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Evaluation of physicochemical, functional and textural properties of rainbow trout (*Oncorhynchus mykiss*) stored at low temperatures

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The physicochemical and functional behaviour of rainbow trout stored at 4 and -20°C for nine days and six months, respectively, were investigated. The combined effect of proteolytic activity and physicochemical changes in the muscle (pH, solubility and proteolytic activity) altered the molecular structure of the proteins. SDS-PAGE showed the degradation of myosin and the formation of peptides of low molecular weight, causing an increase in solubility, which is correlated with changes in the functional properties of meat (water retention, capacity of emulsification and ability of gelling). In a gel obtained from the stored samples, the solubility had an effect on the distribution of the water gel, and significant differences (*P<0.05*) in colour and texture parameters were observed during storage. Regarding correlation analysis, during cooling, all variables studied were dependent on each other, however, in freezing, the correlation diminished in variables such as hardness, solubility and CRA. Surplus meat of trout according to the time of cold storage, can be incorporated into technological processes up to two days at a storage temperature of 4°C and three months at - 20°C, thus could contribute to the functional and textural properties of commercial meat products, generating a value liked trout producers. After this time of storage, the trout flesh can be consumed in the conventional way.

Key words: Rainbow trout, refrigeration, freezing, texture.

INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*) is a species whose production through aquaculture is growing in countries such as France, Italy, Denmark, Germany and Spain and recently in Chile, which ranks first in production for export (FAO, 2005). In Latin American countries and the Caribbean, such as Ecuador, Mexico, Cuba, Brazil and Colombia, is similar and has attained great importance due to the high nutritional and economic value it provides to some regions of these countries. However, this form of marketing is limited. So it is

important to seek alternatives to the storage of the surplus using traditional methods such as low temperature to ensure not only the preservation of the functional (gelling capacity, water holding capacity, emulsifying capacity) and textural properties of muscle of trout but also of its derivatives, such as gels produced from its myofibrillar protein, for possible use in food technological processes. Generally, the conservation of aquaculture species at low temperatures can cause changes to occur in the lipids and myofibrillar proteins of trout, and they can denature during frozen storage (Tejada et al., 2003; Michalczyk and Surówka, 2007; Chen et al., 2007), which leads to loss of integrity of the muscle, modification of the protein solubility (Mohan et al., 2006; Baron et al., 2007) and loss of texture, indicators and factors of functionality such as gellification, emulsification and water holding capacity. Additional studies on trout have demonstrated that the change in texture also resulted from the incorporation of additives (Moreno et al., 2010) and the role of endogenous proteolytic enzymes, specifically the *Lysosomal cathepsins* B, L and D, as well as cytosolic calcium-activated calpains (Godiksen et al., 2009). Both of these enzymes are important proteolytic systems in the softening of the post-mortem meat, and their action involves the breakdown of the myofibrillar structure and is directly correlated with the texture (Bahuaud et al., 2010; Chéret et al., 2007; Delbarre et al., 2006; Yamashita and Konagaya, 1991). With the loss of the protein functional characteristics, the quality of the products obtained from species stored at low temperatures could be affected. Physicochemical characteristics, for example, the colour, are related to the fat content, diet and age, have also been studied to attempt to explain the changes occurring during storage with controlled coatings (Sun et al., 2012; Choubert and Baccaunaud, 2006; Helge et al., 2006). Many studies on rainbow trout involve physicochemical characteristics and proteolytic or oxidation of lipids and proteins; this work aims to assess the effect of storage under cooling and freezing on the physicochemical and functional properties of trout fillets as well as on the textural properties of gel-type surimi obtained from the protein myofibrillar of this species.

MATERIALS AND METHODS

Preparation of the sample

Whole rainbow trout were from a farm located in the municipality of Malinalco, State of Mexico (Mexico). The general appearance of the species was good with a firm flesh texture. Twenty specimens (24 h post-mortem; taking into account the freshness of the fish such as: skin: bright iridescent pigmentation; eye: transparent cornea; gills: bright colour; flesh (cut from abdomen): bluish translucent, smooth, ahining) (Huss, 1995) of approximately 2.5 kg were eviscerated, cleaned, deboned and cut into fillets, and the mean weight were300 ± 10 g (mean ± S.D.). The fish muscle samples were placed in polyethylene food bags, each containing 3 frozen samples. The bags were vacuum packed (Vacuum Packing Machine, Stainless Steel, TVPM-400, Penang, Malaysia). Two batches of samples were divided into 2 lots: 1 was stored at 4°C, and other was stored at -20°C; the latter were stored after a rapid freezing pre-treatment dip in solid-state CO_2 for 20 s. Analysis of the initial sample was performed the day following their capture. The samples were stored at 4°C were analysed every three days for 9 days, and the samples stored at -20°C were analysed each month for 6 months.

Physicochemical and functional properties

pH

The pH measurement was performed according to Owen et al. (1982) as follows: 10 g of meat was homogenised using 90 mL distilled water for 1 min in a domestic blender. After removing the connective tissue, a digital potentiometer (Hanna instrument pH 210, Ukraine) was used to measure the pH.

