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Chemometric tools for a better understanding of fatty acid profile of fishes with potential to diversify fishing farming activity

Ferramentas quimiométricas para o melhor entendimento do perfil lipídico de espécies de peixes com potencial diversificação da piscicultura

Herramientas quimiométricas para una mejor comprensión del perfil lipídico de especies de peces con potencial de diversificación de piscicultura

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Ivane Benedetti Tonial
ORCID: https://orcid.org/0000-0003-2600-7254
Universidade Tecnológica Federal do Paraná, Brazil
E-mail: ivane@utfpr.edu.br
Leandra Schuastz Breda
ORCID: https://orcid.org/0000-0002-0418-7816
Universidade Tecnológica Federal do Paraná, Brazil
E-mail: leandra@utfpr.edu.br
Carla Cristina Lise
ORCID: https://orcid.org/0000-0003-2240-7242
Universidade Tecnológica Federal do Paraná, Brazil
E-mail: carlacristinalise@gmail.com
Davi Costa Silva
ORCID: https://orcid.org/0000-0002-7278-2772
Universidade Tecnológica Federal do Paraná, Brazil
E-mail: davisilva@utfpr.edu.br
Tatiane Cadorin Oldoni
ORCID: https://orcid.org/0000-0001-5471-0145
Universidade Tecnológica Federal do Paraná, Brazil
E-mail: tatianeoldoni@utfpr.edu.br
Naiane Sangaletti Gerhard
ORCID: https://orcid.org/0000-0003-3380-2045
Universidade de São Paulo, Brazil
E-mail: naiane@alumni.usp.br
Marina Leite Mitterer-Daltoé
ORCID: https://orcid.org/0000-0002-6761-9321
Universidade Tecnológica Federal do Paraná, Brazil
E-mail: marinadaltoe@utfpr.edu.br

Abstract
This article evaluates the lipid profile of three fish species with the potential for breeding and diversification in Brazil and worldwide by means of chemometric tools. Grass carp (*Ctenopharyngodon idella*), Pacu (*Piaractus mesopotamicus*), and Catfish (*Ictalurus punctatus*) were captured at 24 months of age and analyzed for lipid profile using gas chromatograph. The multivariate analysis, discriminant and canonical discriminant, were used to better understand the differences in the lipid profiles between the three fish species. The fatty acid profile indicated oleic acid as the major component for all three species, resulting in a high percentage of monounsaturated fatty acids (MUFA). The Pacu presented the lowest content of polyunsaturated fatty acids. Discriminant analysis proved to be an important tool to differentiate lipid profiles between species. The pentadecylic (15:0), palmitoleic (16:1), margaric (17:0), stearic (18:0), arachidonic (20:4), and lignoceric (24:0) fatty acids presented the highest level of discrimination between species. This propagation of fatty acid values from these species reinforces their food quality and expands the possibilities of developing fish farming.
Keywords: Catfish; Discriminate analysis; Grass carp; Pacu.

Resumo
Este trabalho avalia o perfil lipídico, por meio de ferramentas quimiométricas, de três espécies de peixes com potencial de criação e diversificação no Brasil e no mundo. Carpa capim (*Ctenopharyngodon idella*), pacú (*Piaractus mesopotamicus*) e catfish (*Ictalurus punctatus*) foram capturados aos 24 meses de idade e analisados quanto ao perfil lipídico por meio de cromatografia gasosa. Análises estatísticas multivariadas, discriminante e discriminante canônica, foram usadas para entender melhor as diferenças nos perfis lipídicos entre as três espécies de peixes. O perfil de ácidos

graxos indicou o ácido oleico como o principal componente para as três espécies, resultando em uma alta porcentagem de ácidos graxos monoinsaturados (MUFA). O Pacú apresentou o menor teor de ácidos graxos poliinsaturados. A análise discriminante mostrou-se uma importante ferramenta para diferenciar o perfil lipídico entre as espécies. Os ácidos graxos pentadecílico (15:0), palmitoleico (16:1), margárico (17:0), esteárico (18:0), araquidônico (20:4) e lignocérico (24:0) apresentaram os maiores teores de discriminação entre as espécies. Essa divulgação dos valores de ácidos graxos dessas espécies reforça sua qualidade alimentar e amplia as possibilidades de desenvolvimento da piscicultura.
Palavras-chave: Análise discriminante; Carpa capim; Catfish; Pacú.

