Physicochemical Evaluation of Cachama Fillets (*Piaractus brachypomus*) Preserved with Propolis during Storage

Evaluación Fisicoquímica de Filetes de Cachama (*Piaractus brachypomus*) Preservados con Propóleos durante el Almacenamiento

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Abstract. The bioactive compounds that propolis contains present diverse components that can diminish the deterioration of compounds such as fat and certain microorganisms that can affect fish fillets during refrigerated storage. The aim of this study was to evaluate the preserving capacity of ethanol extracts of propolis (EEP) in cachama fish fillets (***Piaractus brachypomus***). The treatments carried out were: (1) ethyl alcohol (96%) as the control; (2) 0.8% EEP; (3) 1.2% EEP; and (4) liquid smoke. Analyses were carried out for total volatile base nitrogen (TVBN), thiobarbituric acid reactive species-TBARS, pH and water loss for 0, 8, 16 and 24 days of storage at 4 ºC with vacuum packaging. The results presented the highest values of the TBARS and TVBN analyses for the liquid smoke treatment and the lowest values for the EEP treatments, demonstrating a significant difference between the treatments ($P<0.05$); however, the best water retention capacity was seen in the fillets treated with liquid smoke. The results for pH did not present significant differences between the treatments ($P>0.05$) during the storage period. The results suggest that EEP can preserve physicochemical characteristics during the shelf life of refrigerated, vacuum packed cachama fillets.

Key words: Biopreservation, fish, ethanol extracts, antioxidant.

Resumen. Los compuestos bioactivos contenidos en propóleos presentan diversos componentes, que pueden disminuir el deterioro de compuestos como la grasa, y la cantidad de ciertos microorganismos, que pueden afectar filetes de pescado durante el almacenamiento bajo refrigeración. El objetivo de este trabajo fue evaluar la capacidad conservante de extractos etanólicos de propóleos (EEP) sobre filetes del pescado cachama (***Piaractus brachypomus***). Los tratamientos realizados fueron: (1) alcohol etílico (96%) como control; (2) EEP 0,8%; (3) EEP 1,2% y (4) humo líquido. Fueron realizados análisis para bases volátiles totales (BVTN), especies reactivas al ácido tiobarbitúrico-TBARS, pH y pérdida de agua durante los días 0, 8, 16 y 24 de almacenamiento a 4 ˚C en empaque al vacío. Los resultados presentan los mayores valores para el análisis de TBARS y BVTN en el tratamiento con humo líquido y los menores valores para los tratamientos con EEP, mostrando diferencia significativa entre tratamientos ($P<0,05$); sin embargo, la mejor capacidad de retención de agua fue para los filetes de cachama tratados con humo líquido. El pH no presentó diferencias significativas entre tratamientos ($P>0,05$) durante el periodo de almacenamiento. Los resultados sugieren que los EEP podrían preservar las características fisicoquímicas durante el tiempo de vida útil de filetes de cachama en empaque al vacío bajo refrigeración.

Palabras clave: Bioconservación, pescado, extractos etanólicos, antioxidante.

The white cachama (*Piaractus brachypomus*), native to the Orinoquía of Colombia, is an important fish species that is cultured in ponds, tanks, and reservoirs. Due to the pleasant sensory characteristics of the meat, it can be consumed in different forms: as a whole fish or fillets. The fresh meat is susceptible to a short shelf life, with bacterial attack being the principal cause of losses. In order to avoid the deterioration of fresh meat, various preservation systems have been used, such as: antioxidant chemicals, biotechnology, microbial polymers and natural antimicrobial agents (Zhou et al., 2011). Preservative compounds contained in the liquid smoke, may offer some advantages in the shelf life of meat and fish, as well as improve the sensory characteristics, although it is not clear conservation benefits in raw products (Stołyhwo and Sikorski, 2005).

Currently, biopreservation is a system that provides security and safety for food; furthermore, it is an alternative to the use of chemical preservatives (Suárez et al., 2008). Food products are vulnerable to microbiological deterioration and alteration of sensory and physicochemical characteristics, for the latter, some chemical changes, such as the autoxidation of lipids, lower the quality of fish and agricultural products, generating undesirable odors, colors, aromas or flavors, and even lowering the

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nutritional value (Fernández *et al*., 1997; Cai *et al*., 2011). Propolis is a natural resinous product, collected by honeybees from plant secretions and used for cover and protection in hives (Kalogeropoulos *et al*., 2009). Propolis is composed of 45% resin, 30% wax and fatty acids, 10% essential oils, 5% pollen and 10% organic compounds and minerals (Mohammadzadeh *et al*., 2007).

