



# PARTIAL CHARACTERIZATION OF LORICARIID CATFISH (Pterygoplichthys disjunctivus, WEBER, 1991) ROE

CARACTERIZACIÓN PARCIAL DE HUEVA DE PEZ ARMADO (Pterygoplichthys disjunctivus, WEBER, 1991)

Elva Janette Guillén-Sánchez<sup>1</sup>, Ramón Pacheco-Aguilar<sup>1</sup>, María Elena Lugo-Sánchez<sup>1</sup>, Susana María Scheuren-Acevedo<sup>1</sup>, María Gisela Carvallo-Ruiz<sup>1</sup>, Gerardo Navarro-García<sup>2</sup> and Juan Carlos Ramírez-Suarez<sup>\*1</sup>

- <sup>1</sup> Laboratorio de Bioquímica y Calidad de Productos Pesqueros. Centro de Investigación en Alimentación y Desarrollo (CIAD). Carretera a La Victoria, Km 0.6, Hermosillo, Sonora, 83304, México.
- <sup>2</sup> Departamento de Investigaciones Científicas y Tecnológicas, Universidad de Sonora, C.P. 83000, Hermosillo, Sonora, México.

# **ABSTRACT**

A partial characterization (proximate, amino acid and fatty acid compositions, electrophoresis,  $a_w$  and color) of loricariid catfish (*Pterygoplichthys disjunctivus*, Weber, 1991) roe, an invasive species of dams and rivers worldwide, was carried out and evaluated as a possible food resource. Mature roe showed high protein and lipid concentrations (26.0  $\pm$  1.5 and 8.2  $\pm$  0.7, respectively), low  $a_w$  (0.89), and a yellow to orange color (hue angle of 68.90  $\pm$  8.5° and chroma of 32.0  $\pm$  0.2). Roe showed that essential amino acids (EAA) prevailed over non-essential (NEAA) (EAA/NEAA ratios of 1.4 and 1.6 for total and free amino acids, respectively) and low  $\omega$ 6/ $\omega$ 3 balance (0.98). Due to its characteristics, loricarid catfish roe can be used as a raw material for caviar-type products.

**Keywords:** Loricariid catfish, Roe, Fatty acids, Amino acids.

# **RESUMEN**

Se realizó una caracterización parcial (análisis proximal, composición de aminoácidos y ácidos grasos, electroforesis, actividad de agua y color) de la hueva del pez Armado (Pterygoplichthys disjunctivus, Weber, 1991), una especie invasiva de presas y ríos en el mundo entero, para su evaluación como posible recurso alimenticio. La hueva madura mostró altas concentraciones de proteína y lípidos  $(26.0 \pm 1.5 \% \text{ y } 8.2 \pm 0.7 \%, \text{ respectivamente}), \text{ baja actividad}$ de agua (0.89), y una coloración amarilla-naranja (ángulo de matiz de  $68.90 \pm 8.5^{\circ}$  y cromaticidad de  $32.0 \pm 0.2$ ). La hueva mostró excelente relación de aminoácidos esenciales/no esenciales con valores de 1.4 y 1.6 para aminoácidos totales y libres, respectivamente, así como un buen balance de ácidos grasos ω6/ω3 (0.98). Debido a las características mostradas, la hueva del pez Armado puede ser utilizada como materia prima para la elaboración de productos tipo caviar.

**Palabras clave:** Pez Armado, hueva, contenido de ácidos grasos y aminoácidos.

### **INTRODUCTION**

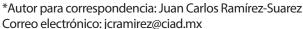
The mature female gonads of many fish species possess thousands of eggs with essential nutrients for the embryo's growth, until the product, in the larvae stage, can

be capable of feeding itself (Plack *et al.*, 1971). However, fish gonads of many species can also be commonly used as a food resource. Their non-fertilized eggs, commonly called roe, represent a product with a high nutritional content (Kirschbaum *et al.*, 2006; Wang *et al.*, 2007).

The loricariid catfish (Pterygoplichthys disjunctivus, Weber, 1991), an endemic species for South America (except Chile), Panamá and Costa Rica (Armbruster and Page, 2006), is an ornamental fish commonly used in North America and other parts of the world to clean fish tanks from algae and other debris. However, the species has made it to the wild and invaded different water corps throughout the world. In Mexico, this species have accidentally invaded reservoirs and rivers with the subsequent ecological problem, impacting some local fisheries (Rueda-Jasso et al., 2013). An example is the invasion of the Adolfo Lopez Mateos reservoir (also known as "El Infiernillo") in the State of Michoacán, México, affecting the economy of its surrounding communities, which depend from the Balsas catfish (Ictalurus balsanus), the redside cichlid (Cichlasoma istlanum) and tilapia fisheries (Mendoza et al., 2009). The loricariids, besides entangling the fishermen's nets, have affected the capture of tilapia in the region. At the time, this species (abundant in the reservoir and its effluents) has been underutilized by the fishermen, discarding all of its catches.

Recently, research in our laboratory has shown the feasibility of loricariid catfish muscle utilization either fresh (Marquez-Rios *et al.*, 2015) or as a raw material for gel production (unpublished data), as a mean to control the locariid population in the reservoir. On the other hand, taken into account the fertilization capacity of the species, going from a mean of 2000 to 2500 eggs (with a maximum capacity of almost 6700 eggs) in the summer months (Rueda-Jasso *et al.*, 2013), its roe could be also used as a food resource. Being a highly reproductive species, it represents a good source of roe since their gonads correspond to up to 30% of the fish weight.

