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Nutritional quality of spray dried protein hydrolysate from Black Tilapia (*Oreochromis mossambicus*)

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Abstract

The nutritional quality of spray-dried protein hydrolysate from black tilapia, a fresh water fish, was evaluated. Hydrolysed protein from *Oreochromis mossambicus* was spray-dried at two different temperatures of 150 °C/76 °C (inlet/outlet temp) and 180 °C/90 °C. Proximate analyses revealed that the dried hydrolysates consisted of 37.7–49.6% protein, 2.6–2.8% fat, 1.6–4.0% moisture and 8.6–8.7% ash. The higher drying temperature used was found to significantly decrease the contents of all amino acids analysed. Nevertheless, the protein quality of both dried hydrolysates was found to be high, with in vitro digestibilities of 88.4 and 92% and protein digestibility corrected amino acid scores of 0.34 and 0.82, respectively. In addition, the predicted protein efficiency ratios of the dried hydrolysates were calculated to be 2.97 and 2.53.

Keywords: Protein hydrolysate; Spray drying; Tilapia

1. Introduction

Interest in fish protein hydrolysates dates back to the 1960s, with most of the work being directed towards the use of fish protein for animal feed (Keyes & Meinke, 1966) and non-dietary purposes (Sen, Sripathy, Lahiry, Sreenivasan, & Subrahmanyan, 1962; Sripathy, Sen, Lahiry, Sreenivasan, & Subrahmanyan, 1962) rather than for human food.

The first investigation into fish protein hydrolysis for human consumption was described by Bertullo and Pereira (1970) and Rutman (1971). The hydrolysates were reconstituted to milk-like products and they had excellent nutritional properties (Yanez, Ballester, & Monckeberg, 1976). In recent years, interest in the use of fish protein hydrolysates for human consumption has been increasing. Yu and Tan (1990) found that a fish cracker, containing protein hydrolysate from *Oreochromis mossambicus* (black Tilapia), was acceptable in terms of appearance, crispiness and colour.

Fish protein hydrolysate is traditionally handled in the liquid form. Spray drying is one of several alternative methods of converting this liquid product into powder form, which has the added advantage of ease of handling and increased stability. Spray-drying of liquid biomaterial into powder is globally employed for various reasons. However, the drying process could cause some detrimental effects to the final product quality. Therefore, the objective of this paper was to develop powdered protein hydrolysates from *O. mossambicus* using spray-drying at two different temperatures. Evaluation of the nutritional qualities of the powdered protein hydrolysates produced was also carried out.

2. Materials and methods

2.1. Raw materials

Black Tilapia (*Oreochromis mossambicus*) was obtained live from a local supplier near the campus. The enzyme used for the hydrolysis was Alcalase, a commercial protease obtained from Novo Nordisk A/S, Denmark.

2.2. Preparation of fish slurry for enzymatic hydrolysis

The fish were gutted and deheaded immediately upon arrival at the laboratory and deboned using a double-drum

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fish deboner. The flesh was then washed 3–4 times to remove water-soluble nitrogenous compounds, minerals, naturally occurring proteolytic enzymes and pigments (Yanez et al., 1976). Excess water was then drained and the flesh was packed in PVC bags and stored at −30 °C until required for further use. Fish meat (813.3 g) was mixed with distilled water (809.2 g) with the ratio of flesh to water fixed according to a pH stat technique, for determination of degree of hydrolysis. The flesh was homogenised with distilled water using a Waring blender for 60 s and the pH adjusted to 8.0 using 2N NaOH and 2N H_2SO_4. The temperature of the slurry was raised to 70 °C for 30 min, then brought down to 55 °C.

