coli) oraz ocenę organoleptyczną takich wyróżników jak: konsystencja, barwa, smak, zapach i ogólna pożyteczność. Twaróg analizowano bezpośrednio po wyprodukowaniu oraz co kolejne 2 doby podczas jego chłodniczego przechowywania przez 24 dni (twaróg pakowany próżniowo) i przez 10 dni (twaróg pakowany w powietrzu).

Na podstawie uzyskanych wyników stwierdzono, że zawinięcie twarogu w Eco Lean i zapakowanie próżniowe w porównaniu z pakowaniem w powietrzu pozwala znacznie ograniczyć niekorzystne zmiany cech fizykochemicznych i sensorycznych podczas przechowywania twarogu w warunkach chłodniczych. Liczba drożdży przekroczyła poziom 10^3 jtk/g po 24 dniach przechowywania twarogu zapakowanego próżniowo i po 10 dniach przechowywania twarogu zapakowanego w powietrzu. Pakowanie próżniowe w porównaniu z pakowaniem w powietrzu pozwala 2,7 razy wydłużyć termin przydatności do spożycia twarogu przechowywanego w temperaturze $4 \pm 1^\circ\text{C}$. Twaróg zapakowany próżniowo wykazuje dobrą jakość przez okres 3 tygodni przechowywania w warunkach chłodniczych.