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Amino acid and fatty acid profiles of materials recovered from Prussian carp, Carassius *gibelio* (Bloch, 1782), using acidic and basic solubilization/ precipitation technique

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ABSTRACT

Isoelectric solubilization /precipitation (ISP) process was used to isolate protein from muscles of Prussian carp, *Carassius gibelio* (Bloch, 1782). Fish protein and lipid were recovered from whole gutted Prussian carp using acidic and basic isoelectric solubilization/precipitation followed by assaying amino acid and fatty acid profile. Essential amino acids content in acidic and basic pH treatment of ISP of Prussian carp were 216.6 and 218.7 $mg.g^{-1}$, respectively. Results showed that identified amino acids in Prussian carp protein isolated by ISP method, could meet all needs of adults, but a supplementary protein must be used for children. Limiting amino acids in both acidic and basic treatments were methionine and cysteine. Nineteen fatty acids of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acid (PUFA) groups were identified in lipid recovered in isoelectric solubilization/precipitation process. Total PUFA in basic treatment was noticeably higher than in acidic one. N-3/n-6 ratio in basic pH treatment was also higher than in acidic one.

Key words: Prussian carp, Amino acid profile, Fatty acid profile, Isoelectric solubilization/ precipitation.

INTRODUCTION

Prussian carp, *Carassius gibelio* (Bloch, 1782) is one of the six species of farmed fish currently in polyculture system in China (Chiu *et al*. 2013). This fish is introduced for some polyculture systems to take maximum benefits of fish production for the food available (Billard & Berni 2004). It can be both unintentionally and intentionally a part of carp polyculture. It is also a strong competitor of common carp, because a large population of young fish can grow in ponds, by consuming both natural and artificial diets of common carp (Woynarovich *et al*. 2010). However, it considered as undesirable fish in Chinese carp polyculture system in Iran by creating some problems in husbandry management (Ghenaat Parast 1995).

Its commercial value is lower than that of other Chinese reared carp due to its small size and low acceptability by consumers. Prussian carp contain a large amount of fine inter-muscular bones, which makes it difficult to eat for people who are not accustomed to these bones. So that, it may limit its desirability to consumers.

Isoelectric solubilization/precipitation (ISP) is a relatively new technique to recover muscle proteins and lipids from various animal sources, including seafood (Jaczynski & Tahergorabi 2015). ISP allows a good recovery of fish protein isolate (FPI) that might be used

in functional foods (Tahergorabi *et al*. 2012), while significantly reducing their fat content (Chen & Jaczynski 2007; Taskaya *et al*. 2009a). ISP allows selective, pH-induced water solubility of muscle proteins with concurrent separation of lipids (Gehring *et al*. 2011). ISP treatment offers several advantages over mechanical processing. It seems to be a useful technology to retrieve functional and nutritious proteins of any eviscerated fish (i.e., without filleting) or fish processing by-products (i.e., frames, heads, etc.) for subsequent development of food products (Tahergorabi *et al*. 2012).

Isoelectric solubilization / precipitation technique has been applied to the whole fish, the fish muscle (Hultin & Kelleher 1999; Taskaya *et al*. 2009a; Taskaya *et al*. 2010; Tahergorabi *et al*. 2012) and the fish processing by-products (Chen & Jaczynski 2007; Chen *et al*. 2007; Gehring *et al*. 2011). Due to the limits in production of capture fisheries as well as blooming human population over the past few years (Taskaya & Jaczynski 2009), conversion of the by-products and low-value species from aquatic resources for human food has been found to create commercial benefits. *C. gibelio* is a nutritious source of protein, but is not accepted in Iranian market because of its small size, boney structure, etc. It has been hypothesized that ISP of *C. gibelio* will result in efficient recovery of high quality protein and lipids to fulfill human nutritional needs. In order to initiate the assessment of its nutritional quality, it is necessary to study the amino acid and fatty acid profiles of its recovered protein and lipid using this novel technique. The objectives of this study were to characterize the amino acid profile of the recovered protein as well as fatty acid profile of the recovered lipid from Prussian carp with isoelectric solubilization / precipitation using basic and acidic pH treatments.