Thus, the objective of the present study was to partially characterize fresh loricariid catfish (*Pterygoplichthys disjunctivus*, weber, 1991) roe, and evaluate it as an approach for possible food utilization.



Recibido: 08 de septiembre de 2015 Aceptado: 23 de noviembre de 2015



# MATERIALS AND METHODS Raw matter

Loricariid catfish (Pterygoplichthys disjunctivus, Weber, 1991) was obtained from a local fisherman in the Marguez River, an important effluent of "El infiernillo" reservoir. Fish was immediately stored in coolers in alternated layers of icefish and transferred to the facilities of the Universidad de San Nicolas of Hidalgo in the State of Michoacán, Mexico, where they were eviscerated and gonads were obtained. Gonads were packed in two plastic containers of approximately 1 Kg each; ice stored in a cooler and transported by airplane, to the CIAD Seafood Products Quality and Biochemistry Laboratory located in Hermosillo, Sonora, Mexico. Raw material was always processed within 24 h post capture. Two samplings trips were conducted (May and November 2008) in order to elucidate the chemical composition of the roe due to season variation. Then, after season was selected two more samplings were conducted (May and July, 2009) for the study. All samplings were conducted under the same sanitary conditions.

### Physicochemical analys

Proximate composition (water, lipids and ash content of roe) was carried out following the AOAC (2000) (Methods 950.46, 960.39, and 938.08, respectively) procedures. Protein content (N  $\times$  5.7) was obtained according to Woyewoda et al. (1986). Carbohydrates were calculated by difference. Roe size and weight were evaluated with a vernier scale and an analytical balance, respectively. Total caloric value was evaluated according to Chizzolini et al. (1999) expressed in kcal 100 g<sup>-1</sup> of sample. pH was determined by direct immersion of electrode in the roe. Water activity (a<sub>w</sub>) was conducted with a PawKit hygrometer (Decagon Corp.). The color of samples was measured using a Konica-Minolta CR-400 Tristimulus Colorimeter (Konica Minolta Sensing, Inc., Japan). Color coordinates were used to measure the degree of lightness (L\*), redness-greenness (+ or - a\*), and yellowness-blueness (+ or – b\*). Additional color traits such as hue angle ( $\Theta$ ) and chroma (C) were calculated from the "L\*", "a\*", "b\*" values (Minolta Corp., 1990). For a better integration and interpretation of a\* and b\* values, hue angles ( $\Theta$  were calculated using the formula:

 $\Theta = Arctang (b*/a*)$ 

The chroma was calculated using the formula:

 $C = (a^{*2} \times b^{*2})^{1/2}$ 

The hardness of samples was evaluated with a TMS-PRO texturometer (Food Technology Corporation) equipped with a 100 N load cell, exerting a 50% compression with a normal stress at 30 mm min<sup>-1</sup> crosshead velocity. Sample (egg) was stabilized at the bottom of a support prepared specially for this purpose. Briefly, the support was made from a 1.5 mL microtube which was carefully cut at the conical section and then placed inside the rest of the cut tube (serving as a support for the conical section). Ten individual eggs were examined for each sample.

# Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Fish roe proteins for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) were obtained from the resulting precipitate of the Folch methodology used in last section. Precipitate (1 g) was homogenized with 9 mL of dissolving solution (5% SDS, 0.1%  $\beta$ -mercaptoethanol) using a Tekmar Tissumizer (Tekmar Co. W. Germany) for 1 min and then heated to 90°C for 50 min to allow maximal protein solubilization and extraction. Subsequently, sample was filtrated with Whatman #4.

SDS-PAGE was performed accordingly to Laemmli (1970) with some modifications (constant current of 110 volts). Discontinuous gels ( $80 \times 60 \times 1.5$  mm, width  $\times$  height  $\times$  thickness) with separating and stacking gels of 13 and 4% acrylamide, respectively, were prepared. Filtrated solution was diluted (1:1 v/v ratio) with sample buffer (4% SDS, 20% glycerol, 10%  $\beta$ -mercaptoethanol, 0.125M Tris, pH 6.8) and dissolved by heating in boiling water for 3 min. Aliquots of 15  $\mu$ g of protein per lane were loaded into the acrylamide gel. Proteins were stained for Coomassie brillant blue R-250 (Bio-Rad) and the molecular weights of protein bands were determine using a high molecular weight standard proteins kit (Bio-Rad).

#### **Amino acids analysis**

Sample preparation for amino acids profile was conducted following the methodology described by Vázguez-Ortíz et al. (1995) with some modifications. Dry and defatted sample (3 mg) was vacuumed hydrolyzed by addition of 6 M hydrochloric acid at 150°C for 6 h, using sodium thioglycolate as antioxidant (1:1 w/w). The hydrolysate was dissolved in 2 mL of 0.5M citrate buffer and used for primary total amino acids determination following the methodology described by Pacheco-Aguilar et al. (1998). For secondary amino acids (proline and hydroxyproline) sample preparation and profile analysis, 125 µL of hydrolyzed sample were diluted in 0.5 mL of 0.4M of borate buffer (pH 10.4) following the methodology of Vázquez-Ortíz et al. (1995). Then, 250 µL of mixture were taken and derivatized with 250 µL of 4-Chloro-7-Nitro-2,1,3-Benzoxadiazole (NBD-Cl, 2.0 mg mL<sup>-1</sup> of methanol) in a water bath at 60°C for 5 min, stopping reaction with 50 μL of 1M HCl.