Fillet water-holding capacity (WHC)

The WHC was determined on 5 g minced muscle as described by Hamm (1975). The storage samples were collected in a centrifuge tube to which 8 mL of 0.6 M NaCl was added. The samples were spun at 1,643 x *g* for 15 min in a centrifuge (5810 R Eppendorf, Hamburg, Germany). The supernatant was decanted, and the unretained volume was measured. The WHC was expressed as the volume of retained 0.6 M NaCl per 100 g of muscle.

Capacity emulsifier (EC)

The EC was measured using the method proposed by Zorba et al. (2006) after homogenisation of 25 g of minced sample with 100 mL of 0.4 M NaCl at pH 6.6 (2 to 4°C). Subsequently, 6 g of the homogenised sample was mixed with 18 mL 0.4 M NaCl at pH 6.6 for 5 s at low speed, and 30 mL of sunflower oil was added to the mixture. The oil was added at a rate of 0.9 - 1.0 mL/s with the help of a homogeniser (Proctor Silex 59735, China), until breakup of the emulsion. The EC was expressed as the average number of mL necessary to affect the change of phase.

Protease extraction

Protease extraction was performed according to the method reported by Okomato et al. (1993) with modifications; the sample was cut finely and homogenised (1:1 w/v) with phosphate buffer 25 mM, pH 7 (with 0.25 M NaCl). Extract centrifugation was performed at 1,643 x *g* for 20 min at 4°C, the supernatant, or enzyme extract (EE), was collected and stored at 4°C until use.

Extraction of acid and alkaline protease and analysis of proteolytic activity

The muscle was homogenised with a phosphate buffer solution 25

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Abbreviations: WHC, Water-holding capacity; **EC,** capacity emulsifier; **SDS PAGE,** sodium dodecylsulphate polyacrylamide gel electrophoresis; **TPA,** textural profile analysis.

mM, pH 7 (with NaCl 0.025 M) at a 1:1 ratio (w/v) and then centrifuged at 1,643 x *g* for 20 min at 4°C (5810 R Eppendorf, Hamburg, Germany). The supernatant was collected and subjected to fractionation with 20 to 60% ammonium sulfate saturation; the precipitate was resuspended in phosphate buffer 25 mM, pH 7 (with NaCl 0.025 M) and dialyzed overnight against the same buffer using a 12,000 kDa cutoff dialysis tube (Sigma-Aldrich, Steinheim, Germany) after this time, was maintained at 4ºC until the subsequent determination of proteolytic activity.

Proteolytic activity

The proteolytic activity was evaluated using the method of Kunitz (1947), with 1% casein in phosphate (20 mM, pH 7) with 0.9% NaCl as a substrate, and by the method of Anson as described in Ouali and Valin (1980), using 1% haemoglobin in a 25 mM universal buffer (phosphoric acid: boric acid: acetic acid; 1:1:1). The substrates were incubated at 35°C for 10 min in a temperature-controlled bath before addition of the enzyme extract. This mixture was then incubated for 10 min to perform hydrolysis. The reaction was halted by adding 0.25 mL of 50% trichloroacetic acid. The reaction mixture was cooled for 10 min at 4°C and centrifuged at 10,822 x *g* for 15 min at 4°C to remove the protein that was insoluble in acid. The soluble protein was determined by measuring the supernatant absorbance at 280 nm using a spectrophotometer (Espectrofotometer Thermo Spectronic 4001/4, USA). We used pH of 2.7 or 7.3 at 35°C as optimal parameters of enzymatic activity present in different aquatic and seafood species according to previous studies (Dublan-Garcia et al., 2006; Gómez-Guillén et al., 1996; Kolodziejska et al., 1994; Konno and Fukazawa, 1993; Wasson et al., 1992; Leblanc and Gill, 1982).

Residual activity was measured after incubation of enzyme extracts with substrate solution pH (2.7 or 7.3) at 35°C; 100% of enzyme activity is the activity of enzyme without storage, and the residual activity is the 100% of enzyme activity less enzyme activity measured during the different sampling intervals. One unit of enzyme activity is defined as the amount of enzyme that produces a change in optical density of 0.001 at 280 nm in 1 min under the conditions established by Yamaguchi et al. (1982) using the following equation (Kunitz, 1947; Ouali y Valin, 1980):

$$PA = (abs * dilution) / ((0.001)\ (time)\ (mL\ of\ extract)) \quad (1)$$

Protein extraction

Myofibrillar protein extraction was performed according to the method described by Ngapo et al. (1992), with modifications. Trout muscle (25 g) was homogenised with ice and cold water in a 1:2:1 ratio (w:w:v) using an Oster commercial blender (Sumbeam Mexican S.A. de C.V. 405-42, Mexico) until the tissue and water were completely integrated. This extract was mechanically shaken for 10 min in an ice bath and then passed through a sieve to remove the connective tissue.

Two volumes (200 mL) of cold water were added, and the sample was shaken once more for 15 min. The homogenate was centrifuged (Eppendorf 5810 R centrifuge, Germany) at 1,643 x *g* for 25 min at 4°C, and the precipitate was re-suspended in a phosphate buffer (25 mM, pH 7, 0.6 M NaCl). After different sampling intervals, the myofibrillar extracts were analysed using SDS PAGE.