2.3. Enzymatic protein hydrolysis

The production of a hydrolysate was performed according to the procedure of Adler-Nissen et al. (1986). Previous work had shown that the optimum enzyme to substrate ratio (E/S) for a tilapia hydrolysate preparation was 2% (Au et al., 1997). Thus, in this study, 2% E/S was used to get optimum hydrolysate production. The enzyme solution was introduced to the fish slurry with continuous stirring using an agitator (IKA-WERK RE 162) maintained at 300 rpm. The pH (8.0) and temperature (55 °C) of the hydrolysis process were maintained constant throughout the process. After 5 h of hydrolysis, the process was terminated by raising the hydrolysate temperature to 90 °C for 20 min (Adler-Nissen et al., 1986). After the mixture cooled down, it was centrifuged at 3000 rpm for 30 min (4 °C) to separate the soluble from the insoluble fraction. The hydrolysate was then placed in a conical flask and kept at −30 °C until spray-drying. A control sample underwent all the above processes without the addition of enzyme solution.

2.4. Production of fish protein hydrolysate powder

The liquid hydrolysate was mixed with 10% maltodextrin (w/w) in a 2l beaker. The mixture was stirred continuously to prevent sedimentation of the maltodextrin and was slightly heated to let the maltodextrin gelatinise and encapsulate the volatile compounds of the hydrolysate. The fluid was then spray-dried, using an Anhydro spray drier (Lab S1 W.O. 1726, Denmark) at two different temperatures of 76/90 °C and 150/180 °C (Jamilah et al., 1999).

2.5. Proximate analysis

Crude protein and fat contents were determined by the micro-Kjeldahl and Soxhlet method of AOAC (1990), respectively. Moisture was determined using the air oven AOAC method (1990) and ash using the basic AOAC method (1990), by heating the samples in the furnace at 550 °C for 8–12 h.

2.6. Degree of hydrolysis (DH)

Calculation of degree of hydrolysis was according to the method of Adler-Nissen (1986).

$$DH = B \times N_b \times 1/\alpha \times 1/MP \times 1/h_{tot} \times 100$$

where

B = volume of base titration NaOH (ml)
N_b = Normality of base
α = average degree of α-NH breakage
$1/\alpha = 1.13$ (Adler-Nissen, 1986)
$h_{tot} = 8.6$ (Adler-Nissen, 1986)

2.7. Amino acid analysis

The PICO TAG method, with modification, was employed for determining the amino acid profile of the hydrolysate (Bidlingmeyer, Cohen, Tarvin, & Frost, 1987). The dry sample (weight equivalent to 4% protein) was added with 6N HCl (15 ml) and placed in the oven at 110 °C for 24 h. Ten millilitres of internal standard was added to the mixture. After derivatisation, 100 μl PICO TAG diluent was added and mixed. 100 μl sample were then injected into the HPLC and analysed with a Water's PICO TAG amino acid analyser.

2.8. In vitro protein digestibility (IVPD)

An in vitro enzymatic pH-stat procedure was used to determine IVPD of the dried hydrolysate (Pederson & Eggum, 1983). A solution containing all three enzymes was prepared as follows: sufficient amounts of porcine pancreatic trypsin (Type IX, Sigma 7-0134), bovine pancreatic chymotrypsin (Type II, Sigma C-4129) and porcine intestinal peptidase (Grade K, Sigma P-7520) were dissolved to give per ml: 23,100, 186 and 0.052 units, respectively. pH was adjusted to 8.0 at 37 °C and maintained for exactly 2.0 min. The mixture was then transferred to an ice bath and kept at 0 °C. The 3-enzymes solution was prepared fresh daily. At the same time, an aqueous suspension of sodium caseinate was allowed to stand at 4 °C for at least 1 h but not longer than 24 h.

2.9. Statistical analysis

All the tests were done in triplicate and data were averaged. Standard deviation was also calculated. All proximate analyses were analysed by using ANOVA and Duncan's multiple range test, using the SAS (Statistical Analysis System Institute, 1989) programme.

3. Results and discussion

3.1. Tilapia hydrolysate production

In the initial stage of hydrolysis, the fish slurry was difficult to stir due to its viscosity. The viscosity, however, dropped rapidly, once the enzyme started to hydrolyse the substrate. As a result, the stirring became easier and the slurry became watery and could then be treated as a free flowing liquid (Mackie, 1983). The optimum degree of hydrolysis obtained after 5-h of hydrolysis at 2% E/S ratio was 14.9%, which was similar to that obtained by Yu and Fazidah (1994).