MATERIALS AND METHODS

Raw material

For this study, a total of 56 pieces of Prussian carp, with an averaged 300 ± 50 g in weight and 20 ± 2 cm in length were purchased from a fish farm (Bandar Turkman, Golestan Province, Iran). The fish were placed in insulated box with ice and delivered within 10 h to Food Science Laboratory in Khorasan-e-Razavi Agricultural and Natural Resources Research Center (Mashhad, Iran). Once arriving, fish were washed, beheaded, eviscerated, re-washed and mechanically filleted. The prepared fillet skinned and then fish meat was minced by a kitchen meat mincer (Biro 812, DCE, USA). Fish mince was randomly divided in 2 homogenous groups for acidic and basic treatment, packaged in polyethylene pouch and kept at -20°C until examination.

Protein isolate from *C. gibelio* mince by ISP method

Frozen mince were thawed in a refrigerator at 4°C during 4h. Thawed minced fish was weighed (140 g) and homogenized with cold (1-3°C) distilled and deionized water at a ratio of 1: 6 (ground fish: water, w: v) using a laboratory homogenizer (Digital, IKA, Germany) set at high speed for 10 min. The homogenized sample was transferred into a beaker. During the entire ISP processing, temperature was carefully set at 4°C. The homogenizing /mixing of the specimens were continued with a laboratory homogenizer during the following pH adjustment steps. The pH of the homogenates was separately adjusted to 2.50 ± 0.05 with 6N HCl, as well as 11.5 ± 0.05 with 10N NaOH in order to solubilize proteins at acidic and basic pH ranges, respectively. These reagents were used for pH adjustments, during both protein solubilization and subsequent precipitation (pH = 5.5). pH was measured by digital pH meter (780, Metrohm, Switzerland). Once the desired pH was obtained, the solubilizing reaction has been allowed to take place for 10 min, followed by centrifugation at 10000 x g and 4°C for 10 min using a laboratory refrigerated centrifuge (Z23HK, HERMLE, Germany). The centrifugation resulted in 3 distinct phases: top phase- lipids, middle phase-muscle protein solution, and bottom phase- insoluble.

The upper layer was collected and kept at-20°C for further fatty acid analysis of profile, while

bottom layer discarded. The solution of solubilized protein was collected for subsequent pH adjustment to encourage isoelectric precipitation of proteins. The pH of the collected supernatant was adjusted to 5.50 ± 0.05 in order to isoelectrically precipitate muscle protein. Once the desired pH was obtained, the solubilizing reaction has been allowed to take place for 10 min. After 10-min precipitation, the solution was centrifuged as described previously.

Precipitated proteins were collected from bottom layer of centrifuge tube and freeze-dried prior to amino acid profile analysis.

Protein content

Crude protein was calculated by converting the nitrogen content determined by Kjeldahl's method (AOAC 2005).

Amino acid profile analysis

The procedure used for the amino acid profile analysis was based on PICO TAG method with slight modification (Shang-gui *et al*. 2004; Matloubi *et al*. 2004). Samples were hydrolyzed in 6M HCl for 24h at 110°C and analyzed by HPLC using a reversed phase column.

Fatty acid analysis

The lipid samples were converted into their constituent fatty acid methyl esters according to Metcalf *et al*. (1966).

Analysis of fatty acid methyl esters was performed by a Unicam 4600 with a bpx 70 capillary column (30.0 m x 0.25 mm i.d) and quantified by FID detector. The split ratio was 10:1.

The GC condition was as follows: injection port temperature was 250°C and FID temperature was 300°C. Oven temperature program was set at an initial temperature of 140°C for 5 min, then raised to 180°C at 20°C min^{-1}, held for 9 min, again was raised to 200°C at 20°C min^{-1} and held for 25min.

The size of the sample injected for each analysis was 1 mL (Metcalf *et al*. 1966). The samples were manually injected into the GC port. Compounds were identified by comparing the retention time of known standards. According to the results of fatty acid analysis, atherogenic (AI) and thrombogenic indices (TI) were calculated with the equations proposed by Ulbricht & Southgate (1991).