Protein content

The protein content was obtained by applying the biuret technique reported in Gornall et al. (1949), which consisted of taking 1 mL of extracted protein and then adding 3 mL of reagent biuret (per liter final volume: 1.5 g copper sulphate x $5H_2O$ ($CuSO_4 \cdot 5H_2O$), 6.0 g sodium potassium tartrate x $4H_2O$ ($KNaC_4H_4O_6.4H_2O$) all dissolved in order in 300mL 10% NaOH before to final volume). The mixture was allowed to rest for 30 min in darkness, and the absorbance was subsequently measured at 540 nm (Espectrofotometer, Thermo Spectronic, 4001/4, USA). The reading obtained correlated to a curve pattern of seroalbumin bovine with a concentration of 0-10 mg/mL.

Solubility

The solubility was determined as proposed by Pilosof (2000). The myofibrillar protein extract at a concentration of 5 mg/mL and pH 7 was centrifuged (Eppendorf 5810 R centrifuge, Germany) at 1,207 x *g* for 20 min at 4°C. The protein content was determined in the supernatant, and the solubility was expressed as the ratio between the protein content in the supernatant and the protein content in the uncentrifuged sample, multiplied by 100. The protein content was determined by the biuret method as described by Alanís-García (1997). 3 mL of the biuret reagent was added to 1 mL of the myofibrillar protein extract. This mixture was stirred gently for a few seconds to homogenise the mixture and was left at room temperature for 30 min in the dark. The absorbance was read at 540 nm using a Beckman spectrophotometer. The results obtained were interpolated on a bovine albumin type curve with a maximum concentration of 10 mg/mL.

SDS-PAGE profile of protein molecular weights

The samples were analysed with SDS-PAGE using the procedure described by García et al. (1993). In total, 15 µL of the sample and 10 µL of the molecular weight markers (10-200 kDa range) (Promega V8491, Madison, USA) were analysed at a constant current of 200 V (Biolec, TC50, Mexico) using Mini-Protean II Cell electrophoresis equipment (Cole Palmer 2857-00, USA) at 4°C. After analysis, the gels were disassembled and stained with 0.1% Coomassie Brilliant Blue R-250 (glacial acetic acid: methanol: water) for 30 to 40 min. They were then placed in a destaining solution (glacial acetic acid: methanol: water; 10:40:50), making the necessary changes (approximately 5 times) in this solution for the protein bands to be clearly observed.

Colour

The colour was measured in the gels based on proteins of rainbow trout. The reflected colours of the gel samples were measured using a Hunter Lab colorimeter (Chroma Meter CR-200, 80025393, Tokyo, Japan) model D25-PC2 using the method described by Little (1975) in the Lab System. The instrument was calibrated using a standard white tile (L = 97.38, a = 0. 17, b = 1.94), to obtain luminosity values (L*), a* and b*; Luminosity (L) is a variable from 0 to 100; where 0 is black and 100 white. Furthermore, for a better assessment of the colour, the two coordinates (a, b) were combined to obtain the chroma (McLaren, 1980). The values of a* and b* serve to determine the hue angle (tonality) (McGuire, 1992) values:

$$\text{Hue angle } (H°ab) = Tan^{-1}\ [b^*/a^*] \quad (2)$$

$$\text{Chromaticity (Chroma)} = (a^2 + b^2)^{1/2} \quad (3)$$

Texture properties

Texture profile analysis (TPA)

The protein extract was adjusted to a concentration of 40 mg/mL and was placed in 3-cm diameter, and 4-cm high glass bottles,

according to "semi-infinite geometry" (Klettner, 1995). The tubes were immersed in a water bath and were gradually heated at 1°C/min from room temperature to 70°C. Once a temperature of 70°C was attained, the tubes were placed in an ice bath for 30 min and stored refrigerated for 12 h.

The textural profile analysis (TPA) was performed at room temperature with a texturometer (Texture Technologies Corp., Scarsdale, NY, USA) using the programme Texture Expert Exceed v2.3 (Stable Micro Systems Ltd, Surrey, England). The conditions of the analysis included: cell load: 5 Kg; type of physical evidence: % compression; speed of tube: 1 mm/s, and instrumental of test: 2.5 cm long x 1 cm diameter acrylic cylinder. Hardness (H) corresponds to the maximum force (N) required to compress the sample. Cohesiveness (A2/A1) is defined as the extent to which the sample could be deformed before rupturing, where A1 is the total energy required for the first compression, and A2 is the total energy required for the second compression. Elasticity (T2/T1) is the ability of the sample to recover its original shape after a force of deformation, where T1 is the first compression peak duration and T2 is the second compression peak duration. Finally, masticability is a parameter obtained from the product of hardness x cohesiveness x elasticity.

Statistical analysis

One-way analysis of variance (ANOVA) was performed. Pearson's correlation coefficients between the means of the parameters studied were also calculated, the correlations were presented as R^2. ANOVAs showing significant differences were followed by a Duncan multiple range tests using the package Statgraphics Plus for Windows 4.1 (Statistical Graphics Corp. 1994-1999, USA). All analysis was performed in triplicate. In all statistical analyses, significance was accepted when $P < 0.05$.