Resumen
Este trabajo evalúa el perfil lipídico, a través de herramientas quimiométricas, de tres especies de peces con potencial de creación y diversificación en Brasil y en el mundo. Se capturaron carpa herbívora (*Ctenopharyngodon idella*), pacu (*Piaractus mesopotamicus*) y bagre (*Ictalurus punctatus*) a los 24 meses de edad y se analizó el perfil de lípidos mediante cromatografía de gases. Análisis estadístico multivariado, discriminante y discriminante canónico, fueron utilizadas para comprender mejor las diferencias en los perfiles lípidos entre las tres especies de peces. El perfil de ácidos grasos indicó que el ácido oleico es el componente principal de las tres especies, lo que resulta en un alto porcentaje de ácidos grasos monoinsaturados (MUFA). Pacu tuvo el contenido más bajo de ácidos grasos poliinsaturados. El análisis discriminante demostró ser una herramienta importante para diferenciar el perfil lipídico entre especies. Los ácidos grasos pentadecílico (15:0), palmitoleico (16:1), margárico (17:0), esteárico (18:0), araquidónico (20:4) y lignocérico (24:0) mostraron los niveles más altos de discriminación entre especies. La divulgación de los valores de ácidos grasos de estas especies refuerza su calidad alimentaria y amplía las posibilidades de desarrollo de la piscicultura.
Palabras clave: Análisis discriminante; Bagre; Carpa herbívora; Pacú.

1. Introduction

The role of aquaculture in providing food, nutrition and employment worldwide is clearly demonstrated by the numbers. In 2018, total global aquaculture production reached the highest level ever recorded at 114.5 million tonnes, employing 20.5 million people (FAO, 2020). In Latin America, the contribution of aquaculture to regional economies has grown substantially in the last 10 years (del Pazo et al., 2021). In Brazil the aquaculture sector showed significant expansion. In 2021 it reached 841 tonnes, a cumulative growth of 45% since 2014. The south of Brazil stands out in the national aquaculture scene, mainly in the production of tilapia (*Pseudocrenilabrinae*). The species represents 86% of all farmed fish in the region. In total, there are 231,900 tonnes in the three southern states: about 43.4% of the national production. Among the southern states, Paraná leads in the production of tilapia, with 182 tonnes (Peixe BR, 2022).

The southwest of Paraná has the potential to improve fish farming. Local authorities plan to increase production but depend on the commitment of small rural producers, who lack incentive. There are many reasons cited as barriers to the development of fish farming chain in the region, primary amongst which are a lack of knowledge and incentive for rearing other species of fish during the off-season for tilapia rearing (Lise et al., 2020; Marques et al., 2019).

Fish are considered a food with high nutritional value and a source of essential omega-6 and omega-3 fatty acids (Matos et al., 2019). These fatty acids are not synthesized in the human organism and play important roles in the structure and function of human tissues (Viriato et al., 2019). The regular consumption of fish is linked to a reduction of the rate of bad cholesterol (low-density protein, LDL), reduction of blood pressure, decreased triglyceride levels, and the prevention of inflammation and thrombosis (Mohanty et al., 2019).

Although the importance of consuming fish for health is recognized, Brazil is characterized by a low consumption of fish. According to FAO data, Brazilians consume 9.6 kg/inhabitant/year, which is less than the recommended value of 12 kg/inhabitant/year and the world average of 20 kg/inhabitant/year (FAO, 2016). Although the Brazilian population does not have the habit of consuming fish, it has the intention to eat (Mitterer-Daltoé et al., 2013), which means there is a potential for increasing consumption of this important source of protein, rich in healthy fatty acids with the correct promotion strategies. In this sense, the stimulus for fish farming emerges as an essential strategy for increasing its consumption (Rosa et al., 2020).

