Use of chitosan coating in increasing the shelf life of liquid smoked Nile tilapia (*Oreochromis niloticus*) fillet

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Abstract The aim of this research was to evaluate the efficiency of liquid smoking and chitosan coating on the shelf life of Nile tilapia (*Oreochromis niloticus*) fillets. Fillets without liquid smoked and chitosan coating (control), liquid smoked fillets (LS), and liquid smoked and chitosan coated fillets (LSCh) were stored at 4 ± 1 °C for 30 days. The physicochemical (pH, moisture content, water activity— a_w , color, texture, total volatile bases nitrogen—TVB-N and thiobarbituric acid reactive substances—TBARS) and microbiological analyses (mesophilic and psychrotrophic counts) and the electrophoresis profile of samples were carried out. Physicochemical parameters, such as TVB-N and TBARS, were reduced in the tilapia fillets with liquid smoking. The presence of the coating of chitosan was effective for the control of the microorganisms during storage. This work showed that the addition of a chitosan coating in liquid-smoked fillets further enhanced the effect of preservation.

Keywords Polyssacharide · Packaging · Coating · Chitosan · Antimicrobial · Liquid smoking · *Oreochromis niloticus*

Introduction

Smoking is a method of food preservation that uses three main parameters: salting, temperature and smoke. The preservation of food is guaranteed by the antioxidant and antimicrobial properties of molecules (Cornu et al. 2006) such as phenolic compounds generated by the combustion combined with the temperature and the conditions of smoking, which can reduce microbiological growth and oxidation (Kjällstrand and Petersson 2001). Smoking is a preeminent process nowadays due to the flavoring and the typical organoleptic qualities it confers to the smoked food (Varlet et al. 2007).

The liquid smoking is a method that uses the components of the smoke in the form of liquid extract; it provides several benefits such as the elimination of carcinogenic compounds formed during combustion. When liquid smoking is used as an alternative to the traditional generated wood smoke, this is normally injected, sometimes combined with brine, to achieve smooth and even coloring of the product (Løvdaal 2015). Liquid smoking is traditionally applied to meat, fish and poultry and has also been used to impart flavor to non-meat items such as cheese, tofu and even pet food (Lingbeck et al. 2014).

Nile tilapia (*Oreochromis niloticus*) is one of the most widely cultivated species of fish in the world. According to Food and Agriculture Organization of the United Nations, the global aquaculture production of *O. niloticus* was 3,436,526 tons (FAO 2013). Fish is an important source of high-quality proteins for humans; however, it is highly

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susceptible to both microbiological and chemical deterioration, due to its high water activity, neutral pH, relatively large quantities of free amino acids, and presence of autolytic enzymes (Jeyasekaran et al. 2006).

Chitosan is a polysaccharide obtained from the alkaline hydrolysis of N-acetyl group of chitin, the main component of the crustacean shells. Chitosan has been reported to have a number of functional properties that make it technically and physiologically useful in nutrition (Gallaher et al. 2002; Shahidi et al. 1999). The use of edible coatings could have a beneficial effect on the preservation of seafood products, since they function as a barrier against moisture and oxygen penetration (Pereira de Abreu et al. 2012). Chitosan is a well-known film-forming biopolymer with strong antimicrobial and antifungal activities (Duan et al. 2010), which has been widely applied to the preservation of seafood products (Duan et al. 2010; Fan et al. 2009; Li et al. 2013; Ojagh et al. 2010).

The aim of this research was to evaluate the efficiency of liquid smoking and chitosan edible coating in enhancing the shelf life of Nile tilapia fillets. The physicochemical and microbiological characterization were evaluated in the fillet samples subjected to the liquid smoking process at 50 °C and to liquid smoked fillets with chitosan coating. The ability of liquid smoking and chitosan to inhibit lipid oxidation was also studied.

Materials and methods

Liquid smoking of fillets

Nile tilapia fillets (142.50 ± 5.59 g) were obtained from a local fish industry of the State of Ceará/Brazil and transported in iceboxes to the Fish Technology Laboratory of the Department of Fisheries Engineering/UFC.