RESULTS AND DISCUSSION

Physicochemical and functional properties

The measurement of these parameters is crucial in ensuring the degree of freshness of the species. In this study, the rainbow trout exhibited a pH of 6.68, coinciding with the value reported by García et al. (2004), who reported a pH of 6.65 for fillets of this species. During storage at 4°C, a significant increase in pH was observed (*P< 0.05*) from the sixth day (Figure 2); this effect could result from the degradation of trimethylamine oxide in the muscle post mortem, as this compound is reduced to trimethylamine by bacterial or enzymatic action (Quitral et al., 2003) and is also responsible for the decarboxylation of amino acids that generates low molecular weight amines (Paczkowski and Schütz, 2011). On the other hand, significant difference was observed in the storage at -20°C, (*p< 0.05*) with a decrease in pH from the second month (Figure 2); this could be explained by changes in glycogen, lactate accumulation and production of inosinic acid phosphorylated as demonstrated in a study conducted in cod stored at 0°C (Fennema, 2000). One of the parameters which presented a good correlation with respect to pH was the WHC, which as shown in Figure 2, increased during storage at 4°C (*p< 0.05*); 67 to 107 mL NaCl 0.6 M per each 100 g of muscle. The amount of water immobilized within muscle tissue depends on how the myofibrillar actin and myosin proteins are organized. The change of the organization of the proteins in storage at 4°C is a reflection of the variation of pH and the action of proteases during the period of conservation, both favor hydrolysis by inducing increased distance between the filaments (Huff and Lonergan, 2005) and exposure of the domain of myosin by induction of depolymerization proteins and promoting the interaction of fractions of the myosin, which results in an increase of the WHC (Moreno et al., 2010). The behaviour of the WHC in storage at -20°C has the same trend during refrigeration storage for 67 to 73 mL (0.6 M NaCl per 100 g of muscle) (*P< 0.05*); however, there was no correlation with respect to pH or the capacity of water retention according to a statistical analysis of correlation at -20°C, which could result from freezing. Another type of chemical interactions, including those between protein carbonyl groups and macromolecules such as carbohydrates and lipids (Dalle et al., 2001; Bader and Grune, 2006; Baron et al., 2007), contributes to the reduction of the holding capacity of water in proportion to the status of storage.

Proteolytic activity and SDS-PAGE profile of protein molecular weights

The endogenous proteolytic activity in the enzyme extract of trout, evaluated at pH of 2.7 and 7.3 at 35°C (Table 1) indicates that there was enzyme activity in both acid proteases, similar to that occurring in neutral proteases. The effect of storage at 4 and -20°C on the proteolytic activity resulted in a significant decrease (*P<0.05*) from 100 to 78 and 81%, respectively, at pH 2.7 and 100 to 57 and 37%, respectively, at pH 7.3 with a linear behaviour with respect to time (Table 1), coinciding with reports by Michalczyk and Surówka (2007), who observed a decrease in cathepsin activity in trout stored at low temperatures. In addition, in some studies on mackerel surimi (*Scomber scombrus*) stored under refrigeration (1 to 2°C) and freezing (-2°C), the proteolytic activity decreased from the first month (Fik et al., 1988; Ming et al., 2000). The decrease of this activity could be due to several factors such as temperature or partial oxidation of thiol groups in the centre of the active enzyme (Fik et al., 1988) or their interactions with salt or sugar in the muscle itself (Reddi et al., 1972). According to the analysis of the electrophoretic profile of the proteins, we observed that although the enzyme activity decreased during storage, the enzyme activity continued during this time, as there was a decrease of the intensity of the band molecular weight 225 kDa, corresponding to myosin. In addition, a thickening and increase in intensity of the 43-kDa actin, 39.5-kDa β-tropomyosin and 37.9-kDa Troponin T bands occurred with increasing time at a storage temperature of 4°C (Figure 3), indicating that hydrolysis of the protein myosin may have occurred. According to Godikse et al.

Table 1. Proteolytic activity in stored trout fillets at 4 and -20°C and pH 2.7 and 7.3.

Sample	Period of storage	pH 2.7 (U/mL)	Residual activity (%)	pH 7.3 (U/mL)	Residual activity (%)
Day					
	0	376.45±1.61^{a}	100	343.03±0.59^{a}	100
	2	335.35±2.64^{b}	89	312.84±3.67^{b}	91
Trout at 4°C	4	305.87±2.64^{c}	81	303.74±1.93^{c}	89
	6	301.51±0.84^{cd}	80	287.99±1.43^{d}	84
	9	295.96±4.99^{d}	79	279.37±5.07^{e}	81
Month					
	0	376.45±1.61^{a}	100	343.03±0.59^{a}	100
	1	328.86±4.28^{b}	87	286.65±1.83^{b}	84
	2	287.01±4.63^{c}	76	255.96±4.70^{c}	75
Trout at -20°C	3	268.60±4.27^{d}	71	226.32±2.19^{d}	66
	4	242.08±3.19^{e}	64	185.20±4.23^{e}	54
	5	217.72±2.54^{f}	58	152.17±1.94^{f}	44
	6	215.36±0.63^{f}	57	127.41±0.56^{g}	37

Values with the different letters in the same column are statistically different at (p < 0.05). Different letters (a, b, c, d, e, f, g) indicate significant difference (p <0.05) in each treatment with storage time.