The aquaculture sector needs to expand the variety of fishes to meet the growing global demand (FAO, 2016). Grass carp (*Ctenopharyngodon idella*); pacu (*Piaractus mesopotamicus*) and catfish (*Ictalurus punctatus*) are species with great potential for cultivation and diversification in Brazil and throughout the world (Marques et al., 2019).

Investigations of the lipid quality composition of fish meat is arousing great interest in the scientific community, as it is directly linked to human health. Furthermore, according to Melo et al. (2019), knowledge of the main characteristics of regional fish increases their viability as a food source. It augments the sector's economy, also generating social benefits.

With this objective, by means of chemometric tools, this study characterizes and evaluates the fatty acid profile of three fish species (grass carp, catfish, and pacu) with the potential for fish farming in the southwest region of Paraná, Brazil.

2. Methodology

2.1 Fish samples

The fish species grass carp (n=6), pacu (n=6), and catfish (n=6) were captured at 24 months of age by fish farmers in the municipality of Pato Branco, Paraná, Brazil. The fish were fed BioBase Linha Bioacqua feed. The grass carp additionally received elephant grass (*Pennisetum purpureum*). The average weights were 4.84 ± 1.24 kg, 2.67 ± 0.38 kg, and 0.91 ± 0.20 kg, for the grass carp, pacu and catfish, respectively, chosen based on preferred market sizes. The fish were subjected to percussive stunning, before being measured, eviscerated, and filleted. The resulting samples were placed in plastic bags and stored in ice chests (ice:fish ratio, 3:1) sent to the Food Technology Laboratory of the Universidade Tecnológica Federal do Paraná (UTFPR), where they were freeze-dried for future analysis.

The portion of fillet for each fish specimen analized, corresponded to the front portion, near the head (Lise et al., 2020). Each portion of fillet contained dorsal, ventral, and lateral line corresponding to the anatomy of each fish. The aim was to simulate a portion as would actually be consumed.

2.2 Fatty acid analysis

Lipids were extracted with a binary mixture of chloroform/methanol (Bligh & Dyer, 1959). Transesterification of total lipids was performed according to method 5509 of ISO (1978). The fatty acid methyl esters (FAME) were separated using a gas chromatograph (SHIMADZU), model CG2010 PLUS, equipped with flame ionization detector (CG-DIC), split injector, and 1:50 sample division ratio. A capillary column with 100 m in length, 0.25 mm internal diameter, and 0.25 µm of film thickness. The chromatographic conditions were a programmed column temperature beginning at 60 ºC for 2 minutes, rising to 160 °C, increasing at 3 °C per minute, remaining at this temperature for 20 minutes, and 240 ºC from 31 to 70 minutes. The carrier gas was hydrogen gas (He) 6.0, used at a flow rate of 2 mL min^{-1}, and nitrogen at 25 mL min^{-1}, with an injector temperature of 270 ºC, detector temperature of 300ºC, and injection volume of 1 µL (AOAC, 1995). The identification of fatty acids was made by comparing the retention times of the samples and standards. Authentic fatty acid methyl ester standards (mix 189-19 from Sigma-Aldrich) were used to identify fatty acids. The results were quantified by normalizing the area of each peak over the total fatty acids.

2.3 Data Analysis

The data obtained were evaluated by analysis of variance (ANOVA) and, to compare the means, the Tukey test was performed with a significance level of 5%. To better understand the differences in the lipid profiles between the three fish species, multivariate statistical techniques were applied using discriminant and canonical discriminant analysis (González et al., 2011). The data were processed using the Statistica 12.7 program.

3. Results and Discussion

3.1 Fatty acid profile

Table 1 shows the fatty acid profile of the three fish species evaluated, the sum of saturated fatty acids (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA), and the ratios between SFA and PUFA.

Table 1 Fatty acid profile, summation and ratios for the freshwater fish Grass Carp, Pacu, and Catfish.