Free amino acids determination in sample was carried out according to Pacheco-Aguilar *et al.* (1998) with a slight modification in buffer flux. Amino acid extraction was carried out by homogenizing 3 g of sample with 6 mL of 7.5% trichloroacetic acid (TCA) (ratio 1:2, roe: TCA) with an Ultra-turrax T25 Basic homogenizer for 2 min at 11,000 rpm in an ice bath. The extract was centrifuged at  $2830 \times g$  for 15 min at 4°C in a Beckman Centrifuge Model J2-21 and the supernatant was filtrated in Whatman # 4 for analysis. Supernatant (100 µL) was brought to 1 mL with 200 µL internal standard (10 µg mL¹, L- $\alpha$ -amine n-butiric acid) and 700 µL HPLC water. A 400 µL aliquot was taken and mixed with 400 µL of O-Phthaldial-

dehyde (OPA, 10 mg of OPA + 250 μL methanol + 37.5 μL de Brij 35 + 25 μL β-mercaptoethanol) and all were diluted to 10 mL with potassium borate buffer, pH 10.4. The mixture was filtered and derivatized for 2 min at room temperature and finally loaded into an HPLC using a 20 µL loop. Amino acid concentration was quantified using a Series 1100 Hewlett Packard HPLC coupled with a fluorescence detector (350 nm excitation and 450 nm emission). Separation was conducted in a C18 octadecyl dimetilsilane reverse phase column (3µM particle diameter, 100 mm long × 4.6 mm ID) (Variant Cat No. R0089200E3). A gradient elution consisting of buffer A (100%) methanol) and buffer B (10% methanol, 90% acetate buffer, pH 6.5) was used for separation at a flow of 1.0 mL min<sup>-1</sup>. A 30 min run was conducted using an initial condition of mobile phase of 80% buffer B and 20% buffer A for 5 min; then a 70% buffer B and 30% buffer A mobile phase was used for 5 min; next, 50% buffer B and 50% buffer A mobile phase was used for 5 min; subsequently, a 20% buffer B and 80% buffer A mobile phase was used for 7 min and finally an 80% buffer B and 20% buffer A mobile phase was used for 8 min. Data (area under the peaks produced by amino acid fluorescence) was analyzed by the CHEM STATION program (Agilent Technologies Inc. Santa Clara, CA, USA). Retention times and peak areas were compared with 16 amino acids standards (Sigma Chemical Co. St. Louis, MO, USA).

# **Fatty acids content**

Lipids were extracted from 6 g of samples (triplicates of different roe) according to the Folch methodology using chloroform:methanol (2:1 v/v). Extracted lipids were flushed with nitrogen and frozen at -80°C, until used for their fatty acid composition analysis. Fatty acids were derivatized to their correspondent methyl-esters using 14% BF<sub>3</sub>-MeOH on 20 mg of oil (in triplicate) (Morrison and Smith, 1964). Briefly, lipid extract (1 mL) was evaporated in a water bath at 60°C under a constant nitrogen flux. Then, 20 mg of oil were diluted with 1 mL of 0.5M NaOH in methanol and 100  $\mu$ L of dichloromethane. Then, nitrogen was flushed and the tube was sealed and heated in a water bath at 90°C for 10 min. Finally, 1 mL of distilled water + 500  $\mu$ L of hexane + 500  $\mu$ L of internal standard (Tridecanoic acid, C13:0) (Sigma Aldrich, Bellafonte, USA) were added and slightly shaken. Identification and quantification of fatty acids methyl-esters (FAME) was obtained by capillary gas chromatography in a Agilent CG 6850, fitted with a capillary Agilent column DB-23 (60m, 0.25 mm id and 0.25  $\mu$ m film) and equipped with a split/splitless injector and a flame ionization detector temperature of 280°C. A 35 min ramp was conducted as follows: Initial oven temperature was 50°C; after 1 min, temperature was raised to 175°C at 25°C/min; then temperature was raised to 230°C at 4°C/min and sustained for 15 min. Individual components were identified by comparing the retention times with those obtained from the FAME mixture standard (Supelco 37, Bellefonte, PA). Results were expressed as a percentage of the total fatty acid methyl esters present in the sample.

#### Statistical analysis

In order to observe for seasonal differences in roe composition, a one way analysis of variance (ANOVA) and Tuckey-Kramer comparison of averages were carried out with a level of significance of 5%, using the statistical package NCSS 2007. For this purpose, two sampling trips were carried out (May and November, 2008). Analysis was conducted at least in triplicate.

