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Research Article

Preservation of fish freshness by edible alginate coating and surface treatment with dry distilled rose petals extract or L-ascorbic acid

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Abstract

Edible coatings are usually used to reduce the drip loss and to inhibit the oxidative processes of muscle foods and when combined with antioxidants to extend their shelf life. The objective of the study was to determine the effect of the incorporation of dry distilled rose (*Rosa damascene* Mill.) petals extract (DDRPE) into an edible alginate coating on the freshness of paddlefish (*Polyodon spathula*). Eight fish samples stored 7 days at 0 - 4°C were tested: C - without coating, 1 - with alginate coating; 2 - with alginate coating incorporated by 2% DDRPE solution; 2 - with alginate coating incorporated by 4% DDRPE solution; 3 - with alginate coating incorporated by 2% L-ascorbic acid solution; 5 - surface treated with 2% DDRPE solution; 6 – surface treated with 4% DDRPE solution and 7 – surface treated with 2% L-ascorbic acid solution. Control sample C was analyzed on the first and seventh day of storage and there were determined the changes in pH, acid value, peroxide value, TBARS, color characteristics and microbial changes of the fish. It was found that using alginate coating with a 2% DDRPE solution preserved the freshness of the paddlefish for up to 7 days at 0 - 4°C.

Keywords

paddlefish fillets, alginate edible coating film, dry distilled rose petals extract; L-ascorbic acid, freshness

Abbreviations

AV – acid value; DDRPE – dry distilled rose (Rosa damascene Mill.) petals extract; pH – the negative log of the hydrogen ion concentration; POV – peroxide value; TBARS – 2-thiobarbituric acid reactive substances

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Introduction

Paddlefish (Pic. 1) is a fast-growing fish of the order *Acipenseriformes*. Its natural habitat is the rivers of North America.

Picture 1. Paddlefish (*Polyodon spathula*)

The European history of the paddlefish began with its introduction to Russia from the United States in 1974. Today, information on the status and trends of paddlefish aquaculture in Europe is scarce (Jarić et al. 2019). This species, as a plankton feeder, has great potential for cultivation in polyculture in various types of water bodies. Paddlefish meat is similar to sturgeon meat and contains up to 26% protein and 8% fat (Kolpanosova et al. 2011). The yield reaches 61% and the meat is suitable for processing into delicacies (Melchenkov and Kanidieva 2015). Oxidation of lipids and pigments is the main factor that reduces quality, shortens shelf life and diminishes the safety of paddlefish. Fresh fish contains relatively more polyunsaturated fatty acids which makes paddlefish lipids more unstable (Kolpanosova et al. 2011). Fish packaging is one of the most commonly used methods to limit oxidative or microbial spoilage. In recent years, are using so-called "edible coatings" (Choulitoudi et al. 2017). Interest in them has been caused by the accumulation and pollution of the environment by conventional polymer packaging. Because of their expensive and laborious decomposing processes (Khan et al. 2013). Edible coatings preserve the quality of fresh fish for a longer period. Moisture evaporation is less, oxidized changes are less pronounced and the color, taste and smell of the fish persist longer (Khan et al. 2013). Food coatings can be combined with various antimicrobial and antioxidant agents. Limiting the access of oxygen to the surface of the fish leads to a slower initiation and development of lipid and myoglobin oxidation. This slows down the fading process and keeps the fish looking natural longer (Volpe et al. 2015). The development of aerobic microflora is hindered. As a result, the shelf life of the fresh fish is extended too (Domínguez et al. 2018). In practice, three categories of food coatings are used.

The first of them has a lipid nature. Representatives of this group are beeswax, paraffin, various vegetable and animal fats. They are in the most part inedible and must be removed before consumption (Khan et al. 2013; Domínguez et al. 2018).

The second group includes coatings based on vegetable or animal proteins. Most often, collagen, gelatin, milk proteins, wheat gluten, corn protein and others are used for this purpose. Similar to polysaccharides, protein coatings have relatively high moisture permeability (Domínguez et al. 2018).

The third group is food coatings based on polysaccharides. Various types of starch, alginates, carrageenan, vegetable gums and chitosan are used for these purposes (Mehdizadeh et al. 2020). Due to their hydrophilic nature, they have limited water vapor barrier properties and have the ability to partially release moisture to the product. Alginates are obtained from brown algae of the *Phaephyceae* class. They can react with divalent calcium or magnesium cations to form a gel-forming layer on the surface of the product to which they are applied. Their main advantage is that they preserve the sensory properties of the fish as much as possible (Song et al. 2011). Alginates are combined with systemic antioxidants which enhances their conservative effect.