Fatty Acids	Grass Carp	Pacu	*Catfish*
	% Peak area		
14:0	$1.67^{b} \pm 0.31$	$2.34^{a} \pm 0.12$	$1.50^{b} \pm 0.12$
14:1	ND	0.32 ± 0.60	ND
15:0	ND	$0.27^{a} \pm 0.12$	$0.20^{a} \pm 0.03$
16:0	$22.2^{a} \pm 0.61$	$22.5^{a} \pm 0.13$	$20.1^{a} \pm 1.00$
16:1	$11.83^{a} \pm 0.68$	$7.60^{b} \pm 0.01$	$5.16^{b} \pm 0.44$
17:0	ND	$0.96^{a} \pm 0.68$	$0.27^{a} \pm 0.05$
18:0	$3.8^{b} \pm 0.46$	$11.3^{a} \pm 0.51$	$5.18^{b} \pm 0.62$
18:1ω-9	$42.7^{a} \pm 1.79$	$42.1^{a} \pm 0.28$	$47.5^{a} \pm 1.49$
18:2ω-6	$11.1^{a} \pm 0.64$	$8.39^{a} \pm 0.57$	$11.0^{a} \pm 0.46$
19:0	$0.25^{a} \pm 0.04$	$0.77^{a} \pm 0.19$	$0.56^{a} \pm 0.05$
18:3ω-6	$1.26^{a} \pm 0.24$	$0.48^{c} \pm 0.15$	$0.93^{b} \pm 0.17$
20:0	$0.15^{a} \pm 0.39$	$0.12^{a} \pm 0.07$	$0.06^{a} \pm 0.08$
20:1	$1.31^{b} \pm 0.28$	$0.89^{c} \pm 0.18$	$1.81^{a} \pm 0.18$
20:4ω-6	$2.52^{a} \pm 0.59$	$0.95^{b} \pm 0.29$	$2.35^{a} \pm 0.91$
22:1	ND	ND	0.09 ± 0.14
24:0	$0.62^{b} \pm 0.17$	$0.56^{b} \pm 0.37$	$2.33^{a} \pm 0.96$
	Sum and ratio		
PUFA	$14.88^{a} \pm 0.96$	$9.82^{b} \pm 2.31$	$14.24^{a} \pm 0.87$
MUFA	$55.87^{a} \pm 1.75$	$50.91^{b} \pm 3.04$	$54.54^{ab} \pm 3.47$
SFA	$28.69^{b} \pm 1.20$	$38.89^{a} \pm 2.65$	$30.23^{b} \pm 2.47$
X	$0.55^{b} \pm 0.34$	$0.38^{b} \pm 0.04$	$0.99^{a} \pm 0.50$
ω-6	$14.88^{a} \pm 0.96$	$9.82^{b} \pm 2.01$	$14.24^{a} \pm 0.87$
PUFA/SFA	$0.52^{a} \pm 0.04$	$0.39^{a} \pm 0.07$	$0.47^{a} \pm 0.02$
Total lipids (g. 100 g^{-1})	$6.80^{b} \pm 2.66$	$12.20^{a} \pm 2.30$	$3.38^{c} \pm 1.92$

X: unidentified fatty acids. ND: Not detected. Values are means ± dp (standard deviation) of analyzes in triplicates. The sums are of fatty acids: SFA (saturated); MUFA (monounsaturated); PUFA (polyunsaturated); ω-6 (omega-6); PUFA / SFA (ratios of the sum of polyunsaturated / saturated fatty acids. Values in the same line followed by the same letters do not differ from each other (p>0.05). N = 6. Source: Authors (2022).