The liquid smoking process was performed initially by individually immersing the fillets in a solution of 20% (w/v) NaCl for 10 min, and then placing them in a stainless steel sieve to drain for 1 min. After this step, the fillets were individually immersed in a solution of 20% (v/v) oil-based liquid smoke (TRIPOBET, Minas Gerais, Brazil) for 10 min and then the excess solution was removed on a stainless steel sieve. The fillets were placed in a drying oven at 50 °C for 30 min, then placed in sterile plastic bags and stored at 4 ± 1 °C. The temperature of the fillets was measured using a digital thermometer during the storage experiment with a value of 5.49 ± 0.36 °C.

Preparation of solutions and application of coating

Chitosan was obtained from shrimp heads of the species *Litopenaeus vanammei* according to the methodology

described by Cahú et al. (2012). The coating solution was prepared by dissolving 1% (w/v) chitosan in 1% (v/v) lactic acid plus 0.1% (v/v) glycerol using a magnetic stirrer for 2 h at room temperature (25 °C), and then sterilized by UV light for 15 min. The fillets were separated into three groups (n = 3) according to the treatment used: fillets without smoking coated with chitosan solution (control), liquid smoked fillets (LS) and liquid smoked fillets coated with chitosan solution (LSCh). The chitosan coating solution was applied by aspersion using a hand sprayer on control and LSCh groups, the samples were placed in stainless steel sieve at 25 °C for 1 min and then in a drying oven at 50 °C for 30 min. The samples of all groups (control, LS and LSCh) were placed in sterile plastic storage bags and stored at 4 ± 1 °C for 30 days. The physicochemical and microbiological analyses of samples were carried out with intervals of 5 days.

Physicochemical analyses

Ten grams of each sample (fish muscle) were blended with 100 mL of distilled water in a blender for 30 s, and the mixture was filtered through Whatman no. 1 filter paper. The pH of the filtrate was measured using a digital pH-meter (PM 608, ANALION, São Paulo, Brazil). The moisture content was determined by weight (10 g) of fillet samples before and after the water evaporation which was performed in a conventional oven (TE-394/1, TECNAL, São Paulo, Brazil) at 105 °C for 12 h, until constant weight (IAL 2008). The moisture content was determined as follows:

$$\% \text{Moisture} = \frac{M_{\text{initial}} - M_{\text{dried}}}{M_{\text{initial}}} \times 100$$

where M_{initial} and M_{dried} are the mass of the sample before and after drying, respectively.

The determination of water activity (a_w) of the samples was performed in Water Activity Analyzer CX-2 (Aqua-Lab) at 25 °C. The color of the fillet fish samples was determined with a colorimeter (Model Chroma Meter CR-400, Konica Minolta, Ltda., Japan). L^* (brightness), a^* (+a, red; −a, green) and b^* (+b, yellow; −b, blue) values of each sample were evaluated by reflectance measurements.

Texture

The texture (surface breaking force) of the sample of tilapia fillets was assessed using a Warner–Bratzler cell at a speed of 2 mm/s to cut through the sample with trigger force set at 0.2 kg m s^{-2} in a Texture Analyzer (TA-XT plus, Texture Technologies Corp., Hamilton, MA, USA). The shear force required to cut the samples is given in kgf. The

following parameter were obtained from force–time curve, using specific software package delivered with the experimental device.

Total volatile base nitrogen (TVB-N)

The analysis of TVB-N was performed by homogenizing 100 g of fillet fish sample with 200 mL of 7.5% (v/v) trichloroacetic acid (TCA) solution. The homogenate was filtered through Whatman no. 1 filter paper. TVB-N was measured by steam distillation of the TCA-fish extract, using the modified method of Malle and Tao (Malle and Poumeyrol 1989). The amounts of TVB-N were calculated from the volume of sulfuric acid used for titration, and the results were expressed in milligrams of nitrogen per 100 g of sample.

Determination of the TBA reactive substances (TBARS)

The lipid oxidation of samples was performed by measuring thiobarbituric acid-reactive substances (TBARS) as described by Buege and Aust (1978). A portion (3 g) of minced fish samples was homogenized with 25 mL of solution containing 0.375% thiobarbituric acid (Baker Analyzer, Austria), 15% trichloroacetic acid, and 0.25 M HCl. The mixture was heated in a boiling water bath (100 °C) for 10 min to develop a pink color, then cooled and centrifuged at 3600g at 25 °C for 20 min. The absorbance of the supernatant was measured by a spectrophotometer at 532 nm, and 1,1,3,3-tetraethoxypropane (TEP) was used as standard. TBARS was expressed as mg malonaldehyde (MDA) equivalents/kg fish (mg MDA eq/kg fish).