Propolis has different bioactive constituents such as terpenoids, flavonoids, phenolic acids, steroids, sugars and amino acids which act as antioxidant, antagonistic factors against the principal bacteria and fungi (Tylkowski *et al*., 2010; Palomino *et al*., 2010). Some polyphenol compounds, derived from caffeic acid and flavonoids in particular, are known to have strong antioxidant properties. The aim of this study was to test the antioxidant and preserving characteristics of propolis in cachama fillets.

MATERIALS AND METHODS

Ethanol extract of propolis (EEP). 100 g of propolis were introduced in a precipitated glass, then 400 mL of ethanol 96% were added. The mixture was shaken during two hours, and was left at rest overnight, and then it was filtered. The residue was subjected to a secondary extraction with the same proportions as the first one. Finally the two extracts were mixed and frozen to precipitate other compounds. The supernatant eep was used to the assays and its solid concentration of 8% was established for the dry oven method. The predominant vegetation around the apiaries where the propolis were obtained consisting of, *Acacia decurrens, Dodonea viscosa, Hesperomeles godotiana, Prunus* sp., *Pirus malus, Fragaria* sp., *Toraxaxum officinalis, Sthacelp salviaefolia*.

Preparation of the fillets. Fish were provided by a commercial fish farm. Fillet samples (60 g) with skin were used. Transversal cuts were used to remove the intramuscular bones from the fillets. The following treatments were used: 1) Control, ethyl alcohol 96%; 2) EEP concentration 0.8 mg mL^{-1}; 3) EEP concentration 1.2 mg mL^{-1}; 4) Liquid smoke was purchased from a commercial smoke flavouring manufacturer (France). Liquid smoke was atomized by pressurized air in the fish fillet at ambient temperature with a vaporization device (Lutetia, France). The fillets were packed in vacuum sealed bags and stored at 4 ºC for 24 days.

Physicochemical analysis

TBA assay. Oxidation of lipids was assessed by the TBA (thiobarbituric acid) assay which is based on the reaction between TBA and MDA (malondialdehyde) and the production of a coloured pigment, the concentration of which can be calculated by measuring the absorbance at 532 nm. Some of the MDA is formed during the oxidation process; however, most of it is generated by the decomposition of lipid peroxides during the acid-heat treatment of the assay (Goulas and Kontominas, 2005).

Preparation of the thiobarbituric acid solution: 0.3 g of TBA (Merck, Germany) were transferred to a 150 mL beaker with 90 mL of distilled water. The beaker was placed in a 80 ˚C water bath until complete dissolution. The solution was then transferred to a 100 mL volumetric flask and brought to volume with distilled water.

Determination of thiobarbituric acid reactive species-TBARS. A 50 g sample of fish meat was minced after the addition of 6 mL of an ethanol solution of butylated hydroxytoluene (BHT, 1 g L^{-1}) to prevent autoxidation. A 10 g homogenized fraction was transferred to a distillation tube and a drop of silicone antifoaming agent was added (Merck, Germany), along with 2.5 mL of 4 N HCL and 97.5 mL of distilled water. This sample was distilled and the first 50 mL of the distillate were collected. Afterwards, 5 mL of the distillate were added to 0.6 mL BHT (1 g L^{-1}) and 5 mL of 0.021 M TBA in a screw-top test tube and heated in a water bath (90 ºC) for 40 min to develop a pink color. Then, absorbance was determined at 532 nm in a Jasco V-530 spectrophotometer (Japan) using a control solution that contained 5 mL of distilled water, 5 mL of the TBA solution and 0.6 mL BHT (1 g L^{-1}). The TBA values were expressed as mg of malonaldehyde (MDA)/kg sample. The MDA concentration was calculated from a calibration curve prepared using 1, 1, 3, 3-tetrametoxypropano (TMP) as a standard compound.

Determination of total volatile base nitrogen (TVBN). The method proposed by Goulas and Kontominas (2005) was used, in which a 10 g fish meat sample was ground with 50 mL of distilled water with a Moulinex™ processor (USA). This material was transferred to a 500 mL beaker with 200 mL of distilled water and distilled after the addition of 2 g of MgO and a drop of silicone antifoaming agent.