Statistical analysis

Data statistical analysis was performed with SPSS software (version 21). The normality of the data verified using Kolmogorov-Smirnov test. Significant difference between the two treatments was determined using t- test with 5% significance.

RESULTS AND DISCUSSION

Amino acid profile

Table 1 shows the protein content in raw and recovered powder from *C. gibelio* using acidic and basic ISP technique. The crude protein content of *C. gibelio* was 19.01% on a wet basis.

By comparing with those of common carp and three Indian carp conducted by Elyasi *et al*. (2010) and Memon *et al*. (2011), Prussian carp in the present study was characterized by high level of protein content, indicating that it has a sufficient amount of protein to be recovered. Protein recovered from *C. gibelio* by ISP technique resulted in a protein recovery yield of 96.92 and 85.77% on dry basis in acidic and basic treatments, respectively (Table 1).

The protein recovery yield in both acidic and basic treatments in the present study was higher than those for rainbow trout (Chen & Jaczynski 2007) and common carp (Azadian & Moosavi-Nasab 2013). Crude protein reported by Taskaya *et al*. (2009b) for whole carp was concentrated to 89−90% at acidic treatment and to 94−95% at basic one. Chen & Jaczynski (2007) pointed out that the slight differences in protein recovery by ISP method may probably be attributed to different methods used to determine the protein concentration. Similar to previous studies (Kristinsson & Liang 2006; Chen & Jaczynski 2007) on ISP isolation, in the present study, the higher protein recovery yields was found at acidic than at basic pH. Based on the results, the isoelectric solubilization / precipitation technique allowed successful isolation of *C. gibelio* protein. To assess the quality of recovered

proteins, the amino acid composition was determined. The essential and non-essential amino acid composition is shown in Tables 2 and 3, respectively.

The predominant amino acids among the nonessentials were glutamic and aspartic acids, while among essentials were lysine and leucine. Similar results were reported by Usydus *et al*. (2009) for amino acid profiles of fish products available in Poland.

Although the total amino acid (TAA) content was higher in acidic pH treatment compared to basic one, the latter showed higher EAAs content. Similar result was reported by Chen *et al*. (2007). ISP at basic pH allows the recovery of muscle protein of higher nutritional quality, assessed by a greater amount of essential amino acids (EAA) comparing to acidic one (Chen *et al*. 2007; Gigliotti *et al*. 2008).

Although acidic solubilization resulted in a slightly higher protein recovery yield, it is a general consensus that solubilization at basic pH gives overall better results (Jafarpour & Gorczyca 2008). Higher ($P > 0.05$) isoleucine and threonine content was found in recovered protein by basic treatment than those by acidic one, whereas the methionine and phenylalanine content were higher ($P < 0.05$) in basic treatment. In the present study, the total content of EAAs ranged from 216.60 to 218.70 $mg.g^{-1}$ of protein, which is lower than the values reported by Chen *et al*. (2007) for the trout processing by-products. The total content of EAAs in isolated protein of *C. gibelio* constituted approximately 39% and 40% of total amino acids in acidic and basic treatments, respectively (Table 2). Chen *et al*. (2007) have reported TEAA/TAA ratios in their experiments to be 38.9%, 45.9% and 48.8% for the by-products and the protein recovered at acidic and basic pH, respectively.

Protein recovered from *C. gibelio* by ISP method at basic pH resulted in higher content of EAAs and lower content of total non-essential amino acids (TNEAAs) than at acidic one (Tables 2 and 3).

The ISP at basic pH generally yielded a higher amount of EAA than ISP at acidic pH (Chen *et al*. 2007; Taskaya *et al*. 2009a). This has probably been due to the less pH-induced by proteolysis at basic pH. Proteolysis can lead to partially hydrolyzed proteins and eventually releasing free amino acids.

The lysine content, as a very essential amino acid, was approximately 45.30 and 44.70 $mg.g^{-1}$ in protein recovered by ISP method at acidic and basic pH, respectively, which was significantly lower than that of whole egg (70 $mg.g^{-1}$) and those reported by Chen *et al*. (2007) (67 to 78 $mg.g^{-1}$).