Microbiological analysis

25 g samples of each treatment were aseptically homogenized with 225 mL of 0.85% (w/v) NaCl solution. Serial dilutions were performed for each sample and 1 mL of each dilution was placed in petri dishes. Pour-plate method using plate count agar was applied to determine the total plate and psychrotrophic counts in the fish samples. The inoculated agar plates were incubated at 37 °C for 48 h for determining total plate counts, and at 4 °C for 10 days for psychrotrophic counts. Microbiological data were converted into logarithms of the number of colony-forming units (CFU/g).

Electrophoresis

Proteins from fillets of *O. niloticus* were analyzed by sodium dodecyl sulphate polyacrylamide gel

electrophoresis (SDS-PAGE) (Laemmli 1970) in 0.75 mm vertical gel slabs of 12.5% polyacrylamide separation gel consisting of 0.33 M Tris–HCl, pH 8.8, ammonium persulfate (100 mg/mL), tetramethylethylenediamine (TEMED) and 1% SDS buffer, and 4% polyacrylamide stacking gel consisting of 0.2 M Tris–HCl, pH 6.8, ammonium persulfate (100 mg/mL), TEMED and 4% SDS buffer, using a Mini-Protein II apparatus (Bio-Rad; Milan, Italy). Samples (0.5 mg/mL) were dissolved in 0.065 M Tris–HCl, pH 6.8, 1% SDS buffer, 0.02% bromophenol blue and 10% glycerol in the presence of β -mercaptoethanol (2%), and then incubated at 100 °C for 5 min. The electrophoresis was conducted at a constant current of 25 mA for 90 min. The protein bands were visualized by staining with 0.1% (w/v) Coomassie Brilliant Blue R-250. The molecular markers were phosphorylase b (97 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa) and α -lactalbumin (14.4 kDa).

Statistical analysis

Statistical differences within treatment groups were established by analysis of variance (ANOVA). Differences between treatments were assessed with the Tukey test. Differences were considered to be significant at $p < 0.05$. The software used was Origin 6.0 Professional.

Results and discussion

Physicochemical analyses

Figure 1 presents pH values of tilapia fillets during the 30 days of storage at 4 °C. The smoking process caused a

Fig. 1 pH evolution of tilapia fillets during cold stored at 4 °C. Different letters in the same day indicate a statistically significant difference (Tukey test, $p < 0.05$)

reduction in pH on the initial day of storage when compared with the control group. LS and LSCh presented values of 6.14 ± 0.10 and 6.18 ± 0.08 respectively, while the control pH was 6.57 ± 0.06 on day 0. This behavior can be explained by the acid compounds from the liquid smoke solution. The pH of control samples was stable until the 15th day and increased with storage time (20 days), reaching a value of 7.47 ± 0.08 at the end of 30 days storage. The LS and LSCh groups maintained low pH during the storage. The pH values were similar for the smoked fillets on the last day of storage (LS, 6.56 ± 0.04 ; LSCh, 6.60 ± 0.06). The increase in pH can be justified by bacterial growth in the fish, as discussed further. This behavior also has been related in some studies with other fish species, such as lingcod (Duan et al. 2010), sardine (Ababouch et al. 1996; Campos et al. 2005) and hake (Ruiz-Capillas and Moral 2001). The increase in pH has a pronounced effect on the quality of the product during storage, especially in terms of sensorial characteristics such as odor, color and texture, which have a negative effect on fish products (Shenderyuk and Bykowski 1989). Spoilage bacteria utilize low molecular weight compounds such as amino acids present in fish muscle and induce the accumulation of alkaline ammonia components, resulting in the increased of pH (Campos et al. 2005).