A 250 mL Erlenmeyer flask was used to collect the distillate, which contained 25 mL of a 3% boric acid solution and 3 drops of Tashiro indicator (red methyl and blue methylene mixture). 125 mL of the distillate were collected. The boric acid solution turned green when it became alkaline due to the distillate of total volatile base nitrogen (TVBN). Afterwards, the solution was assessed with a 0.1 N hydrochloric acid solution. The quantity of TVBN at mg/100 g of fish meat was calculated from the volume of hydrochloric acid (V) and its concentration (C), using the following equation:

$$TVBN, mg / 100g = \frac{(V \times C \times 14 \times 100)}{10}$$

pH. The pH was determined with a Schott potentiometer (Lab 850, USA) with a combination electrode, with direct insertion into the sample. Three readings were taken at different points.

Water loss. Water loss was determined by modifying the equation proposed by Suárez *et al.* (2008), where the fillets were weighed on the different sampling dates and related to the initial weight with the following equation:

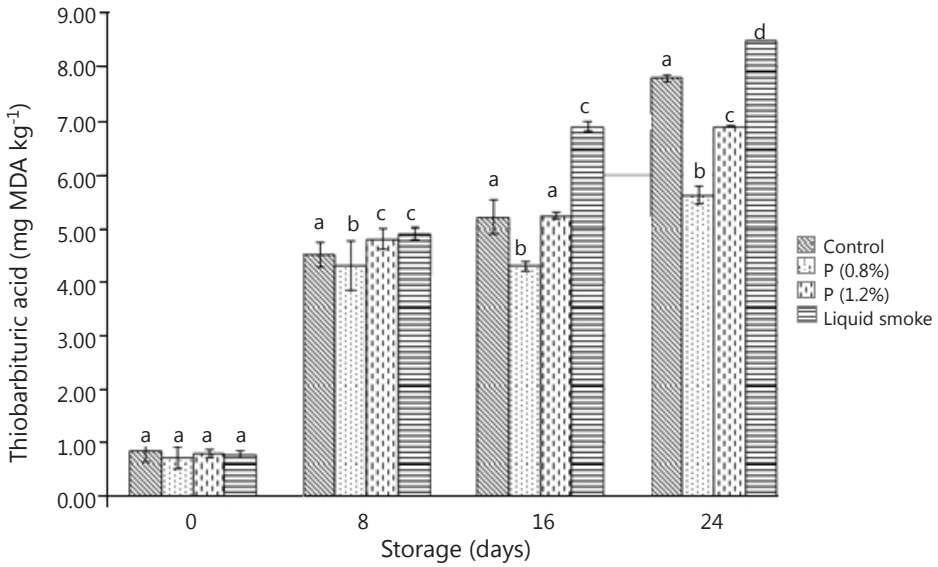
$$\text{Water loss} = \frac{\text{Initial fillet weight-Final fillet weight}}{\text{Initial fillet weight}} \times 100$$

Experimental design and analysis of the data. For the study of the preserving effects of propolis and liquid smoke in the treatments and storage time on the quality attributes of the fish fillets, a factorial design with two factors was used (time and preservation). Four levels of preservation (EEP 0.8, 1.2; liquid smoke and control) and seven levels of storage time (0, 4, 8, 12, 16, 20 and 24 days) were employed. The data were analyzed according to an ANOVA one-way, in conjunction with a Tukey multiple comparison test, with statistical significance defined as ($P \leq 0.05$). Different statistical analyses were carried out with MATLAB software V. 7.9 (Mathworks, U.S.A.).

RESULTS AND DISCUSSION

Determination of thiobarbituric acid reactive species-TBARS. Figure 1 presents the results for the thiobarbituric acid analysis (TBA). The thiobarbituric acid values increased throughout the storage time in the three treatments, with the 0.8 mg mL^{-1} of EEP treatment presenting the lowest values, followed by the 1.2 mg mL^{-1} of EEP treatment.

The TBA values indicated that the oxidation of lipids increased until 24 days, with significant differences between the treatments ($P<0.05$). The treatments with 0.8 and 1.2 mg mL^{-1} EEP demonstrated a lower



Columns belonging to the same set of data with different letters are significantly different (LSD, $P \leq 0.05$, n = 3 analyses).