(2009), who observed that degradation of the electrophoretic profile of the proteins appeared to be similar in trout muscle extract mixed with commercial cathepsins B, L and D compared with an extract without additives. This result might suggest that the enzymes act *in situ*, followed by the hydrolysis of muscle proteins.

Solubility

The solubility in samples stored at 4°C positively correlated with the WHC and the EC; in contrast, a negative correlation was observed with luminosity, hardness and chewiness. A significant linear increase (*P<0.05*) in solubility with respect to time was observed (Table 2). For samples stored at -20°C, no correlation was observed. The solubility increased from the second month and decreased from the third (Figure 2). The change in solubility of the proteins was closely associated with the change in their secondary and tertiary structures (Michalczyk and Surówka, 2007), which were themselves affected by various factors such as pH and proteolytic action. According to Mohan et al. (2007), by moving away from the isoelectric point pH amending the electric charge of the protein, the resulting electrostatic repulsion between the molecules contributed to the solubility of the proteins, coupled with the hydrolysis of the myofibrillar proteins derived from the endogenous proteolytic activity; as a result, low molecular weight peptides, which are more apt to dissolve, are produced. Furthermore, in terms of the effect of storage at -20°C on the solubility of the myofibrillar proteins, freeze denaturation exposes the carbonyl groups of the proteins and generates aggregation reactions, such as those occurring with lipids (Dalle et al., 2001), which are compounds observed in high proportions in trout. As mentioned above, these reactions diminish the WHC and the solubility of proteins, such that these interactions generate insoluble products in saline solutions and generate non-linear solubility behaviour with respect to time (Table 2).

Capacity emulsifier (EC)

The emulsification capacity of extracted proteins (Figure 2) increased with storage time at 4°C (*P<0.05*), most likely due to dissociation of muscle protein complexes (Farouk et al., 2003), which would have allowed increasing molecular flexibility and hydrophobicity, favouring solubility. The latter plays an important role in emulsifying properties because a greater quantity of soluble protein increases the ability of emulsification stabilisation coupled to it by the effect of pH (Fennema, 2000), as it is located away from the isoelectric point of protein emulsifiers. The effect of the emulsifying capacity upon storage at -20°C decreased significantly (*P<0.05*) over time. This result could be due to the decreased pH and protein solubility (Figure 2), or, according to Ramirez et al. (2000), to the formation of intramolecular disulphide bridges resulting the conformational changes that occur during the storage period, such as the formation of disulphide, hydrogen and hydrophobic bonds, which associate with the oil added in what is known as the aggregation phenomena (Benjakul and Bauer, 2000).

Colour

At a storage temperature of 4°C, the values of light and shade (Table 3) indicate a significant difference from the

Table 2. Proteolytic activity, colour, texture and physicochemical variables compared with time of storage at 4 and -20°C.

Sample	Value	pH 2.7 (U/mL)	pH 7.3 (U/mL)	L*	H°ab	Chroma	Hardness (N)	Elasticity	Cohesiveness	Chewiness (N)	pH	Solubility	WHC	EC
	Ordinate	358.89	333.83	81.41	79.14	8.93	6.04	0.70	0.48	1.90	6.65	2.92	63.81	103.26
Trout at 4°C	Slope	-8.54	-6.77	-0.55	0.39	-0.07	-0.39	0.00	0.00	-0.12	0.02	0.73	4.33	3.78
	R^2	-0.89	-0.95	-0.95	0.56	-0.55	-0.91	-0.96	0.11	-0.98	0.92	0.98	0.97	0.87
	Ordinate	356.99	330.95	82.41	78.66	7.92	6.49	0.70	0.46	2.08	6.70	3.45	64.43	92.33
Trout at -20°C	Slope	-26.80	-35.24	-1.83	1.28	0.73	-0.16	0.00	0.00	-0.07	-0.03	0.21	1.38	-1.43
	R^2	-0.97	-1.00	-0.94	0.77	0.77	-0.77	-0.76	-0.14	-0.84	-0.96	0.57	0.87	-0.91

Table 3. Colour and texture parameters in trout gel stored at 4 and -20°C.