The moisture content of the samples of LS and LSCh groups showed initial a moisture content lower than the control group, and further, for LSCh group this value was slightly lower than the LS (Table 1). This was due to the smoking process, by passing through a drying stage in an oven, and for the LSCh group it was also due to the chitosan coating that protected from water loss. The control group

presented a decrease in moisture of approximately 1.5% after 30 days of storage. However, on the last day of storage (after 30 days) at 4 °C, the groups did not present significant differences in moisture content (control $74.87 \pm 0.36\%$; LS $75.52 \pm 0.84\%$; LSCh $74.10 \pm 0.27\%$).

Fish fillets present a high water activity (a_w) in the tissue, favoring the proliferation of microorganisms. The reduction of water content is a way to increase the shelf life of fish. The control samples showed initial values of a_w of 0.963 (Table 1) while the a_w values of tilapia fillets after the process of liquid smoking were reduced (LS, 0.955; LSCh, 0.951). The chitosan coating in the liquid-smoked fillets maintained the a_w during 30 days of storage. The a_w of the control was reduced after 30 days to 0.939, probably due to water loss during storage.

Some variations in the color parameters between treatments and during cold storage (Table 1) were observed. On day 0, there were no differences in L^* values between the groups. However, after 30 days of storage, LS group showed significant increase ($p < 0.05$) in L^* , though no variation was observed in a^* . Higher values of L^* indicated more luminosity, brightness and transparency and, consequently, lower darkening. The b^* values of the LS and LSCh did not change with storage time, but were higher than the control. This characteristic may be explained by the aggregation of the compounds of liquid smoke in the fillets, that gave a yellowish color to the fish.

Texture

The fish texture is an important quality characteristic and is influenced by several factors. These include fish age and

Table 1 Moisture, water activity (a_w), texture and color of tilapia fillets during cold storage at 4 °C

	Moisture (%)		a_w		Texture (kgf)	
	D0	D30	D0	D30	D0	D30
Control	76.24 ± 0.47^{Aa}	74.79 ± 0.38^{Ab}	0.963 ± 0.002^{Aa}	0.939 ± 0.004^{Ab}	0.277 ± 0.063^{Aa}	0.253 ± 0.045^{Aa}
LS	74.82 ± 0.53^{Ba}	75.52 ± 0.84^{Aa}	0.955 ± 0.002^{Ba}	0.943 ± 0.001^{Ab}	0.407 ± 0.034^{Ba}	0.400 ± 0.060^{Ba}
LSCh	73.45 ± 0.17^{Ca}	74.27 ± 0.44^{Ba}	0.951 ± 0.003^{Ba}	0.953 ± 0.001^{Ba}	0.358 ± 0.029^{Ba}	0.656 ± 0.053^{Cb}
Color						
	L^*		a^*		b^*	
	D0	D30	D0	D30	D0	D30
Control	49.94 ± 1.83^{Aa}	51.70 ± 2.15^{ABa}	-0.11 ± 0.26^{Aa}	-1.45 ± 0.58^{Ab}	0.62 ± 0.24^{Aa}	2.14 ± 0.48^{Ab}
LS	47.99 ± 2.27^{Aa}	54.55 ± 1.72^{Ab}	-0.76 ± 0.53^{ABa}	-0.43 ± 0.71^{ABa}	3.19 ± 0.72^{Ba}	3.56 ± 0.79^{Ba}
LSCh	51.88 ± 1.94^{Aa}	49.76 ± 0.98^{Ba}	-1.12 ± 0.29^{Ba}	-0.27 ± 0.52^{Bb}	2.83 ± 0.55^{Ba}	3.38 ± 0.76^{ABa}

Values followed by different capital letters in the same column are significantly different ($p < 0.05$)

Values followed by different lowercase letters in the same row are significantly different ($p < 0.05$)

D0—day 0

D30—day 30

size, fat content and distribution of muscle fat, protein profile and concentration, and processing conditions (Hultmann et al. 2004). The texture of *O. niloticus* fillets were evaluated by shear force (kgf) in the initial day (D0) and final day (D30) (Table 1). The lowest value on D0 was found in the texture of control fillets (0.277 ± 0.063 kgf), while the liquid smoked fillets presented a firmer texture of muscle fibers (LS, 0.407 ± 0.034 kgf; LSCh, 0.358 ± 0.029 kgf) and no significant differences ($p < 0.05$) were observed. The smoking process changed the microstructure and texture of the fillets, which was demonstrated by the increased shear force. The temperature applied during liquid smoking decreases the amount of water in the myofibrillar network, resulting in reduced water activity and moisture content in fish fillets. The reduction of liquid holding capacity in the muscle and the increased salt content leads to a more rigid texture. On the last day of the analysis, LSCh had a greater shear force (0.656 ± 0.053 kgf), while treatment LS and control had no change in texture. The interaction of fish proteins with chitosan forms a protection against external agents (moisture, oxygen, microorganisms) that can cause deterioration of fish, although this coating can change the muscle texture.