Recently, the attention of manufacturers and consumers has been focused on the use of natural substitutes. Thus, the use of natural antioxidant extracts can inhibit the oxidation of lipids and pigments in fresh fish (Choulitoudi et al. 2017). Despite their drawbacks food coatings represent an ecological solution to the problem of increasing polymer waste in the ecosystem (Volpe et al. 2015). The shelf life of fresh rainbow trout was extended by applying combined food coatings of carboxymethyl cellulose with thyme (*Zataria multiflora*) essential oil or grape seed extract (Raeisi et al. 2014). Such edible coatings can inhibit lipid

oxidation. It was found that after 7 days of refrigerated storage TBARS almost doubled from 4.5 to 8.0 nmol/g meat (Lou et al. 2000). Therefore, it is advisable to look for ways to prevent oxidative deterioration of lipids in fresh paddlefish. In the literature available to us there is no data on the use of combined food coatings from alginates and dry distilled rose (*Rosa damascena* Mill.) petals extract (DDRPE) in order to extend the shelf life of fresh fish. Therefore, the aim of the present study was to determine the effect of incorporating a DDRPE and L-ascorbic acid in an edible alginate coating on preservation the fresh paddlefish (*Polyodon spathula*).

Materials and Methods

Materials. The study was carried out using a five-year-old paddlefish (*Polyodon spathula* Walbaum, 1792). The fish was polyculture with carp, silver carp and European catfish on a full-scale hot water farm located in Central Bulgaria. The farm's pools are earthen with a soft muddy bottom and the average depth is about 1.3 m. After fishing the fish were placed in storage pools from which five individuals were randomly selected and immediately sent to the laboratory of the University of Food Technologies, Plovdiv for analysis. Transportation, and the electrical stunning were done according the Council Regulation (EC) No 1099/2009. The average live weight of the paddlefish was 8910 g. Immediately after collection the fish fillets were placed in plastic bags and stored for 12 hours at 0 - 4°C. For the experiment 2 controls and 7 experimental samples were prepared, packed in polymer sealed bags which were stored in a refrigerator at 0 - 4°C for a period of 7 days.

C1 - uncoated control samples, stored 1 day after death;

C7 - uncoated control samples, stored 7 days after death;

1 - experimental samples covered by alginate coating, stored 7 days after death;

2 - experimental samples covered by alginate coating after a surface treatment with 2% DDRPE extract solution, stored 7 days after death;

3 - experimental samples covered by alginate coating after a surface treatment with 4% DDRPE extract solution, stored 7 days after death;

4 - experimental samples covered by alginate coating after a surface treatment with 2% solution of L-ascorbic acid, stored 7 days after death;

5 - experimental samples, the surface of which was treated with a 2% DDRPE extract solution without an alginate coating, stored for 7 days after death;

6 - experimental samples, the surface of which was treated with a 4% DDRPE extract solution without an alginate coating, stored for 7 days after death;

7 - experimental samples, the surface of which was treated with a 2% solution of L-ascorbic acid without alginate coating, stored for 7 days after death;

For analysis of pH, AV, POV and TBARS from each sample, an average laboratory sample was prepared by grinding in a meat grinder with a cell diameter of 3 mm and subsequent homogenization until a homogeneous pasty mass was obtained. From the average sample prepared in this way, 9 repetitions of each of the studied indicators were made.

Methods. Sensory analysis of fish meat was carried out by a panel of 5 experts on the hedonistic scale with 5 points in accordance with the recommendations of Meilgaard et al. (1987).

The color characteristics (L*, a*, b*) of the muscular surface of fish fillets were determined using a Konica Minolta colorimeter, model CR-410 (Konica Minolta Holding, Inc., Ewing, NJ, USA) according to the method described by Hunt et al. (2012).

The pH value of the samples was measured by the electro potentiometric method (Korceala et al. 1986) using a Microsyst MS 2004 pH meter (Microsyst, Plovdiv, Bulgaria) equipped with a temperature sensor and a combined pH electrode Sensorex Combination Recorder S450 CD (Sensorex pH Electrode Station, Garden Grove, California, USA).