#### **RESULTS AND DISCUSSION**

#### Physicochemical analysis

Fish roe, like all ovum prepared to develop life, is characterized to have an important nutritional content. However, its composition depends of its maturation. Thus, a physicochemical analysis of fish roe was conducted at two different seasons, one in May (spring) and another in November (fall). Table 1 shows the physicochemical results at the two different sampling periods. Although no significant difference was found in their proximate composition (except for protein content, p < 0.05), May roe showed higher values in all parameters evaluated except for ash and carbohydrate contents. Besides, May roe was heavier and harder (p < 0.05) than November roe. Carbohydrate content on roes from both seasons gave them a characteristic sweet flavor. There are no reported values in the literature for proximate composition of loricariid catfish roe in other regions; however, Bledsoe et al. (2003) reported similar values for channel catfish (Ictalururs punctatus) and within the range of Caspians sturgeons (Huso sp) and Chinook salmon (O. tshawytscha). Mol and Turan (2008) showed similar protein contents on

**Table 1.** Physicochemical parameters of loricariid catfish (*Pterygoplichthys disjunctivus*, Weber, 1991) roe at two different catching months (Proximate parameters are shown in %).

**Tabla 1.** Parámetros fisicoquímicos de la hueva del pez Armado (*Pterygoplichthys disjunctivus*, Weber, 1991) en dos diferentes meses de captura (Parámetros proximales en %).

month	Moisture	Protein	Lipid	Ash	Carbohydrates*	Weight (mg)	Texture ( gf )**
May roe	63.0 ± 1.7 <sup>a</sup>	26.0 ± 1.5 <sup>a</sup>	$8.2\pm0.7^{a}$	$1.7\pm0.6^{a}$	2.8	$20.0\pm0.0^{a}$	30.0 ± 1.7 <sup>a</sup>
Nov. roe	60.8 ± 3.2°	21.5 ± 1.9 <sup>b</sup>	7.9 ± 1.2 <sup>a</sup>	2.1 ± 0.1 <sup>a</sup>	7.7	12.0 ± 0.0 <sup>b</sup>	25.7 ± 1.0 <sup>b</sup>

a-b Means with different letter on each parameter are significantly different (p < 0.05). n = 3. \*Obtained by difference. \*\*n = 6.



different black caviars, salmon roe (Salmo trutta labrax) and waxed mullet (Mugil cephalus) roe when comparing it with the loricariid catfish May roe. On the other hand, protein, lipid and ash contents in the present study were higher than data shown for roe of several marine species such as skipjack (Katsuwonous pelamis), tongol (Thunnus tonggol) and bonito (Euthynnus affinis) (Intarasirisawat et al., 2011) and similar in protein and lipid values with blue fish (Scombrops boops) and pacific flounder (Paralichthys olivaceus) (Iwasaki and Harada, 1985). Loricariid catfish May roe showed similar and higher weights than Beluga (Huso huso) and Osetra (Acipenser persicus) caviars, respectively (Mol and Turan, 2008).

The variation in physicochemical composition of loricariid catfish roe in the present study is related to the reproductive season, which for this type of fish can be considered that starts in May (Rueda-Jasso, 2013). Although November is also considered within the reproductive season (Rueda-Jasso, 2013), reproduction of loricariid catfish is at its doom, thus showing lower values in most of its proximal composition. Besides, variation inside (and between) species can be due to different reasons such as feeding condition of the fish, habitat, reproduction stage, energy needs, among others factors (Bledsoe *et al.*, 2003; Schubring, 2004).

In terms of caloric value, loricariid catfish roe provided a caloric value of 189 and 187.9 kcal 100 g<sup>-1</sup> for May and November roe, respectively. These values are smaller than the one reported for mullet (*Mugil cephalus*) roe (231.8 kcal 100 g<sup>-1</sup>) but bigger than the one reported for whiting (*Merlangius merlangus euxinus* Nordmann, 1840) roe (Kocatepe *et al.*, 2012).

Overall results indicated that loricariid catfish roe obtained in May showed a chemical composition and egg size ( $3.25\pm0.4$  mm) similar to commercial species, making this roe a favorable raw matter for the possible development of a product of commercial interest. Thus, roe obtained in two more sampling trips, in May and July 2009, were used to continue the present research.

pH is a good food quality index since its value can be a sign of food spoilage related to the growth of microorganisms. Regarding this parameter, loricariid catfish roe showed a value of  $5.87 \pm 0.1$ , value similar to the reported for trout (*Oncorhynchus mykiss*) (pH 6.02) (Schubring, 2004), and mature salmon roes (*O. tshawytscha*) (pH 5.62) (Bekhit *et al.*, 2009). In a study conducted by Bekhit *et al.* (2009), authors found that roe pH was influenced by its maturation state as well as by the species. During its maturation, gonad shows a pH decrease in the ovarian fluid as well as in moisture content due to the increase in protein, lipids and mass of the eggs (Lahnsteiner, 2007). Thus, the low pH found in loricariid catfish roe is an indication that gonads used for the present study were mature.

One important food stability index related to the shelf life of a product is its water activity (a<sub>w</sub>). Regarding this parameter, fresh loricariid catfish roe showed a value of 0.89, low enough to prevent the growth of deteriorative bacteria such as mesophiles and psychrophiles (Labuza *et al.*, 1970),

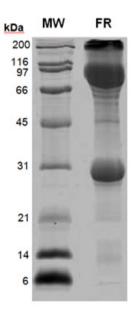
as well as some yeast and pathogenic microorganisms. Very few pathogens such as *Staphylococcus aureus* may overcome such condition (Notermans and Heuvelman, 1983; Valero *et al*, 2009). However, the hurdle effect of NaCl, a<sub>w</sub> (0.89), pH (5.87) and storage temperature (0-4°C) in fresh loricariid catfish roe suggests a safety control.