Total volatile base nitrogen (TVB-N)

One of the biochemical methods used to evaluate fish freshness is the total volatile basic nitrogen (TVB-N) (Saloko et al. 2014). TVB-N, a parameter that quantifies the compounds composed of ammonia and primary, secondary, and tertiary amines, is widely used as an indicator of deterioration of muscle tissues (Fan et al. 2009).

All groups showed low values on the initial day, ranging from 1.75 ± 0.09 to 1.79 ± 0.12 mg TVB-N/100 g sample (Fig. 2). The control group significantly increased the concentration of TVB-N during storage, presenting a value of 13.92 ± 0.07 mg/100 g on day 15, and 31.58 ± 1.05 mg/100 g after 25 days of storage, which exceeded the limit recommended by MAPA of 30 mg TVB-N/100 g (MAPA 1952). However, on the last day, the values of TVB-N of liquid smoked samples were 8.12 ± 1.11 mg/100 g to LS and 4.17 ± 0.83 mg/100 g to LSCh.

It was also observed that the chitosan coating in liquid smoked samples (LSCh) reduced about 51% the value of TVB-N, demonstrating a better contribution of this coating to maintaining the quality of the fish. Using whole cod fillets and different types of soluble chitosan coatings, Jeon et al. (2002) reported a reduction of 33–50% in the formation of TVB-N at the end of the 12th day of storage. Souza et al. (2010) also demonstrated the reduction of TVB-N in salmon (*Salmo salar*) fillets coated with chitosan for 18 days at 0 °C.

Fig. 2 Total volatile base nitrogen (TVB-N) of tilapia fillets stored at 4 °C. The horizontal line represents the limit for characterization of fresh fish determined by MAPA, which is 30 mg of TVB-N/100 g. Different letters in the same day indicate a statistically significant difference (Tukey test, $p < 0.05$)

Determination of the TBA reactive substances (TBARS)

The lipids present in the food can deteriorate during time due to the action of hydrolytic enzymes, the oxidation of the samples by contact with oxygen or by the presence of microorganisms. One of the widely used index to evaluate lipid oxidation is the TBARS value (Souza et al. 2010).

In Fig. 3, the initial TBARS values were higher in the control than in LS and LSCh. The liquid smoking may have removed some compounds of lipid oxidation during the process. TBARS values continued to increase and showed maximum value on day 30 (0.30 ± 0.01 mg MDA eq/kg fish). The smoked fillets (LS and LSCh) had similar values until day 15. On day 30, the value LS (0.19 ± 0.01 mg MDA eq/kg fish) was greater than LSCh (0.15 ± 0.01 mg MDA eq/kg fish). Chitosan showed an

Fig. 3 Thiobarbituric acid-reactive substances (TBARS) of tilapia fillets during cold storage at 4 °C. Different letters in the same day indicate a statistically significant difference (Tukey test, $p < 0.05$)

antioxidant effect decreasing the oxidation of lipids in tilapia fillets. The chitosan coating applied on the fish fillets acts as a barrier against the passage of oxygen from the external environment to the surface of the fish, resulting in decreased lipid oxidation and maintaining the quality of the fish fillet.

The obtained results are in agreement with published works. Jeon et al. (2002) found lower TBARS values in chitosan-coated herring and Atlantic cod samples throughout to 12 days of cold storage. Salmon coated with chitosan showed TBARS values (1.08 mg MDA/kg fish) lower than the uncoated fillets (1.76 mg MDA/kg fish) after 18 days of storage at 0 °C (Souza et al. 2010).