The essential amino acid (EAAs) content of protein recovered by acidic and basic pH treatment of ISP has been compared to values recommended by the FAO / WHO (1990) for adults and infants (Table 4).

The protein isolated from *C. gibelio* met the recommended energy and protein needs of FAO/WHO (1990) amino acid reference pattern established for humans.

The limiting amino acid in protein isolated from *C. gibelio* has been found to be methionine + cysteine.

Table 1. Protein content in raw and recovered powder from *C. gibelio* using acidic and basic ISP technique.

Treatment	Protein content
Raw fish	19.01 ± 0.01% wet basis
Acidic treatment	96.92 ± 0.01% dry basis
Basic treatment	85.77 ± 0.02% dry basis

Table 2. Essential amino acid (mg.g^{-1}) in protein recovered from *C. gibelio* using acidic and basic ISP technique.

Amino acid (AA)	Treatments	
	Acidic ISP process	**Basic ISP process**
Valine	28.10 ± 0.26 [a]	28.90 ± 0.35 [a]
Lysine	45.30 ± 0.23 [a]	44.70 ± 0.24 [a]
Leucine	46.00 ± 0.20 [a]	46.40 ± 0.00 [a]
Isoleucine	24.50 ± 0.20 [b]	26.10 ± 0.36 [a]
Methionine	10.90 ± 0.15 [a]	9.30 ± 0.54 [b]
Threonine	25.10 ± 0.33 [b]	28.90 ± 0.84 [a]
Histidine	13.10 ± 0.13 [a]	12.90 ± 0.12 [a]
Phenylalanine	23.60 ± 0.48 [a]	21.50 ± 0.43 [b]
Total Essential Amino Acid (TEAA)	216.60	218.70
Total Amino Acid (TAA)	549.60	546.50
TEAA/TAA	0.394	0.400

Data are given as mean and S.D. (n=2).
Means value in rows with different small letters indicate significant differences ($P < 0.05$).

Table 3. Non-essential amino acid (mg.g^{-1}) in protein recovered from *C. gibelio* using acidic and basic ISP technique.

Amino acid (AA)	Treatments	
	Acidic ISP process	**Basic ISP process**
Aspartic acid	55.80 ± 0.50 [b]	61.20 ± 0.98 [a]
Glutamic acid	100.50 ± 1.69 [a]	101.20 ± 2.92 [a]
Serine	26.30 ± 0.68 [a]	26.60 ± 1.21 [a]
Glycine	28.30 ± 0.33 [a]	24.60 ± 0.48 [b]
Arginine	45.20 ± 0.20 [a]	40.70 ± 0.07 [b]
Alanine	23.60 ± 0.28 [a]	23.80 ± 0.42 [a]
Proline	19.00 ± 0.46 [a]	17.50 ± 0.24 [b]
Tyrosine	27.10 ± 0.15 [a]	24.30 ± 0.14 [b]
Cysteine	7.20 ± 0.13 [a]	7.90 ± 0.25 [a]
Total Non-essential Amino Acid (TNEAA)	333.00	327.80
Total Amino Acid (TAA)	549.60	546.50
TNEAA/TAA	0.606	0.600

Data are given as mean and S.D. (n=2).
Means value in rows with different small letters indicate significant differences ($P < 0.05$).

Table 4. Essential amino acid composition (mg.g^{-1}) of recovered protein from *C. gibelio* compared with FAO/WHO pattern of energy and protein requirements.

Amino acid (AA)	Treatments		FAO/WHO Pattern	Percentage for Acidic pH	Percentage for basic pH
	Acidic pH	Basic pH			
Valine	28.10	28.90	50	56.2	57.8
Lysine	45.30	44.70	55	82.3	81.3
Leucine	46.00	46.40	70	65.7	66.3
Isoleucine	24.50	26.10	40	61.2	65.2
Methionine + Cysteine	18.10	17.20	35	51.7	49.1
Threonine	25.10	28.90	40	62.7	72.2
Histidine	13.10	12.90	20	65.5	64.50
Phenylalanine + Tyrosine	50.70	45.80	60	84.5	76.3