Sixteen fatty acids were identified in the fatty acid profile, being the majority of the three fish species, oleic acid (18:1ω-9) followed by palmitic acid (16:0), both showing no significant difference (p>0.05) between species. According to Swapna et al. (2010), the predominance of these acids seems to be characteristic of freshwater fish. Monounsaturated fatty acids were the majority in all three species studied, with levels that ranged from 50 to 56%, followed by saturated (29 - 39%) and polyunsaturated (10 - 15%) fatty acids. This means that the meat of the evaluated fish is more vulnerable to sensory changes caused by lipid oxidation, which reinforces the need for care with the conservation of this food. Among the species evaluated, the Pacu presented the highest percentage of saturated fatty acids (SFA) and the lowest percentage of MUFA and PUFA, suggesting a meat with greater oxidative stability of lipids and prolonged storage period. The ratios between polyunsaturated/saturated fatty acids (PUFA/SFA) ranged from 0.39 to 0.52, with no statistical difference between species. The nutritional quality of a lipid profile of a food can be evaluated by the PUFA/SFA ratio. The higher the proportion, the greater the reduction in total cholesterol in blood plasma Saldaña et al. (2015). Values below 0.45 are not recommended for a healthy diet since they can present a potential to induce an increase in blood cholesterol, which suggests the moderate consumption of pacu meat (HMSO, 1984).

The content of ω-6 was determined based on the sum of linoleic (18:2ω-6), gamma-linolenic (18:3ω-6), and arachidonic (20:4ω-6) polyunsaturated fatty acids. The pacu species had the lowest percentage (9.8%) of ω-6, differing significantly from the carp and Catfish, which in turn presented the highest sums (14.8 and 14.2% respectively), with linoleic acid being the major contributor of the class. No fatty acids representing the ω-3 family were identified in any of the species evaluated. Such results corroborate previous studies found in the literature that suggest that freshwater fish have a higher percentage of ω-6 fatty acids. In contrast, saltwater fish have a high content of ω-3 fatty acids (Fernandes et al., 2014).

3.2 Discriminating fatty acids between species

It is considered indispensable to classify food products that wish to be placed on the market (Mitterer-Daltoé et al., 2012). The multivariate techniques of discriminant and canonical discriminant analysis have been applied to multivariate biological data, indicating the parameters that are essential to separate interest groups (Marques et al., 2021).

The multivariate discriminant and canonical discriminant analysis techniques were employed to better understand the differences in lipid profiles between the three fish species (Table 2). The primary objective of discriminant analysis is to understand group differences and predict the likelihood that an entity (individual or object) will belong to a specific class or group based on independent metric variables (Mitterer-Daltoé et al., 2012). The data submitted to this analysis showed the value of Lambda Wilks used to indicate the statistical significance of the discrimination power of each variable when value = 1 (no discriminatory power) and value = 0 (perfect discriminatory power). The saturated fatty acids that best discriminated the fish were the pentadecylic (15:0), palmitoleic (16:1), marginal (17:0), stearic (18:0), arachidonic (20:4ω-6), and lignoceric (24:0) fatty acids. Palmitoleic acid 16:1 (ω-7) is an essential fatty acid and may present an anti-inflammatory activity. It was found in high quantities in the grass carp species (11.8%), while stearic acid (18:0) was identified in higher concentration in the Pacu species (11.3%).

Table 2. Statistical significance of each variable discrimination and Canonical analysis function.