Microbiological analysis

Microbial activity is largely responsible for the deterioration of fish. The main factors for bacterial contamination of seafood are contamination of the raw material from the environment and from the processing, and the conditions used during storage that influence the bacterial growth (temperature, a_w , pH and microbial interactions) (Løvdaal 2015). In the natural habitat, fish can be contaminated by certain pathogens such as *Salmonella* spp., *Escherichia coli*, among other mesophilic microorganisms (Almeida Filho et al. 2002). Most bacteria in the fish microbiota are psychrotrophic (*Acinetobacter* spp., *Aeromonas* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Vibrio* spp.) (Cardoso et al. 2003). The International Commission on Microbiological Specifications for Foods establishes the maximum limit of 7.0 log CFU/g for aerobic microorganisms to fish (ICMSF 1986).

In the microbiological analysis, results showed a lower initial mesophilic count in LSCh than in LS and control groups (Fig. 4a). After 30 days of storage at 4 °C, the LS and LSCh groups showed the lowest values (4.54 and 3.61 log CFU/g respectively). Psychrotrophic counts of the control increased from 6.42 log CFU/g on day 0 to 8.05 log CFU/g on day 30. The liquid smoked fillets with and without chitosan coating also presented the lowest psychrotrophic counts on day 30 (LS, 7.45 log CFU/g; LSCh, 7.36 log CFU/g) (Fig. 4b).

The results demonstrate that liquid smoked Nile tilapia fillets have a significant reduction in mesophilic and psychrotrophic counts. Several studies have demonstrated the efficacy of the antimicrobial activity of smoking in fish fillets (Saloko et al. 2014; Løvdaal 2015), mainly due to the presence of phenols, carbonyls and organic acids (Lingbeck et al. 2014). The salt content and the decrease of a_w are also responsible for inhibiting the growth of microorganisms, however the number of microorganisms was

Fig. 4 Microbiological analysis of tilapia fillets. **a** Total plate count during cold storage at 4 °C; **b** psychrotrophic count during cold storage at 4 °C

better controlled in the presence of the chitosan coating. Jeon et al. (2002) reported that chitosan coatings resulted in 2–3 log reductions in total plate counts of herring and cod samples after 12 days of refrigerated storage. Chitosan is well known for its excellent film-forming property and broad antimicrobial activity against bacteria and fungi (Rabea et al. 2003). One possible explanation is that chitosan mainly acts on the outer surface of bacteria; the antimicrobial property of chitosan can be mediated by the interactions between the positively charged chitosan and negatively charged microbial cell membranes, which causes the leakage of cellular proteins and other intracellular constituents (Dutta et al. 2009). Chitosan also inhibits microbial growth by the chelation of nutrients and essential metals, spore components, as well as the penetration of the nuclei of the microorganisms, which causes interference with protein synthesis by binding with DNA. The chitosan coatings also act as an oxygen barrier and can inhibit the growth of aerobic bacteria (Devlieghere et al. 2004).

Electrophoresis

The electrophoretic profile of tilapia fillets on days 0 and 30 are shown in Fig. 5. A higher number of bands after 30 days of storage were observed between 30 and 45 kDa for all groups. The deterioration of the fish by microorganisms may result in the formation of polypeptide chains of low molecular weight. In addition, lysosomal catheptic enzymes are involved in the deterioration of muscle texture, and different cathepsins may act in concert to autolyze fish muscle during the storage (Ashie et al. 1996a, b). The smoked fillets (LS and LSCh) had lower intensity bands between 30 and 45 kDa, which shows a lower degradation of proteins in these samples. Lund and Nielsen (2001) studied changes in myofibrillar proteins from smoked salmon (at 28 °C) after different storage periods using SDS-PAGE, and observed that several bands appeared or increased in intensity in the 43–150 kDa range.

Conclusion

This study demonstrated the beneficial effects of liquid smoking and chitosan coating on fillets of *O. niloticus*. LS and LSCh showed less variation of physicochemical parameters when compared to the control group. A

reduction in the levels of TVB-N and TBARS was observed in liquid smoked fillets. Antimicrobial and antioxidant activities of chitosan enhanced the quality of coated fillets. The liquid smoking associated with the chitosan coating may promote greater shelf life of fish.

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Fig. 5 SDS-PAGE of tilapia fillets during cold storage at 4 °C. Lanes MM—molecular mass markers—phosphorylase b (97 kDa), albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa), α -lactalbumin (14.4); D0—day 0, D30—day 30

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