Figure 1. Values obtained from determination of thiobarbituric acid reactive species-TBARS in cachama fillets treated with EEP and liquid smoke during 24 days of storage.

increase in comparison with the control and liquid smoke, showing the antioxidant activity of propolis already reported by Chaillou and Nazareno (2009). TBA analysis has been widely used to calculate the degree of oxidation of lipids and the presence of substances reactive to TBA, taking into account factors of the second stage of autoxidation where peroxides are generally produced, which are oxidized to aldehydes and ketones.

In this sense, other studies (Kalogeropoulos *et al*., 2009) have determined that the principal components responsible for the antioxidant activity of propolis from Greece and Cypres correspond to phenolic acids, flavonoids, and anthraquinones.

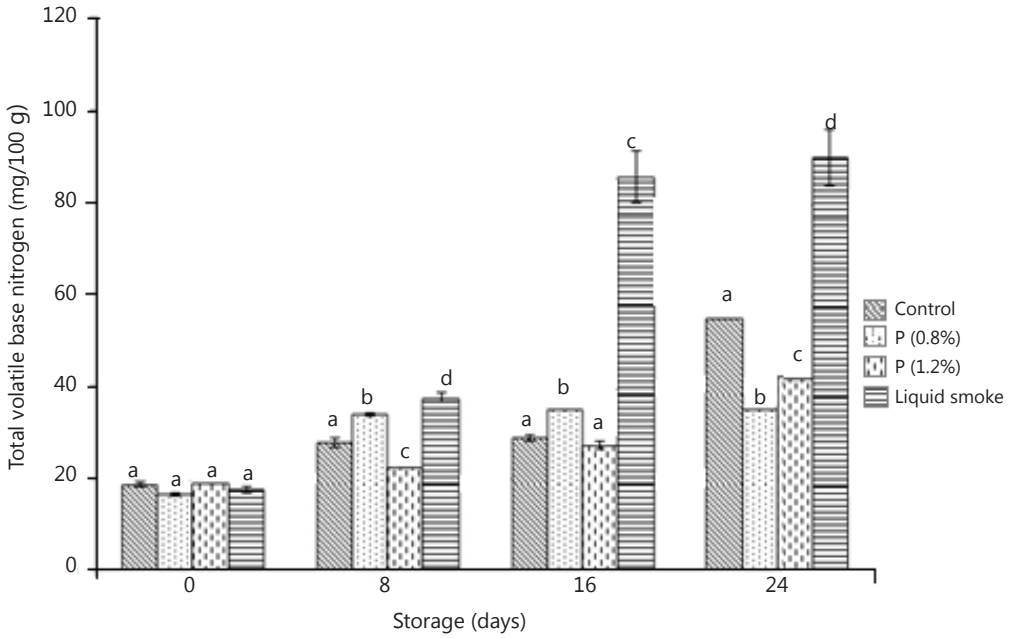
Similarly, other authors have stated that the antioxidant properties of propolis are mainly due to caffeic acid and its derivatives CAPE and DMAC. In addition, galangin, kaempferol and quercetin are compounds that are high in antioxidants. These results agree with the reports of Ahn *et al*. (2007).

On the other hand, the hydrophobic characteristics and cellular structure are responsible for the antioxidant activity of propolis, where they are located in the lipid bilayer region, without affecting the ordinate structure of the cellular membrane (Gregoris and Stevanato., 2010).

Analysis of total volatile base nitrogen TVBN. TThe TVBN values of the stored fish products indicated the formation of nitrogenated compounds produced by the activity of proteolyctic bacteria and native proteases in the fish meat, in this way, the TVBN value is one of the most used quality indices for fresh meat and refrigerated fish products (Kilincceker *et al*., 2009; Kostaki *et al*., 2009).

The changes in TVBN values for the cachama fillet samples during the storage period are seen in figure 2. All of the cachama fillet samples presented increases in TVBN levels. The highest TVBN values were seen in the liquid smoke treatment, surpassing the maximum acceptable value of 35-40 mg/100 g (Kostaki *et al*., 2009) at 6 days of storage. The lowest TVBN values were seen in the treatment with 0.8% propolis. Only the treatments with propolis maintained acceptable levels until 24 days of storage.

TVBNs mainly include nitrogen from ammonia in freshwater fish, along with trimethylamine (TMA) and dimethylamine (DMA) in ocean fish (Limbo *et al*, 2009); these values reflect the extent of the degradation of proteins and non-protein nitrogen compounds (Kilincceker *et al*., 2009). The TVBN values found in this study, after 6 and 14 days of storage for the liquid smoke treatment and the control, respectively, were



Columns belonging to the same set of data with different letters are significantly different (LSD, P ≤ 0.05, n = 3 analyses).