Extraction of total lipids was carried out according to the method of Bligh and Dyer in accordance with the recommendations of Güntersperger and Escher (1994).

The degree of hydrolytic rancidity of total lipids was determined by the acid value (AV). Acid value was determined by neutralization of free fatty acids with

a potassium-based solution using a phenolphthalein indicator according to the method described by Kardash and Tur'yan (2005).

The primary products of lipid peroxidation expressed as a peroxide value (POV) were determined spectrophotometrically according to the procedure of Schmendes and Holmer (1989) using a Camspec dual beam UV-VIS spectrophotometer, model M 550 (Camspec Ltd., Sawston, Cambridge, UK).

The determination of the lipid peroxidation by-products expressed by TBARS was performed according to the method of Botsoglou et al. (1994) with Camspec Dual Beam UV-VIS Spectrophotometer Model M 550 (Camspec Ltd., Sawston, Cambridge, UK).

The microbiological status of the fish samples was established by bacteriological examinations carried out in accordance with the microbiological criteria of Regulation (EC) No 1441 of 05.12.2007, in accordance with the methods set out in ISO 4833: 2003.

Statistical analysis was carried out using SAS software (SAS Institute Inc.) by the method of one-way analysis of variance (ANOVA) according to Student's test with the probability of accepting the null hypothesis ($p < 0.05$).

Results and Discussion

Sensory analysis. The comparison of the sensory analysis data of fresh paddlefish samples after 7 days storage at 0 - 4ºC shows that the highest scores were evaluated by the sensory properties of the alginate-coated fillet treated with a 2% DDRPE solution (samples 2, Fig. 1). The texture and appearance of the fillet from samples 2 were awarded with a maximum score of 5 and its smell and taste - with score above 4. For the last two indices similar scores were evaluated to the paddlefish fillets which were externally processed only with 2% and resp. 4% DDRPE solutions (samples 5 and 6 - Fig. 1).

With the lowest smell and taste scores the panel evaluated the paddlefish fillet superficially treated with 2% L-ascorbic acid solution only (sample 7) and control samples C7 (Fig. 1).

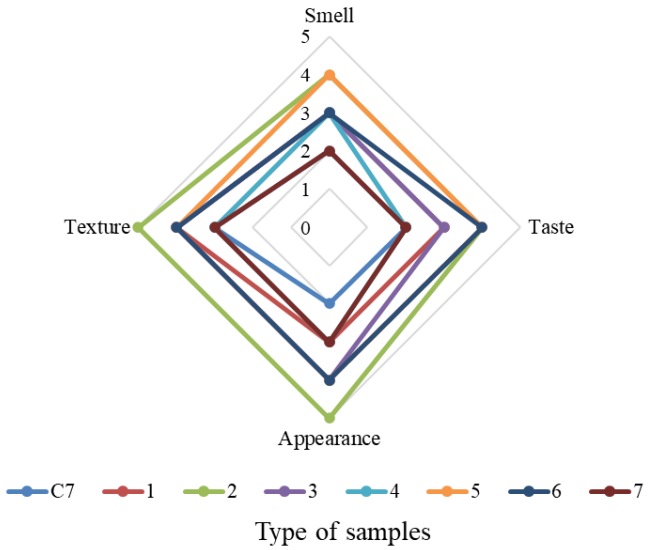

Figure 1. Scores for smell, taste, texture and appearance, awarded after sensory analysis of paddlefish fillets, after 7 days of storage at a temperature of 0 - 4°C

Color characteristics. Compared to control samples C7 the color brightness (L*) of alginate-coated paddlefish fillets remained higher for the period of seven days of refrigerated storage (samples 3, 1 and 2, respectively) (Table 1). The lowest color brightness (L*) values were found in the experimental samples of paddlefish fillets without an alginate coating (samples 7 and 6). Their L* values did not differ significantly ($p < 0.05$) in comparison with those of the control samples C1. Quite the opposite, after incorporating a 4% DDRPE solution into an edible alginate surface coating (samples 3) the color brightness (L*) value increased dramatically by 16.55%. A similar increase in color brightness (L*) value was found in paddlefish fillets superficially treated with 4% or 2% DDRPE solution only, uncoated with alginate films (experimental samples 7 and 6, Table 1).