3.2. Powdered protein hydrolysate

The dried protein hydrolysate produced with 10% w/w maltodextrin was powdery, had a mild fish aroma and did not stick to the drying chamber of the spray drier. The average yields of the spray-dried products of enzymatic hydrolysis and control were 9.6 and 4.1%, respectively. Typical yields of fish protein hydrolysates have been reported to be 10–15%, based on fresh fish substrate (Hale, 1972; Quaglia & Orban, 1990). The lower yields obtained in this study are probably due to the fact that only the soluble fraction was spray–dried. Losses also occurred as a result of small batch drying, particularly in the case of the high capacity spray drier. Yields of fish protein hydrolysates were also consistent with the degree of hydrolysis, since lower DH of control hydrolysis gave a lower yield of spray-dried product (Nana & John, 1994).

3.3. Effect of spray drier temperatures on proximate composition of hydrolysate powder

Table 1 shows the proximate composition of two types of hydrolysate powders produced. A significant ($P<0.05$) difference in the crude protein content was observed between the samples. A decrease of 23.9% in crude protein, when the drying temperature was increased from 150 °C to 180 °C, was noted. The fat content, however, was not significantly affected by spray-drying. As expected, higher drying temperature also caused a 59.8% reduction in moisture content of the hydrolysates. The carbohydrate content of the samples is mainly due to the added maltodextrin. The ash content was not affected by the different temperatures used.

3.4. Protein quality evaluation

3.4.1. Effect of temperature on amino acid profile of hydrolysate powder

Table 2 shows the amino acid profile of the dried hydrolysates. As expected, a higher temperature was found to significantly decrease the content of all amino acids tested. This result revealed that all the essential amino acids of type B (higher temperature) hydrolysate were severely affected by the temperature used, except methionine. Lysine and threonine contents were markedly affected, decreasing by 62.2 and 56.3%, respectively. However, the reduction in leucine content was insignificant. A decrease of 50.8% occurred in the isoleucine content. Phenylalanine and tyrosine contents decreased by 29.7% and 47.6%, respectively.

Table 3 shows that Type A (lower temperature) hydrolysate contents of isoleucine, leucine, methionine and cystine, phenylalanine and tyrosine are comparable with that of the FAO/WHO amino acid reference pattern (1973) that has been established for humans. The limiting amino acid of hydrolysate is found to be threonine. The increase in hydrophobic amino acids, such as isoleucine, leucine and lysine, is important, due to the effects that these have on the physical and functional properties of food proteins. The hydrolysed Tilapia protein powder, type A, supplied a higher proportion of the amino acid requirements of humans than the non-hydrolysed fish protein powder (control), and the quantities of essential amino acids, isoleucine, leucine and lysine covered 84.10 to 100% of the FAO 'ideal' (FAO, 1973).

3.4.2. In vitro protein digestibility

The multienzyme system used in determining the protein digestibility in this paper could reduce the effect caused by a specific enzyme inhibitor. Consequently, using multienzymes instead of trypsin alone could avoid under estimating digestibility of proteins containing trypsin inhibitor. Secondly, a single enzyme system that attacks at a specific peptide bond may give different results for proteins containing different concentrations of the specific amino acid. The multienzyme system did reduce the limitations that were evident for a single enzyme system, and gave a better approximation of protein degestibility (Hsu, Vavak and Miller, 1977).

The in vitro digestibility of the powdered hydrolysate is shown in Table 4. The type A hydrolysate powder exhibited the highest digestibility of 92.1%. The thermal

Table 1
Proximate composition of hydrolysate powders

Composition	Inlet/outlet temperature	
	150 °C/76 °C (Type A)	180 °C/90 °C (Type B)
Protein	49.6±0.19a	37.7±0.05c
Fat	2.80±0.13a	2.56±0.10a
Moisture	3.93±0.15a	1.58±0.12b
Ash	8.65±0.01a	8.56±0.03a
Carbohydrate[a]	35.0	49.6

Means in the same row with different letters are significantly different at 5% level, as determined by Duncan's multiple range test.