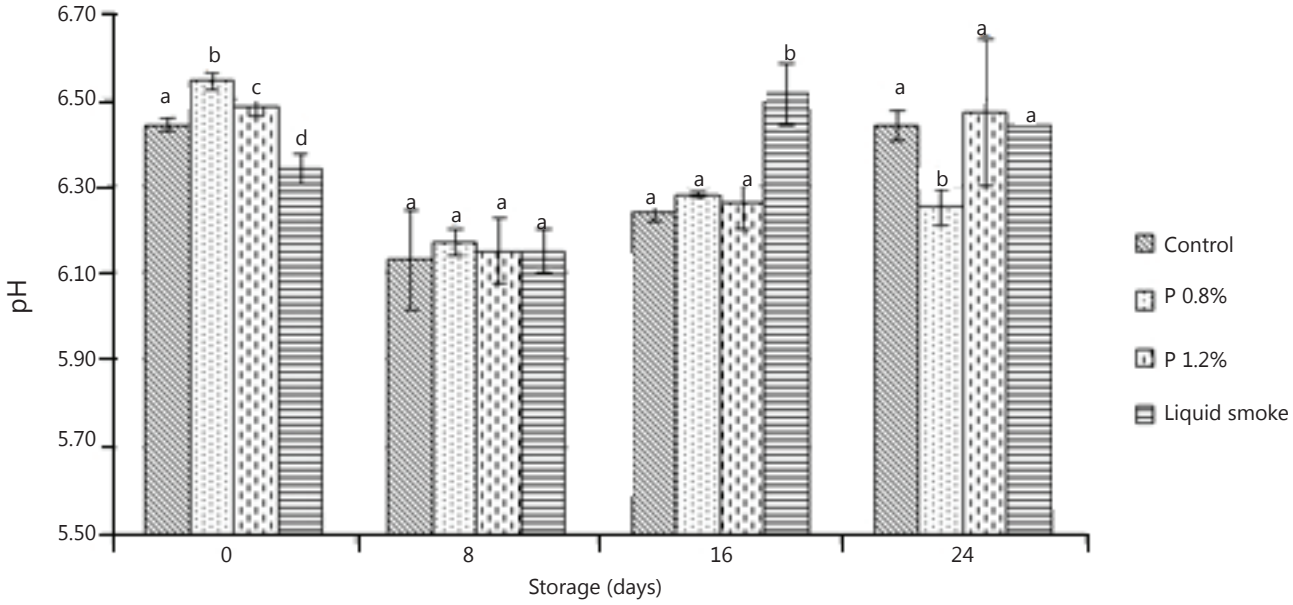
Figure 2. TVBN values in cachama fillets treated with EEP and liquid smoke during 24 days of storage.

unacceptable and were associated with unpleasant odors from the meat (Kilincceker *et al*., 2009). In the present study, the gradual increase in TVBN was accompanied by a gradual decrease in fillet quality.

As seen in Figure 2, the TVBN levels of the cachama fillets treated with propolis increased more slowly than the control and the liquid smoke, although the difference between the treatments with propolis was not significant (P>0.05). This finding could be due to the antimicrobial activity of the propolis and the reduction of the capacity of the bacteria to carry out oxidative deamination of non-protein nitrogen compounds (Fan *et al*., 2009). The ammonia compounds arising from decomposition is one of the principal ingredients responsible for the putrification of fish and has a typical fish odor (Kostaki *et al*., 2009). The results obtained for the treatment of cachama fillets treated with liquid smoke do not agree with the results obtained by Alcicek (2011). The short duration of the liquid smoke treatment could be explained by the high values generated for TVBN by proteolysis, caused by the enzymatic activity and generated microbes (Kolsarıcı and Ozkaya, 1998), since the smoking was carried out by aspiration at room temperature, without the use of thermal processes.

Determination of pH. Figure 3 presents the results of the pH analysis. During the storage period, the pH values increased, oscillating between 6.1 and 6.5. No significant difference was found between the treatments (p>0.05), however, high values were obtained for all the treatments except for the 0.8% propolis treatment. The results showed a decrease for all the treatments until 8 d of storage. Afterwards, the values were stable except for the liquid smoke treatment.

The decrease in pH until 8 d in all cases suggests the presence of lactic acid bacteria, due to the anaerobic conditions generated by the vacuum packaging producing lactic acid, which was responsible for the reduction in pH. From 16 days, the increase started



Columns belonging to the same set of data with different letters are significantly different (LSD, P ≤ 0.05, n = 3 analyses).