Sample	Period of storage	L*	H°ab	Chroma	Hardness (N)	Elasticity	Cohesiveness	Chewiness (N)
Day								
	0	81.99±0.09^{a}	80.29±0.52^{abc}	8.58±0.17^{a}	6.87±0.09^{a}	0.69±0.04^{a}	0.42±0.03^{a}	1.98±0.13^{a}
	2	80.04±0.54^{ab}	78.28±0.56^{a}	8.89±0.57^{a}	4.43±0.11^{b}	0.69±0.02^{a}	0.53±0.11^{a}	1.61±0.32^{ab}
Trout at 4°C	4	79.01±0.78^{bc}	82.53±0.70^{bc}	9.24±0.21^{a}	4.15±0.20^{b}	0.68±0.01^{a}	0.53±0.02^{a}	1.31±0.06^{b}
	6	77.27±0.09^{c}	78.81±2.63^{ab}	8.36±0.01^{a}	3.71±0.05^{c}	0.66±0.01^{a}	0.50±0.01^{a}	1.22±0.02^{bc}
	9	77.13±1.90^{c}	83.97±1.77^{c}	8.13±1.00^{a}	2.88±0.12^{d}	0.65±0.01^{a}	0.46±1.04^{a}	0.85±1.08^{c}
Month								
	0	81.99±0.09^{a}	80.29±0.52^{ab}	8.58±0.17^{ab}	6.87±0.09^{a}	0.69±0.04^{a}	0.42±0.03^{a}	1.98±0.13^{a}
	1	80.76±1.08^{a}	80.41±1.74^{ab}	9.09±0.53^{bc}	6.09±0.047^{ab}	0.70±0.02^{a}	0.50±0.03^{b}	2.16±0.36^{a}
	2	79.95±0.25^{ab}	79.80±0.21^{a}	9.49±0.03^{c}	5.88±0.33^{b}	0.68±0.01^{a}	0.48±0.01^{ab}	1.92±0.07^{a}
Trout at -20°C	3	77.80±0.84^{b}	82.37±0.89^{b}	9.18±0.26^{bc}	5.82±0.33^{b}	0.69±0.01^{a}	0.48±0.05^{ab}	1.94±0.30^{a}
	4	72.78±0.07^{c}	79.47±0.13^{a}	8.30±0.04^{a}	5.93±0.35^{ab}	0.66±0.02^{a}	0.43±0.03^{ab}	1.68±0.19^{a}
	5	71.92±0.29^{c}	87.57±0.10^{c}	12.81±0.04^{d}	6.08±0.42^{ab}	0.67±0.02^{a}	0.40±0.01^{a}	1.64±0.18^{a}
	6	73.18±1.50^{c}	87.58±0.53^{c}	13.28±0.32^{d}	5.34±0.25^{b}	0.67±0.01^{a}	0.48±0.02^{ab}	1.72±0.02^{a}

Values with the different letters in the same column are statistically different at (p < 0.05). Different letters (a, b, c, d) indicate significant difference (p <0.05) in each treatment with storage time.

second day (*P<0.05*). Because the amount of light determines the white component in the sample, when the values obtained were decreased from 82 to 77, the chromaticity did not differ.

For storage at -20°C, the values of brightness, hue and chroma indicate a difference (*P<0.05*) with respect to the values of the initial sample from the second month. The brightness decreased to 72. These variations may be attributed to the distribution of water in the sample, as water from the surface will cause the sample to appear whiter. Qiao et al. (2001) reported that according to a correlation analysis, this particular variable is an excellent value for predicting the responses of physicochemical and functional variables. In this study, this variable correlated with the luminosity of most variables upon storage at a temperature of 4°C, while at

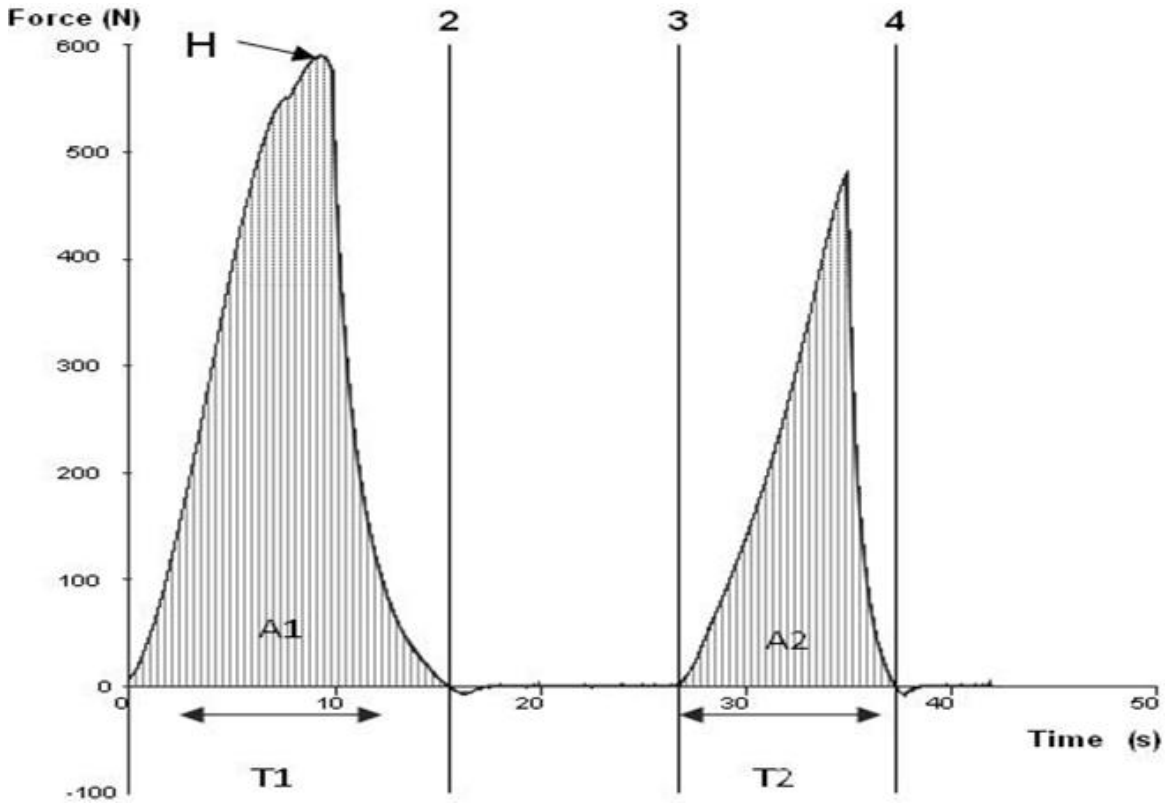

Figure 1. Texture profile analysis curve. Hardness=H; cohesiveness=A2/A1; elasticity=T2=T1; chewiness= hardness x cohesiveness x elasticity.