[a] Carbohydrate was not determined but determined by difference.

treatment undergone by Type A hydrolysate seemed to improve digestibility of the protein by destroying protease inhibitors and enhance unfolding of the protein, resulting in unmasking of the peptide bonds. However, the higher spray-drying temperature used in type B hydrolysate powder resulted in a decrease of protein digestibility, possibly through thermal cross-linking of protein (Hsu et al., 1977).

3.4.3. Protein digestibility-corrected amino acid scoring (PDCAAS)

In calculating the PDCAAS of a food protein, any score above 1.0 is rounded down to 1.0 for further calculation. There is absolutely no nutritional advantage in consuming proteins with scores greater than 1.0, since excess amino acids are not utilised by the body as amino acids, per se. Instead, excess amino acids are deaminated by the body and the nitrogen excreted as urea, while the remaining carbon skeleton can be utilized as energy or stored. Table 5 shows the example of the calculation of type A hydrolysate powder.

Proteins with a PDCAAS of 1.0 are considered high quality proteins or complete proteins that meet the essential amino acid requirements of humans (Henley & Kuster, 1994). In this study, hydrolysate A exhibited a high PDCAAS of 0.82 (Table 6). PDCAAS of the control hydrolysate was 39.0% lower than that of type A, due to low protein digestibility and a low essential amino acid content. The low essential amino acid content of the control hydrolysate may be due to the low

Table 2
Amino acid profile of fish protein hydrolysate[a]

Amino Acids (mg/g crude protein)	Treatment					
	150 °C/76 °C		% Increase	180 °C/90 °C		% Increase
	AC	A		BC	B	
Aspartic acid (Asp)	45.4±0.16a	67.2±5.30b	47.8	15.6±0.99c	24.5±2.41d	56.5
Glutamic acid (Glu)	141±6.48a	151b±7.05b	7.58	73.7±3.16c	74.5±8.28c	1.03
Serine (Ser)	21.5±0.78a	24.0a±0.10	11.3	17.0±1.38b	18.3±1.24b	7.79
Glycine (Gly)	31.8±3.16b	40.9±1.66b	28.4	18.5±2.77a	19.6±4.05a	5.90
Arginine (Arg)	34.4±2.42a	49.1±0.50b	42.9	22.11±0.59c	26.9±0.23d	21.4
Threonine (Thr)	20.1±6.42a	30.2±6.89a	50.2	10.2±3.11c	13.2±8.13b	29.5
Alanine (Ala)	32.4±0.99b	37.7±2.01a	16.6	23.7±2.33a	30.4±1.05ab	28.6
Proline (Pro)	17.5±2.42b	22.6±2.98a	29.3	19.0±1.66a	21.8±2.02a	14.8
Tyrosine (Tyr)	16.4±2.69a	25.8±1.08a	57.1	13.0±2.03b	13.5±5.07b	4.00
Valine (Val)	23.0±0.25a	31.9±0.56a	38.9	20.0±0.04a	21.1±0.78a	0.38
Methionine (Met)	19.9±2.09a	27.6±3.01b	38.6	23.7±1.36c	31.7±1.09d	33.8
Isoleucine (Iso)	24.4±3.15a	33.6±1.26a	38.2	15.0±2.33b	16.6±4.67b	10.6
Leucine (Leu)	56.0±0.78a	68.9±0.11a	22.9	50.9±1.26a	58.1±1.24a	14.1
Phenylalanine (Phe)	52.0±2.43a	59.1±2.81a	13.7	38.6±0.31b	41.6±2.58b	7.70
Lysine (Lys)	50.3±0.04a	75.9±1.11b	50.9	25.4±2.51c	28.7±4.59c	13.2
Total	588	741	26.5	386	440	140

Mean values in the same row with different letters are significantly different at 5% level, as determined by Duncan's multiple range test.

[a] A and B, hydrolysate powders from hydrolysis with enzyme Alcalase; AC and BC, hydrolysate powders from control hydrolysis without enzyme Alcalase.