Fatty acid profile

Table 5 shows the fatty acid compositions of *C. gibelio* in the acidic and basic pH treatments. A total of 19 fatty acids were detected by using GC. There were some differences in the fatty acid profile between acidic and basic treatments. In the acidic treatment, SFA was the most abundant group of fatty acids, followed by MUFA, while in basic one, MUFA was the most abundant group of fatty acids followed by the SFA. A similar pattern of fatty acids in acidic and basic treatments was found in silver carp (Zakipour Rahimabadi & Dad 2012) and common carp (Zakipour Rahimabadi *et al*. 2011) respectively. It is well documented that, in addition to the environmental and the biological factors, processing has detrimental effect on lipid content and fatty acid composition (Sigurgisladóttir & Pálmadóttir 1993; Aubourg 1999). In the acidic and basic treatments, the quantity of fatty acids in descending order were C16:0 >C18:1c > C18:0 and C16:0 >C18:1c > C16:1, respectively.

It has pointed out that the FA composition of the lipids recovered during isoelectric solubilization / precipitation of trout processing by-products at acidic and basic treatments was similar (P > 0.05) to the FA composition in the starting material, except for ALA and LA (P < 0.05). The pH treatments during solubilization of the protein in trout processing by-products did not affect (P > 0.05) affect the composition of n-3 and n-6 PUFAs and, consequently, the ratio of n-3/n-6 in trout recovered lipids (Chen *et al*. 2007).

Basic treatment recovered higher total n-3 fatty acids and EPA+DHA content compared to acidic one. Ratios of n-3/ n-6 were 0.40 and 1.35 in acidic and basic treatments, respectively. In the present study, higher ratio of n-3/ n-6 in basic treatment is in disagreement with the results of Chen *et al*. (2007) who recovered lipids from rainbow trout processing by-products using isoelectric solubilization /precipitation. The atherogenic (AI) and thrombogenic (TI) indices were 1.05 and 1.17 in acidic and 0.75 and 0.60 for basic treatments, respectively. Values obtained in the present study are slightly higher than those reported for horse mackerel and bouge by Orban *et al*. (2011), for raw materials roes of blue fin tuna by Garaffo *et al*. (2011), and for red porgy by García-Romero *et al*. (2014).

Pepping (1999) pointed out that the human body's optimal balance between omega-6 and omega-3 fatty acids is 2:1 to 4:1. Thus, the fish can help this balance between omega-6 and omega-3 due to high content of the n-3 fatty acids. The atherogenic (AI) and thrombogenic (TI) indices, as proposed by Ulbricht & Southgate (1991), give an indication of the attitude of a composite diet or a food for the protection of atherosclerosis and platelets aggregation. Better values of atherogenicity

(AI) and thrombogenicity (TI) indices in basic treatment of *C. gibelio* could promote a positive effect on nutritional value for human consumption. It is reported by Taskaya *et al*. (2009b) that ISP has no impact on the fatty acid (FA) composition.

Table 5. Fatty acid profile (g.100g^{-1}) in lipid recovered from *C. gibelio* during ISP technique.