-20°C, the variables only correlated with pH, WHC, EC, elasticity and chewiness. The variation of the colour with respect to the key variable is expressed in degrees, where 0° = red, 90° = yellow, 180° = green and 270° = blue (McGuire, 1992). In the present study, for both storage temperatures, 78 values were obtained with respect to 88°. Notably, the values greater than 88° correspond to samples from storage at -20°C because at this temperature, other types of reactions occur, such as oxidation of lipids and interaction with the pigments that colour. According to Hunt (1980), in meat products, it is desirable to have values less than 90°; higher values indicate a poor meat. The increase is attributed to the key changes that exist in the pigments myoglobin and oxymyoglobin to metmyoglobin (Choubert and Baccaunaud, 2006). The chromaticity (chroma) is a dimensionless value that expresses the intensity of a hue (H°ab). In the present work, the values of this intensity (Table 3) are in a range that is considered tenuous, with significant differences (*P<0.05*) occurring at a storage temperature of -20°C, with higher values in months 5 and 6.

Textural properties

The TPA analysis parameters are presented in Table 3. A linear decrease was observed in the hardness (Table 2) until the end of storage time for both temperatures (*P<0.05*), indicating that the muscle becomes softer; this result could be due to endogenous enzyme activity (Figure 1), which may act on structural proteins such as myosin (Bahuaud et al., 2010) that provide firmness in meat products (Godiksen et al., 2009). A downward trend was also observed for chewiness, demonstrating a significant difference (*P<0.05*) in storage at 4°C but not for storage at -20°C; this result occurs because chewiness is a derived parameter of hardness, which decreased 22.3% upon storage at -20°C.

For samples stored at 4°C, a decrease in hardness of 58.1% occurred, indicating a direct relationship with chewiness. No significant difference was observed in cohesiveness (*P<0.05*). Upon storage at 4°C, the obtained value is near 0.5, which indicates that the gel recovered 50% of its original structure after the first compression. Upon storage at -20°C, a significant difference (*P<0.05*) was observed, taking values near 0.5.

The elasticity parameter was not changed significantly during the storage time for both temperatures, with average values of 0.7, indicating that the product has a good degree of recovery after compression. It follows that the changes in texture and functionality could be mainly