On the other hand, color represents one major food quality attribute for its acceptability. In this regard, loricariid catfish roe showed a lightness (L\*) of  $35.76 \pm 4.2$ , a hue angle of  $68.90 \pm 8.5^{\circ}$  and a chroma of  $32.0 \pm 0.2$ , setting its color within the first (red-yellow) quadrant, inside the orange to yellow area.

Regarding texture, loricariid catfish roe showed a hardness of 19.61 gf, which is inside the range (12.4-47.9 gf) found by Craig and Powrie (1988) for Chum salmon roe of similar size (2.5-4 mm).

#### **SDS-PAGE**

Protein pattern of loricariid catfish roe is shown in Figure 1. Three main bands were observed: a high molecular weight (MW) protein band that did not even enter the separating gel, which can correspond to β-ovomucin and / or collagen (Al-Holy and Rasco, 2006; Bekhit *et al.*, 2009); another band with a MW of approximately 77 kDa, which can correspond to ovotransferrin, a bactericidal and antioxidant glycoprotein (Nakamura and Doi, 2000); an additional 30 kDa MW band corresponding to either ovomucoid, a glycoprotein rich in sulfur amino acids (Doi and Kitabatake, 1997; Intarasirisawat *et al.*, 2011) or fosvitin, a bactericidal protein (Al-Holy and Rasco, 2006).



**Figure 1.** Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) profile of loricariid catfish (*Pterygoplichthys disjunctivus*, weber, 1991) roe. MW = Molecular weight standards. FR = Fresh roe.

**Figura 1.** Perfil electroforético (SDS-PAGE) de las proteínas de la hueva de pez Armado (*Pterygoplichthys disjunctivus*, weber, 1991). MW = Estándar de masa molecular. FR = Hueva fresca.



Among other protein that can be identified between 45 and 70 kDa are: a 45 kDa band similar to ovalbumin, a phoshoglycoprotein; ovoglobulins (G2 y G3) and/or ovoinhibitor at 49 kDa; and avidin (at 68 kDa), a protein with bactericidal and fungicidal action (Nakamura and Doi, 2000). Besides, minor proteins (in concentration) were observed at low MW, one at approximately 20 kDa corresponding most probably to ovoglycoprotein (Nakamura and Doi, 2000) and another band at approximately 14 kDa, which belongs to lysozyme, an anti-bacterial protein (Yousif *et al.*, 1994). In general, results provide information about the set of proteins that protect the egg during its development, characteristic in favor for its possible use as a raw material.

#### **Amino acid content**

According to literature, fish roe represents a well balanced food due to its essential nutrients, such as proteins of high biological value, composed of essentials amino acids of high digestibility and availability (Intarasirisawat et al., 2011). Table 2 shows the amino acid composition (total and free) of loricariid catfish roe. As it is shown, its protein possesses a high quantity of essential amino acids. Major essential amino acids found were arginine (113 mg g<sup>-1</sup> of roe), lysine (95.4 mg g<sup>-1</sup> of roe), leucine (87.9 mg g<sup>-1</sup> of roe) and valine (70.5 mg g<sup>-1</sup> of roe). Actually the first three amino acids showed the highest concentration only after glutamic acid (125 mg g<sup>-1</sup> of roe), which showed the highest concentration. A very small amount of proline (8.4 mg g<sup>-1</sup> of roe) was found on the roe. However, proline is not an abundant amino acid in roes. Amino acid content agrees with Iwasaki and Harada (1985), who studied amino acid content of roe from 14 different species finding these same amino acids as predominant (although not in the same order). According to Seagran et al. (1953) arginine is an amino acid that helps in the egg's maturation. Thus, results indicate that loricariid catfish roe used in the present study corresponded to roe in a high state of maturation. Differences from the literature may be related with the species, age, diet, and habitat of the fish (Mol and Turan, 2008). However, it is clear that this species uses several essential amino acids for its roe development.

Iwasaki and Harada (1985) established an essential/ non-essential amino acids (EAA/NEAA) ratio of 0.74 for a high quality protein in roe from 14 species. In the present study, a ratio almost twice higher (1.4, Table 2) was observed for loricariid fish roe. Taking into account that most high quality protein, as in milk and meat, possesses around 40% of EAA (by content) (Phillips, 2012), then this fish roe showed higher EAA values. Therefore, loricariid fish roe can be considered a good source of essential amino acids.

Free amino acids can be responsible for roe flavor development; in this regard, free amino acid content on sample showed low values in all amino acids tested. However, its EAA/NEAA ratio was higher than the ratio given by amino acids found as part of the primary structure. The free amino acids detected (arginine, leucine, lysine and alanine) might conferred the sour-sweet characteristic taste of loricariid cat-

**Table 2**. Total and free amino acids composition (mg of amino acids sample-g<sup>-1</sup>) of loricariid catfish (*Pterygo-plichthys disjunctivus*, Weber, 1991) roe.

**Tabla 2**. Composición de aminoácidos libres y totales (mg de aminoácido g-muestra<sup>-1</sup>) de la hueva de pez Armado (*Pterygoplichthys disjunctivus*, Weber, 1991).