Table 3
Essential amino acid composition of *O. mossambicus* hydrolysate compared with the FAO/WHO pattern[a]

Amino acids	*O. mossambicus* hydrolysate A (mg/g crude protein)	FAO/WHO pattern[a]
Isoleucine	33.6	40
Leucine	68.9	70
Lysine	75.9	55
Methionine + cystine	27.6	35
Phenylalanine + tyrosine	85.0	60
Threonine	30.2	40
Valine	31.9	50
Total	353	360

[a] From Refs. 28 and 29 (Copyright Food and Agriculture Organization of the United Nations, 1973).

Table 4
In vitro protein digestibility of Tilapia protein hydrolysate powder

Sample	In vitro protein digestibility (%)
A	92.1[a]
AC	84.6[a]
B	88.4[a]
BC	76.9[a]

[a] Average of duplicate runs.

Table 5
PDCAAS of Tilapia protein hydrolysate, Type A

I Essential amino EAA	II Tilapia protein hydrolysate[a] EAA profile (mg/g protein)	III FAO/WHO 2–5 year old ref. pattern (mg/g protein)	IV Uncorrecte amino acid score[b]	V PDCAAS[c]
Histidine	–	19	–	–
Isoleucine	33.6	28	1.20	1.11
Leucine	68.9	66	1.04	0.96
Lysine	75.9	58	1.31	1.21
Met + Cys	27.6	25	1.10	1.01
Phe + Tyr	85.0	63	1.35	1.24
Threonine	30.2	34	0.89	*0.82*
Tryptophan	–	11	–	–
Valine	31.9	35	0.91	0.84

PDCAAS, protein digestibility-corrected amino acid scoring.
[a] Based on essential amino acid profile of Tilapia protein hydrolysate. Essential amino acid profile and protein digestibility of Tilapia protein hydrolysate are based on actual analysis in this paper.
[b] Uncorrected amino acid score = column II/column III.
[c] PDCAAS = uncorrected amino acid score (column IV)×92.1%(protein digestibility of type A); PDCAAS = 0.82 (lowest corrected amino acid score).

Table 6
PDCAAS of Tilapia protein hydrolysates

Hydrolysate type	PDCAAS
A	0.82
AC	0.50
B	0.34
BC	0.23

PDCAAS, protein digestibility-corrected amino acid scoring.

Table 7
PER values for Tilapia protein hydrolysates

Sample	PER value
A	3.0
AC	2.4
B	2.5
BC	2.2

PER, protein efficiency ratio.

degree of hydrolysis. On the other hand, the PDCAAS of type B dropped dramatically and this is probably caused by the high temperature spray-drying, which destroyed some of the amino acids in the sample.

3.4.4. Protein efficiency ratio (PER)

The most important disadvantage of the PER test is that PER values of protein have no proportional relationship to one another or to the suitability of the protein source analyzed for maintenance of protein nutrition. It also lacks precision, has poor reproducibility and is expensive. Nevertheless, PER was predicted, based on amino acid composition, for comparison purposes (Alsemeyer et al., 1974). PER of spray-dried hydrolysates was found to range from 2.2 to 3.0 (Table 7). Generally we can see that PER of type A was significantly higher than that of type B which was obtained at a higher drying temperature. The PER of type B hydrolysate was 14.8% lower than that of type A hydrolysate which was probably because of the destruction of some of the essential amino acids, due to the higher temperature used.

4. Conclusion

Proximate analyses revealed that the spray-dried hydrolysates consisted of 37.7–49.6% protein, 2.6–2.8% fat, 1.6–4% moisture and 8.6–8.7% ash. A significant reduction in the contents of all amino acids was seen with higher temperatures of spray-drying. Nevertheless, the protein qualities of both hydrolysates were found still to be high, with in vitro digestibilities of 88.4 and 92% for type B and Type A, respectively. Protein digestibility corrected amino acid score for was found to be 0.82 Type A and 0.34 for type B hydrolysates, with the limiting amino acid for both being threonine. In addition, predicted PERs were calculated to be 3.0 and 2.5 for powdered hydrolysates type A and type B, respectively.

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