Changes in pH value. During the refrigerated storage (7 days, 0 to 4°C) the pH value of all paddlefish fillets tested decreased by approximately 0.7 to 1.0 pH units (Fig. 2). At the end of the study period the fillets treated with a 2% solution of dry distilled rose petals extract, with and without an alginate coating (samples 2 and 5), had the lowest pH values. They are followed by the fillets treated with 4% DDRPE extract solution, both with and without an alginate coating (samples 3 and 6) (Fig. 2).

Table 1. Instrumentally recorded color characteristics (L*, a*, b*) of paddlefish fillets after 7 days of storage at 0 - 4°C

Samples	C1	C7	1	2	3	4	5	6	7
L*(C)	^{a} 49.18 ± 0.21	^{b} 51.93 ± 0.01	^{d} 55.77 ± 0.04	^{c} 53.75 ± 0.16	^{e} 57.32 ± 0.01	^{b} 51.28 ± 0.09	^{b} 51.16 ± 0.04	^{a} 49.94 ± 0.15	^{a} 49.11 ± 0.14
a*(C)	^{b} 17.47 ± 0.32	^{a} 16.42 ± 0.01	^{a} 15.30 ± 0.13	^{b} 17.24 ± 0.18	^{a} 15.20 ± 0.23	^{c} 19.33 ± 0.10	^{a} 16.12 ± 0.24	^{c} 18.13 ± 0.15	^{c} 18.40 ± 0.06
b*(C)	^{a} 6.54 ± 0.03	^{a} 8.05 ± 0.25	^{c} 10.63 ± 0.09	^{c} 10.25 ± 0.08	^{d} 11.58 ± 0.23	^{b} 9.30 ± 0.03	^{b} 9.35 ± 0.30	^{c} 10.43 ± 0.08	^{a} 7.79 ± 0.10

$^{a, b, c, d, e}$ - indices showing significant differences ($p < 0.05$) between the mean values in the rows

It has been established that the change in the pH value of the paddlefish fillet during its 7-day storage at 0 - 4°C practically does not depend on the presence of an alginate coating and a surface treatment with DDRPE or L-ascorbic acid solutions.

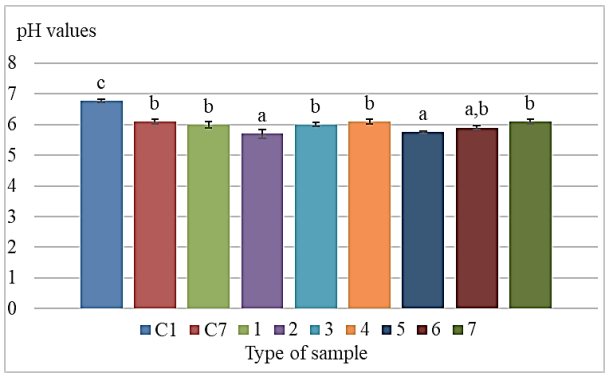

Figure 2. pH values of paddlefish fillets after 7 days of storage at 0 - 4°C

Lipolytic changes. During the 7-day storage of paddlefish fillets at 0 - 4°C, control samples C7 showed an almost two-fold increase in the amount of AV compared to control C1 samples (Fig. 3). Contrary to that, no significant ($p \geq 0.05$) differences of AV were found in experimental samples 2 (with an alginate coating and 2% DDRPE solution), experimental samples 6 (surface treated with 4% DDRPE solution without an alginate coating) and experimental samples 4 (with an alginate coating and a 2% L-ascorbic acid solution) in comparison with control samples C1. In experimental samples 7, 3, 1 and 5 the AV was significantly ($p < 0.05$) higher than the one in control samples C1 but approx. 2 times as low as the one in the control samples C7.

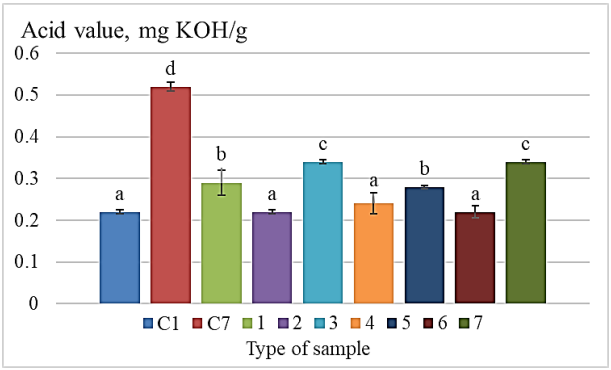

Figure 3. Acid value of total lipids of paddlefish fillet after 7 days of storage at 0 - 4°C

The results obtained allow to be concluded that the surface treatment with a 2% DDRPE solution or L-ascorbic acid in combination with an alginate coating or a 4% DDRPE solution only can be successfully applied for effective inhibition of the lipolytic changes of the paddlefish fillet.