	Total amino acids	Free amino acids
Aspartic acid	82.6 ± 5.9	0.07 ± 0.0
Glutamic acid	125 ± 5.1	$0.25 \pm 0.0$
Asparagine	Nd	$0.08 \pm 0.0$
Serine	$6.7 \pm 3.8$	0.11 ± 0.0
Glutamine	$7 \pm 0.4$	$0.11 \pm 0.0$
Glycine	$32.9 \pm 3.3$	$0.05 \pm 0.0$
Tyrosine	$24.9 \pm 2.2$	$0.12 \pm 0.0$
Taurine	$3.5 \pm 0.0$	0.25 ± 0.0
Alanine	$76.3 \pm 4.7$	$0.15 \pm 0.0$
Histidine*	4.9 ± 1.4	$0.08 \pm 0.0$
Threonine*	17.5 ± 2.1	$0.09 \pm 0.0$
Arginine*	113 ± 7.3	0.42 ± 0.1
Methionine*	$28.7 \pm 0.7$	$0.10 \pm 0.0$
Valine*	70.5 ± 4.2	$0.13 \pm 0.0$
Phenylalanine*	43.1 ± 2.7	$0.12 \pm 0.0$
Isoleucine*	55.6 ± 4.7	0.11 ± 0.0
Leucine*	$87.9 \pm 5.0$	$0.21 \pm 0.0$
Lysine*	95.4 ± 5.5	$0.28 \pm 0.0$
Proline	$8.4 \pm 0.7$	Nq
Hydroxyproline	Nd	Nq
EAA/NEAA	1.4	1.6
Total	883.9	2.73

Data represent mean  $\pm$  standard deviation of 2 replications (n=2). \*essential amino acids; EAA/NEAA: essential and nonessential amino acids ratio; Nq not quantified; Nd not detected. Tryptophan and cysteine were not quantified.

fish roe. Besides, glutamic acid is considered a flavor enhancer, imparting a flavor described as fresh in meat products.

#### **Fatty acid content**

One of the most important characteristics fish roe possesses, and which is highly valued for, is its lipids content and composition. According to Tocher and Sargent (1984), fish roe is characterized to contain polyunsaturated fatty acids (PUFA´s), such as eicosapentaenoic (EPA C20:5,  $\omega$ 3), araquidonic (AA C20:4  $\omega$ 6) and docosahexaenoic (DHA C20:6  $\omega$ 3) acids, due to their presence within membrane phospholipids, which are easily mobilized for cell proliferation during the egg´s maturation and also used for energy reserve during the organisms development.

In this respect, Table 3 shows the fatty acid composition of lipids extracted from loricariid catfish roe, showing a

**Table 3.** Fatty acid composition (% total FA) in the loricariid catfish (*Pterygo-plichthys disjunctivus*, Weber, 1991) roe.

**Tabla 3**. Composición de ácidos grasos (% del total de ácidos grasos) en la hueva del pez Armado (*Pterygoplichthys disjunctivus*, Weber, 1991).

FA (%)	Roe
SFA	
C4:0	$3.5 \pm 0.0$
C14:0	$3.3 \pm 1.0$
C16:0	23.5 ± 1.8
C17:0	$2.6 \pm 0.5$
C18:0	9.7 ± 0.8
Others	5.1 ± 0.0
Total	47.6 ± 0.1
MUFA	
C18:1 <b>ω</b> 9 c	17.3 ± 1.5
C16:1	$7.0 \pm 1.0$
Others	$4.3 \pm 0.2$
Total	$28.6 \pm 0.0$
PUFA	
C18:2 <b>ω</b> 6 t	$2.3 \pm 0.6$
C18:2 <b>ω</b> 6 c	$1.2 \pm 0.0$
<b>γ</b> C18:3 <b>ω</b> 6	4.6 ± 1.7
C20:3 <b>ω</b> 6 c	$2.0 \pm 0.5$
C20:4 <b>ω</b> 6	$1.6 \pm 0.0$
C18:3 <b>ω</b> 3	2.7 ± 1.0
C20:3 <b>ω</b> 3	$0.3 \pm 0.0$
C20:5 <b>ω</b> 3 c	$0.7 \pm 0.3$
C22:6 <b>ω</b> 3	$8.2 \pm 0.9$
Others	$0.2 \pm 0.0$
Total	23.8 ± 0.0
ω6/ω3 ratio	0.98

Data represent the mean  $\pm$  SD of 6 replications (n=6). SFA = saturated fatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids. c = configuration cis, t = configuration trans.

high content of saturated fatty acids (47.6%), mainly from palmitic acid (23.5%). Monounsaturated fatty acids were found in a relatively high quantity (28.6%) from which oleic acid (C18:1  $\omega$ 9 with 17.3%) represented the highest concentration on sample. Regarding to PUFA's, sample showed about one fourth of its total (23.8%), being the docosahexaenoic acid (C22:6  $\omega$ -3, DHA) the predominant PUFA in sample with 8.2%. Concerning the eicosapentaenoic acid (C20:5  $\omega$ -3, EPA) content, sample showed a characteristic content for fresh water species (as loricariid catfish) with a low value of 0.7%. Similar fatty acid contents were found in roe from Rohu (*Labeo rohita*) and Murrel (*Channa striatus*) (Prabhakara Rao *et al.*, 2010), both fresh water species with a low eicosapentaenoic acid (EPA) content (1.5 and 0.6%, respectively). Domination by the

four fatty acids listed above was more extreme in lumpfish (*Cyclopterus lumpus*) roe, for which Basby *et al.* (1998) reported the four fatty acids to comprise 73.5% (versus practically 50% in the present study).