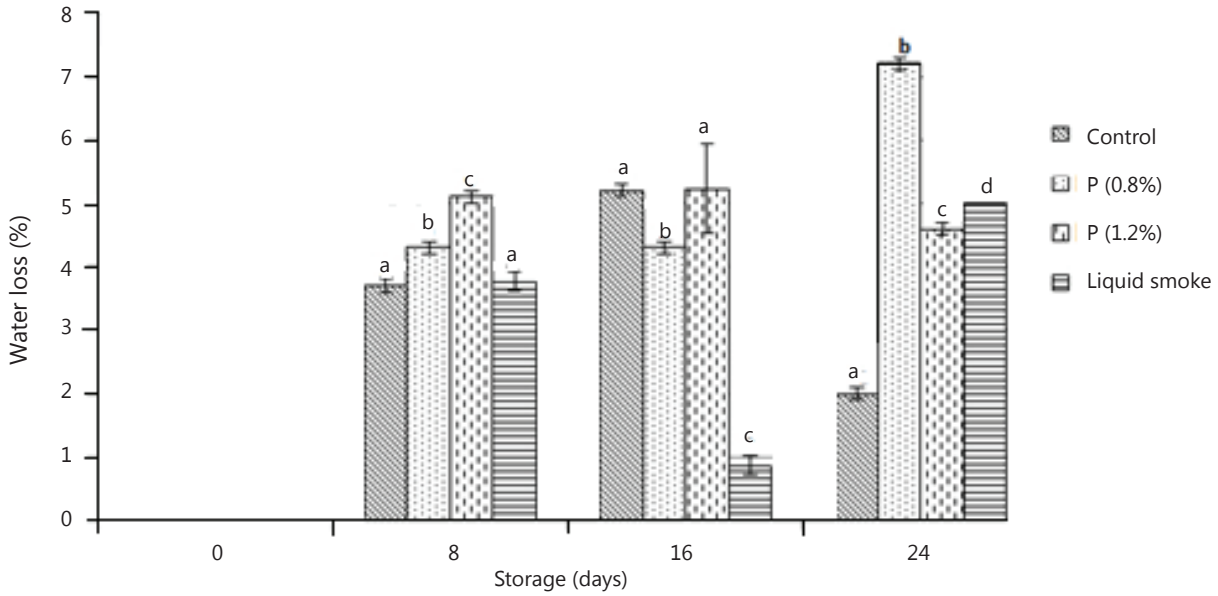
Figure 3. Values obtained in the pH analysis of cachama fillets treated with EEP and liquid smoke for 24 days of storage.

due to the enzymatic denaturation of proteins which basically increased the pH values as storage time elapsed.

Similar results have been reported for other fish (Ozogul *et al*., 2005; Ocano-Higuera *et al*., 2009). This phenomenon is presumably due to the production of basic nitrogen compounds such as ammonia during bacterial growth (Mahmoud *et al*., 2007; Fan *et al*., 2009; Ayala *et al*., 2010). Furthermore, the increase in pH of a food matrix is related to the decomposition caused by microbial activity (Mahmoud *et al*., 2006).

Water loss. The results of the water loss analysis are presented in Figure 4. Significant differences were found ($P<0.05$) during the storage period, presenting losses of 7% for the control and propolis concentrations of 0.8%, in contrast to the smaller losses for the treatments with 1.2% propolis and liquid smoke, 5% and 4.5% respectively.

The properties of fish meat can be affected by myofibrillar protein denaturation. These changes depend on the original muscle conditions (Ocano-Higuera *et al*., 2006). Fish muscle usually has a decrease in pH and water retention capacity during storage (Flores and Bermell, 1984). The best results obtained in the liquid smoke treatment could be due to the phenol



Columns belonging to the same set of data with different letters are significantly different (LSD, $P \leq 0.05$, $n = 3$ analyses).

Figure 4. Values for the water loss of cachama fillets treated with EEP and liquid smoke during 24 days of storage.

content of liquid smoke; these phenol derivatives can form dihydrogen bonds with water, which can influence the water retention capacity of meat (Martinez *et al*., 2004).

CONCLUSIONS

Ethanol extracts of propolis in concentration of 1.2 mg/ml can be an option in the conservation of fish chilled fillets as alternative to the use of chemical preservatives.

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