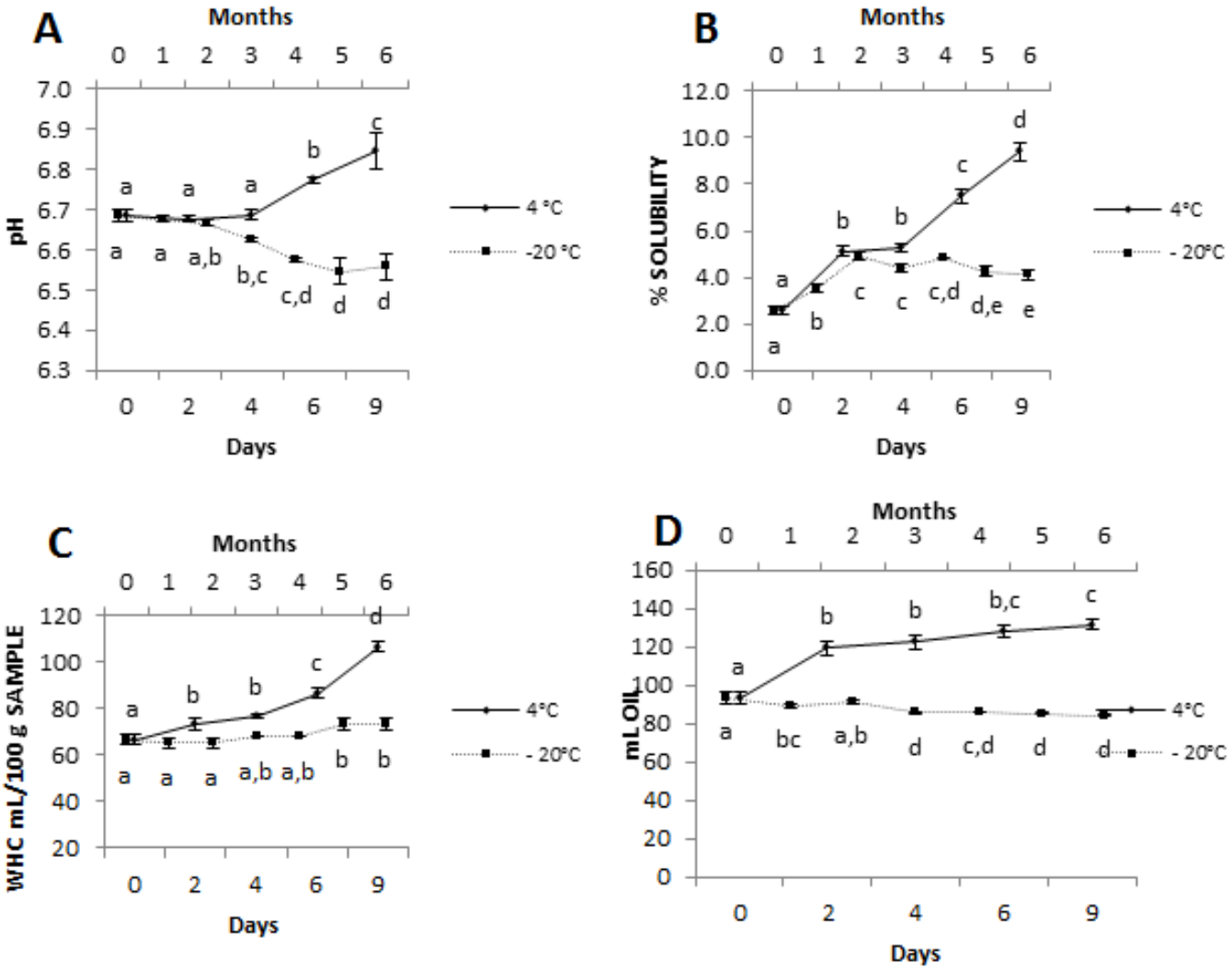

Figure 2. Physicochemical and functional properties as a function of time. A) pH, B) % solubility, C) and WHC D) emulsification capacity. Different letters (a, b, c, d, e) indicate a significant difference ($p < 0.05$) in each treatment with storage time.

attributed to changes in myofibrillar proteins due to the action of proteases; as a result, physicochemical changes changes are produced by the effect of cold storage, triggering the behaviour of the variables described in this investigation.

Conclusions

The study demonstrates that both storage temperatures significantly affected the physicochemical, functional and textural properties of beef and trout. Once the product is captured, the recommended storage is for up to six days; however, the use of this technology for processing meat stored at 4°C may be more feasible to two days and a temperature of -20°C is possible for storing in a period of six months and the technology can be used to at least three months.

After this time, it may be possible to use conventional consumption. Because during the study, some properties were observed to be maintained and others, such as the WHC and EC, were improved, one could say that the storage can be beneficial depending on the desired application that is, longer storage, which increases the EC, although altering the myosin, could be used in emulsified foods such as sausages. The functional and textural properties, such as the emulsifying capacity and gel strength, of the trout suggest its incorporation into commercial meat products.

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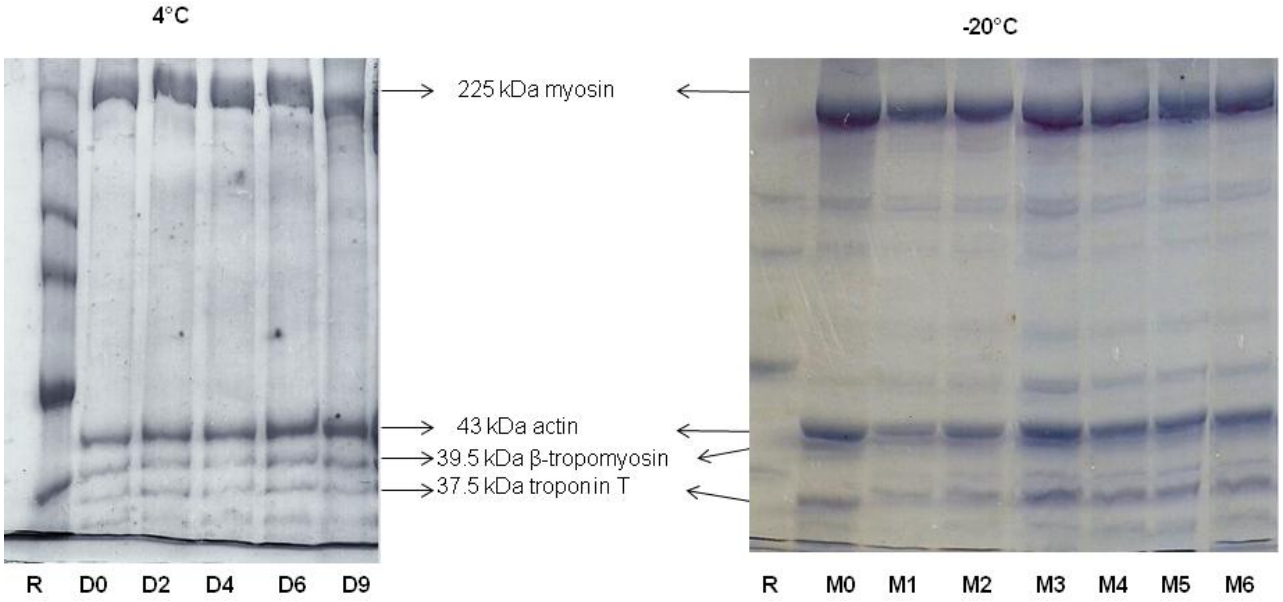

Figure 3. Electrophoretic profile extract of trout stored at 4°C and -20°C. R=reference, D= day and M= month.

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