According to Prabhakara Rao *et al.* (2010), the high amount of DHA in fish roe lipids helps on the development of fish larvae. The effect of habitat, gonadal maturation, physical activity and feeding on the lipid content can be observed in the noticeable difference found between fresh and marine water species, the fore ones presenting higher levels of  $\omega 6$  fatty acids than marine species (Bledsoe *et al.*, 2003; Prabhakara *et al.*, 2010; Intarasirisawat *et al.*, 2011).

Polyunsaturated fatty acids are responsible for vital functions in organisms, therefore they require being in equilibrium on the diet. In this context, an imbalance in the  $\omega 6/\omega 3$  ratio consumption has been observed in the western diet with a ratio of 15-20:1, instead of 1/1 as suggested by Simopoulos (2008) for wild animals and human beings. In the present study, the  $\omega 6/\omega 3$  ratio found for loricariid catfish roe was 0.98, indicating that this roe is close to the suggested 1:1 ratio, showing a  $\omega 6/\omega 3$  balance which is very important for health.

Thus, the present research is the first approach to partially know the characteristics of this roe; however, further research is needed to complete its characterization.

#### **CONCLUSIONS**

Loricarid catfish (*Pterygoplichthys disjunctivus*, Weber, 1991) roe can be used as a raw material for caviar-type products of commercial interest due to its chemical composition (good source of protein with high essential amino acids and fatty acid composition with a well  $\omega 6/\omega 3$  balance) and physical characteristics (appealing yellowish color, sweet flavor, size and hardness).

#### **ACKNOWLEDGEMENTS**

Authors wish to thank to Fondo Mixto CONACYT-Gobierno del Estado de Michoacán for the support to the project "Desarrollo Tecnológico para el Aprovechamiento e Industrialización del Pez Diablo en la Región del Bajo Balsas en Michoacán, FOMIX # 37147.

#### REFERENCES

Al-Holy, M.A. and Rasco, B.A. 2006. Characterization of salmon (*Oncorhynchus keta*) and sturgeon (*Acipenser transmontanus*) caviar proteins. Journal of Food Biochemistry. 30: 422-428.

AOAC. 2000. Official Methods of Analysis. Association of Official Analytical Chemist. USA.

Armbruster, J.W. and Page, L.M. 2006. Rediscription of *Pterygoplicthys punctatus* and description of a new species of *Pterygolplicthys* (Siluriformas: Loricariidae). Neotropical lchthyology. 4(4): 401-409.

Basby, M., Jeppesen, V.F. and Huss, H.H. 1998. Chemical composition of fresh and salted lumpfish (*Cyclopterus lumpus*) roe. Journal of Aquatic Food Product Technology. 7(4): 7-21.



- Bekhit Alaa, E.A., Morton, J.D., Dawson, C.O., Zhao, J.H. and Lee, H.Y.Y. 2009 Impact of maturity on the physicochemical and biochemical properties of Chinook salmon roe. Food Chemistry. 117: 318-325.
- Bledsoe, G., Bledsoe, C.D. and Rasco, B. 2003. Caviars and fish roe products. Critical Reviews in Food Science and Nutrition. 43(3): 317-356.
- Chizzolini, R., Zanardi, E., Dorigoni, V. and Ghidini, S. 1999. Calorific value and cholesterol content of normal and lowfat meat and meat products. Food Science & Technology. 10: 119-128.
- Craig, C.L. and Powrie, W.D. 1988. Rheological properties of fresh and frozen chum salmon eggs with and without treatment by cryoprotectants. Journal of Food Science. 53(3): 684-687.
- Doi, E. and Kitabatake, N. 1997. Structure and functionality of egg proteins. En Food proteins and their applications. S. Damodaran y A. Paraf (ed.) pp 325-340. Marcel Dekker. Inc. New York, NY.
- Intarasirisawat, R., Benjakul, S. and Visessanguan, W. 2011. Chemical compositions of the roes from skipjack, tongol and bonito. Food Chemistry. 124: 1328-1334.
- Iwasaki, M. and Harada, R. 1985. Proximate and amino acid composition of the roe and muscle of selected marine species. Journal of Food Science. 50(6): 1585-1587.
- Kirschbaum, J., Krause, C. and Brückner, H. 2006. Liquid chromatographic quantification of synthetic colorants in fish roe and caviar. European Food Research & Technology. 222: 572–579.
- Kocatepe, D., Turan, H., Kaya, Y., Erden, R. and Erdoğdu, F. 2012. Proximate chemical composition of whiting (*Merlangius merlangus euxinus* Nordmann, 1840) roe. Journal of Aquatic Food Product Technology. 21: 362–368.
- Labuza, T.P., Tannenbaum, S.R. and Karel, M. 1970. Water content and stability of low-moisture and intermediate-moisture foods. Food Technology. 24(5): 35.
- Laemmli, K. 1970. Cleavage of structural proteins during assembly of the head bacteriophage T4. Nature. 217: 680-685.
- Lahnsteiner, F. 2007. First results on a relation between ovarian fluid and egg proteins of salmon trutta and egg quality. Aquaculture Research. 38: 131-139.
- Marquez-Rios, E., Pacheco-Aguilar, R., Ramirez-Suarez, J.C., Ocaño-Higuera, V., Garcia-Sifuentes, C., Scheuren-Acevedo, S. and Mazorra-Manzano, M.A. 2015. Postmortem biochemical and microbiological changes in loricariid catfish (Pterygoplichthys disjunctivus) muscle during ice storage. Journal of Aquatic Food Product Technology DOI:1 0.1080/10498850.2013.828146.
- Mendoza, A.R., Escalera, G.C., Contreras, B.S., Koleff, O.P., Ramírez, M.C., Álvarez, T.P., Arroyo, D.M. and Orbe, M.A. 2009. Invasión de plecos en la presa El Infiernillo, México: análisis de efectos socioeconómicos (relato de dos invasores). En Directrices trinacionales para la evaluación de riesgos de las especies acuáticas exóticas invasoras. pp: 42-51. Commission for Environmental Cooperation (CEC).
- Mol, S. and Turan, S. 2008. Comparison of proximate, fatty acid and amino acid compositions of various types of fish roes. International Journal of Food Properties. 11: 669–677.
- Morrison, W.R. and Smith, L.M. 1964. Preparation of fatty acid methyl esters and dimethylacetates from lipids with boron fluoride-methanol. Journal of Lipid Research. 5: 600-608.