Fatty acids	Treatments	
	Acidic ISP process	Basic ISP process
C14:0	3.83 ± 0.20 ^{b}	4.19 ± 0.15 ^{a}
C16:0	30.64 ± 0.50 ^{a}	26.27 ± 0.55 ^{b}
C17:0	0.77 ± 0.01 ^{a}	0.42 ± 0.03 ^{b}
C18:0	10.85 ± 0.25 ^{a}	4.76 ± 0.09 ^{b}
C24:0	0.00 ± 0.00 ^{b}	0.24 ± 0.02 ^{a}
Σ Saturated fatty acids (SFA)	46.09	35.88
C16:1	6.24 ± 0.20 ^{b}	13.78 ± 0.70 ^{a}
C17:1	0.64 ± 0.05 ^{b}	1.21 ± 0.07 ^{a}
C18:1 *cis*	27.18 ± 0.45 ^{a}	25.38 ± 0.90 ^{b}
C18:1 *trans*	3.80 ± 0.18 ^{a}	5.38 ± 0.15 ^{b}
Σ Mono-unsaturated (MUFA)	38.04	45.75
C18:2 n-6 *cis*	6.97 ± 0.30 ^{a}	5.51 ± 0.20 ^{b}
C18:3 n-6	0.00 ± 0.00 ^{b}	0.19 ± 0.03 ^{a}
C18:3 n-3	0.41 ± 0.07 ^{b}	1.00 ± 0.12 ^{a}
C20:3 n-9	0.21 ± 0.05 ^{b}	0.61 ± 0.15 ^{a}
C20:3 n-3	0.37 ± 0.09 ^{a}	0.10 ± 0.05 ^{b}
C20:4 n-6	4.12 ± 0.14 ^{a}	1.41 ± 0.08 ^{b}
C20:5 n-3 (EPA)	1.59 ±0.03 ^{b}	3.59 ± 0.05 ^{a}
C22:4 n-6	0.00 ± 0.00 ^{b}	0.39 ± 0.04 ^{a}
C22:5 n-3	0.37 ± 0.03 ^{b}	1.73 ± 0.02 ^{a}
C22:6 n-3 (DHA)	1.76 ± 0.08 ^{b}	3.76 ± 0.10 ^{a}
Σ Poly-unsaturated (PUFA)	15.80	18.29
Σ EPA + DHA	3.35	7.35
Σ n-3	4.50	10.18
Σ n-6	11.09	7.50
Σ n-3/ Σ n-6	0.40	1.35
AI	1.05	0.75
TI	1.17	0.60

Data are given as mean and S.D. (n=2).

Means value in rows with different small letters indicate significant differences ($P < 0.05$).

Isoelectric solubilisation /precipitation (ISP) is an efficient method for the recovery of functional and nutritional proteins and lipids of Prussian carp. In addition to the use of this low-value fish, protein and lipid extracted by ISP method could be used in functional foods. Although the acidic treatment has resulted in a higher protein recovery, but ISP method at basic pH resulted in higher content of EAAs and lower content of total non-essential amino acids (TNEAAs). Basic treatment showed higher total n-3 fatty acids as well as n-3/n-6 ratio compared to acidic one. The present study indicates that proteins and lipids recovered during isoelectric solubilization /precipitation of whole Prussian carp may be useful in several food and nonfood applications, contributing to more responsible utilization of aquatic bio-resources.

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ترکیب اسید آمینه و اسید چرب پروتئین و چربی بازیافت شده از ماهی کاراس معمولی (*Carassius gibelio*) با استفاده از تکنیک انحلال و ترسیب اسیدی و بازی

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چکیده

در این تحقیق، از روش انحلال و ترسیب ایزوالکتریک جهت ایزوله کردن و استخراج پروتئین از ماهی کاراس معمولی (*Carassius gibelio*) استفاده شد. پروتئین و چربی بازیافت شده از ماهی کاراس معمولی در جریان استفاده از ISP اسیدی و بازی برای بررسی پروفایل اسید آمینه و اسید چرب بررسی شد. محتوای اسیدهای آمینه ضروری در ISP اسیدی و بازی به ترتیب ۲۱۶/۶ و ۲۱۸/۷ میلی‌گرم در هر گرم بود. نتایج نشان دادند که پروفایل اسید آمینه به دست آمده از پروتئین بازیافت شده از کاراس معمولی به روش ISP، تامین کننده تمامی نیازهای افراد بالغ است، ولی کودکان نیازمند مکمل پروتئینی هستند. اسیدهای آمینه محدود کننده در پروتئین‌های استخراجی در هر دو تیمار اسیدی و بازی متیونین و سیستئین بودند. نوزده اسید چرب از انواع اشباع (SFA)، تک غیر اشباعی (MUFA) و چند غیر اشباعی (PUFA) در چربی بازیافتی از پروسه ISP شناسایی شدند. مقدار PUFA در تیمار بازی به طور قابل ملاحظه‌ای بیش از تیمار اسیدی بود. نسبت اسیدهای چرب امگا-۳ به امگا-۶ نیز در تیمار بازی بیش از این نسبت در تیمار اسیدی بود.

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