Changes caused by the initiation and progression of lipid oxidation. During a seven-day period of storage of paddlefish fillets at 0 - 4°C the peroxide value of two control samples C1 and C7 and all seven experimental samples changed within a relatively small range. The most pronounced change by 0.21 μeq O_2/g was found in experimental samples 2 with an alginate coating and a 2% DDRPE solution and in experimental samples 3

with an alginate coating and a 4% DDRPE solution. The differences between them were not significant ($p \geq 0.05$) (Fig. 4). Therefore, after 7 days of storage of the paddlefish fillets at 0 - 4°C the surface treatment with alginate coatings and/or antioxidant solutions (dry distilled rose petals extract or L-ascorbic acid) does not have a significant effect on the lipid oxidation processes initiation expressed by the formation, accumulation and transformation of lipid hydroperoxide radicals studied by the POV.

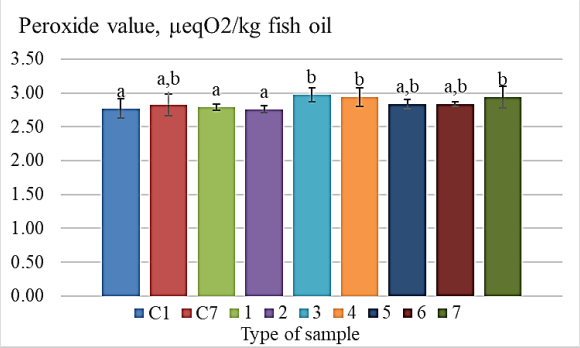

Figure 4. Peroxide value (POV) of total lipids of paddlefish fillets after 7 days of storage at 0 - 4ºC

It is likely that the shelf life is very short or that some of the primary lipid oxidation products formed break down very rapidly into lower molecular weight by-products. A similar assumption is confirmed by our data on changes in the levels of the secondary products of lipid oxidation expressed by TBARS (Fig. 5). In contrast to our data, Choulitoudi et al. (2017) studying the effect of a carboxymethyl cellulose coating with the addition of rosemary extract 200 - 800 ppm on smoked eel fillet found some effective inhibition of both primary and secondary products of lipid oxidation. Compared to the first day of the experiment (control sample C1) after 7 days of storage of paddlefish fillets at 0 - 4°C (control sample C7) TBARS increased by 8.6 times ($p < 0.05$) and reached values up to 3.96 mg MDA/kg (Fig. 5). It was found that the application of surface coatings of alginates and/or antioxidant solutions with dry distilled rose petals extract or L-ascorbic acid is an adequate technological approach to significantly limit the development and formation of the secondary products of lipid oxidation.

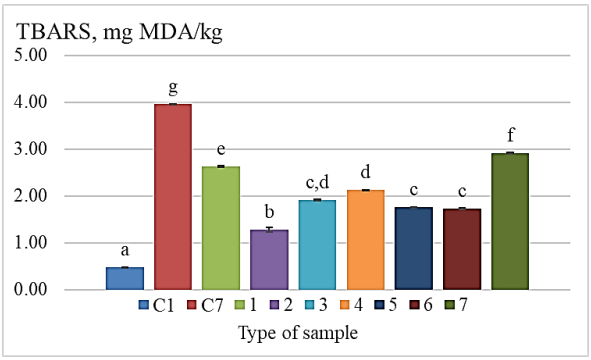

Figure 5. TBARS of total lipids of paddlefish fillets after 7 days of storage at 0 - 4°C

In this regard, the smallest increase in TBARS was found in experimental samples 2 (alginate coated and treated with a 2% DDRPE solution). In these samples TBARS increased by 2.78 times ($p < 0.05$) and reached values up to 1.28 mg MDA/kg.