- Nakamura, R. and Doi, E. 2000. Egg Processing. En Food Proteins: Processing Applications. S. Nakai, and H.W. Modler (ed). pp: 171-207. Wiley-VCH. New York, NY.
- Notermans, S. and Heuvelman, C.J. 1983. Combined effect of water activity, pH and sub-optimal temperature on growth and enterotoxin production of *Staphylococcus aureus*. *Journal of Food Science*. 48:1832–1835.
- Pacheco-Aguilar, R., Lugo-Sánchez, M.E., Villegas-Ozuna, R.E. and Robles-Burgueño, R. 1998. Histamine quantification in Monterrey Sardine Muscle and canned products from Northwestern Mexico. Journal of Food Composition and Analysis. 11(2): 188-195.
- Plack, P.A., Pritchard, D.J. and Fraser, N.W. 1971. Egg proteins in cod serum. Journal of Biochemistry. 121: 847-856.
- Prabhakara Rao, P.G., Jyothirmayi, T., Karuna, M.S.L. and Prasad, R.B.N. 2010. Studies on lipids profiles and fatty acid composition of roe from Rohu (*Labeo rohita*) and Murrel (*Channa striatus*). Journal of Oleo Science. 59: 515-519.
- Phillips, S.M. 2012. Dietary protein requirements and adaptive advantages in athletes. British Journal of Nutrition. 108:S158–S16.
- Rueda-Jasso, R.A., Campos-Mendoza, A., Arreguín-Sánchez, F., Díaz-Pardo, E. and Martínez-Palacios, C.A. 2013. The biological and reproductive parameters of the invasive armored catfish *Pterygoplichthys disjunctivus* from Adolfo López Mateos El Infiernillo Reservoir, Michoacán-Guerrero, Mexico. Revista Mexicana de Biodiversidad. 84: 318-326.
- Schubring, R. 2004. Differential scanning calorimetric (DSC) measurements on the roe of rainbow trout (*Oncorhynchus mykiss*): influence of maturation and technological treatment. Thermochimica Acta. 415: 89-98.
- Seagran, H.L., Mobey, D.E. and Dassow, J.A. 1953. The amino acid content of roe at different stages of maturity from the five species of pacific salmon. Journal of Nutrition. 139-149.
- Simopoulos, A.P. 2008. The omega-6/omega-3 fatty acid ratio, genetic variation, and cardiovascular disease. Asia Pacific Journal of Clinical Nutrition. 17(Suppl. 1): 131–4.
- Tocher, D.R. and Sargent, J.R. 1984. Analyses of lipids and fatty acids in ripe roes of some Northwest European Marine Fish. Lipids. 19: 492-499.
- Valero, A., Pérez-Rodríguez, F., Carrasco, E., Fuentes-Alventosa, J.M., García-Gimeno, R.M., and Zurera, G. 2009. Modelling the growth boundaries of Staphylococcus aureus: Effect of temperature, pH and water activity. International Journal of Food Microbiology. 133:186–194.
- Vázquez-Ortíz, F.A., Caire, G., Higurea-Ciapara, I., and Hernández, G. 1995. High performance liquid chromatographic determination of free amino acids in shrimp. Journal of Liquid Cromatography. 18(10): 2059-2068.
- Wang, W., Batterman, S., Chernyak, S. and Nriagu, J. 2007. Concentrations and risks of organic and metal contaminants in Eurasian caviar. Ecotoxicology and Environmental Safety. 1-11.
- Woyewoda, A.D., Shaw, S.J., Ke, P.J. and Burns, B.G. 1986. Recommended Laboratory Methods for Assessment of Fish Quality. Canadian Technical Report of Fisheries and Aquatic Sciences No. 1448, 156.
- Yousif, A.N., Albright, L.J. and Evelyn, T.P.T. 1994. In vitro evidence for the antibacterial role of lysozyme in salmonid eggs. Diseases of Aquatic Organisms. 19: 15-19.