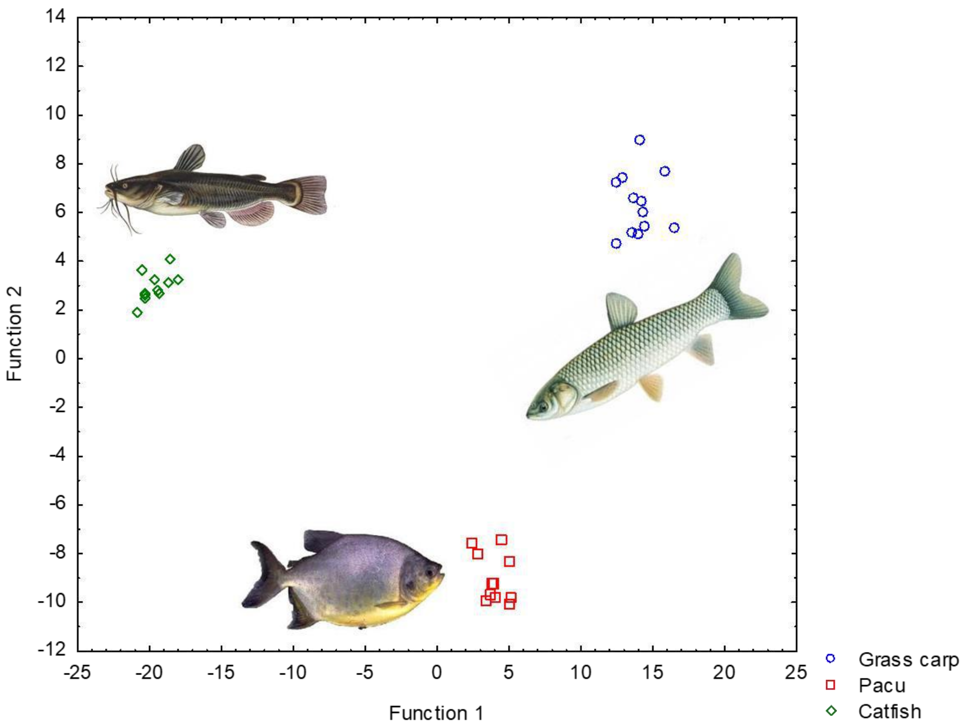
Fatty acids	Lambda Wilks	F	P
14:0	0.817510	2.00905	0.163093
14:1	0.754035	2.93579	0.078799
15:0	0.613397	5.67239	0.012294
16:0	0.726298	3.39162	0.056238
16:1	0.142745	54.04944	<0.0001
17:0	0.427419	12.05661	0.000476
18:0	0.388965	14.13832	0.000204
18:1ω-9	0.986953	0.112366	0.894375
18:2ω-6	0.957518	0.377122	0.691428
19:0	0.949208	0.454835	0.642054
18:3ω-6	0.822072	1.94794	0.171471
20:0	0.790723	2.38198	0.120843
20:1	0.767092	2.73262	0.091966
20:4ω-6	0.192513	37.75021	0.000000
24:0	0.373482	15.09755	0.000141
	Canonical R	**Chi square**	**P**
Function 1	0.997651	231.8590	<0.0001
Function 2	0.989957	97.8183	<0.0001

Source: Authors (2022).

The results of the canonical discriminant analysis revealed the total explained variation of the fatty acids that best discriminate between fish species of 99% for function 1. Canonical discriminant functions are linear combinations of the original variables aimed at defining which of these cause maximum separation between groups, which is equivalent to minimizing variability within classes. The functions obtained allow for discrimination of the elements belonging to the different classes of the groups in the best possible form (González et al., 2011). Other works showed good results from canonical discriminant analysis applied with the aim of better resolving the main variables that differentiated the food samples (Mitterer-Daltoé et al., 2013; Marques et al., 2021). Mitterer-Daltoé et al. (2012) showed the squared value of the canonical R for the function explains 90% of the quality of the meat of different breeds. Marques et al. (2021) demonstrated a total variation, explained by Function 1 of 99%, to explain bioactive properties of yacon juice during long-term refrigerated storage.

Figure 1 corroborates the high discrimination power of fatty acids between the three species and validates the canonical discriminating functions. The joint graph of the sample element in the plane of the first two functions shows how well the fish species were separated.

Figure 1. Graph of the first two functions of the canonical discriminant analysis.



Source: Authors (2022).

4. Conclusion

The fish species studied are important sources of fatty acids for Brazilians. Significant differences were recorded in the total lipid content, resulting in a classification of fatty fish for Pacu, intermediate fish for grass carp, and lean fish for Catfish. Oleic acid was the majority in the fatty acid profile for all three species, resulting in a high percentage of monounsaturated fatty acids. The Pacu presented the lowest content of polyunsaturated fatty acids. The lipid profile proved to be an important parameter of discrimination between species, as also discriminant analysis proved to be an important tool to differentiate lipid profile between species. The pentadecylic (15:0), palmitoleic (16:1), margaric (17:0), stearic (18:0), arachidonic (20:4), and lignoceric (24:0) fatty acids presented the highest power of discrimination between the species studied.

Thus, in a country like Brazil, characterized by low fish consumption, but with the intention of increasing consumption, grass carp, pacu, and catfish are important alternative species to increase fish consumption and diversify fish farming in specific regions of the country.

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