Similar of our results were obtained by Mehdizadeh et al. (2020) who studied the effects of chitosan film and starch combined with a pomegranate peel extract and a thymus (*Thymus kotschyanus*) essential oil for a period of 21 days of chilled beef storage. Significant inhibition of lipid oxidation and better sensory characteristics were found in beef coated with a film of 1% chitosan starch, a pomegranate extract and a 2% thymus essential oil.

Our results are similar to those determined by Song et al. (2011) who found the coating of alginates, vitamin C and natural polyphenols successfully inhibit secondary oxidative changes in the lipid fraction of chilled bream after 21 days of refrigeration. A similar effect was reported by Volpe et al. (2015) using carrageenan coating with a lemon essential oil on rainbow trout (*Oncorhynchus mykiss*) fillets for 15 days when refrigerated at 4 ± 1°C).

Microbiological analysis. It was found that during the 7 days of storage at 0 - 4ºC of paddlefish fillets control samples (C1 and C7) the six microbiological parameters studied showed a significant increase (Table 2). The edible coating of paddlefish fillets with alginates alone (experimental samples 1) or in combination with surface treatment with antioxidant solutions (experimental samples 2, 4 and 3) demonstrates a certain bacteriostatic effect. It

was established that the use of a combination of an alginate coating and a surface treatment of paddlefish fillets with a 2% DDRPE solution (experimental samples 2) most effectively inhibited the development of mesophilic aerobic microorganisms. Representatives of the genus *Enterobacteriaceae* and coliforms as well as *E. coli* bacteria are not even detected (Table 2). This combination ranked second compared to the other experimental samples in terms of suppression of the development of the total count of psychrophilic bacteria, moulds and yeasts (Table 2). The total count of psychrophilic bacteria was found to be the lowest in sample 4 (paddlefish fillet coated with alginate in combination with a surface treatment with a 2% L-ascorbic acid solution) and the moulds and yeasts count was lowest in experimental samples 6 (the surface of the paddlefish fillet was treated with a 4% DDRPE solution without an alginate coating). Similar results were obtained by Mehdizadeh et al. (2020) when studying the effect of a chitosan and starch film in combination with a pomegranate peel extract and a thymus (*Thymus kotschyanus*) essential oil during a 21-day storage of chilled beef and found inhibition of the development of *L. monocytogenes* in the samples with 1% a chitosan starch and a pomegranate extract as well as in the samples coated with a pomegranate extract and a thymus essential oil only.

Results similar to ours in suppressing the growth of microorganisms after 15 days of storage at 4 ± 1°C of rainbow trout (*Oncorhynchus mykiss*) fillets coated with carrageenan with addition of a lemon essential oil were found by Volpe et al. (2015) as well.

Conclusions

The application of an alginate coating in combination with surface treatment with a 2% DDRPE solution has led to the conclusion that it is the most suitable one. The combination preserved the sensory properties and the red color component (a*) for the time of storage. Also inhibits the development of lipolytic and lipid oxidation processes and the microbial growth after a 7-day storage of paddlefish fillets at 0 - 4°C.

Table 2. Changes in microflora in paddlefish fillets after 7 days of storage at 0 - 4ºC

Samples	C1	C7	1	2	3	4	5	6	7
Total microbial count, cfu/g	9.0×10^4	5.0×10^7	3.6×10^7	8.7×10^6	4.0×10^7	5.7×10^7	2.4×10^7	1.9×10^7	1.4×10^7
Coliforms, cfu/g	3.0×10^3	3.3×10^7	6.0×10^6	1.5×10^6	3.5×10^7	3.0×10^7	1.0×10^7	4.1×10^7	3.1×10^7
Enterobacteriaceae, cfu/g	6.0×10^2	5.0×10^5	4.1×10^5	9.3×10^4	1.1×10^6	1.5×10^6	5.8×10^5	7.2×10^5	2.1×10^6
Escherichia coli, cfu/g	2.5×10^2	4.0×10^5	2.5×10^4	-	1.6×10^5	7.0×10^4	9.5×10^4	1.9×10^5	1.1×10^6
Psychrotrophic bacteria, cfu/g	9.2×10^3	8.0×10^8	3.0×10^7	2.0×10^7	2.0×10^7	1.0×10^7	9.0×10^8	1.0×10^8	1.0×10^8
Yeast and mould, cfu/g	-	8.4×10^4	5.2×10^4	2.0×10^4	2.5×10^4	5.4×10^4	5.5×10^3	1.4×10^4	8.7×10^4

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