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Determination of protein quality of rainbow trout (*Salmo irideus*) by *in vitro* protein digestibility-corrected amino acid score (PDCAAS)

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Protein digestibility and protein quality of raw rainbow trout, broiled rainbow trout and smoked rainbow trout were studied by *in vitro* assay, Amino acid score (AAS) and protein digestibility corrected amino acid score (PDCAAS). Protein digestibilities of samples were determined using an *in vitro*, three-enzyme method in a pH-stat and three- and four-enzyme pH-drop methods. Amino acid score was based on the amount of the single most limiting amino acid, and its calculation included the use of the requirement pattern suggested by FAO/WHO/UNU for pre-school children. Protein digestibilities of raw, broiled and smoked rainbow trout were found to be 87.1, 84.0 and 83.4% using the three-enzyme pH-drop method, 84.7, 81.4 and 80.8% using the four-enzyme pH-drop method, and 95.5, 93.9 and 91.2% using the three-enzyme pH-stat method, respectively. When the amino acid score was corrected for *in vitro* (three-enzyme pH-stat method) protein digestibility, the resulting values of 99.8, 97.1 and 93.9% were obtained. Amino acid score corrected for protein digestibility seems to predict, accurately, the nutritional quality of fish protein when *in vitro* values are used.

INTRODUCTION

Since 1919, the protein efficiency ratio (PER) method, which measures the ability of a protein to support growth in young, rapidly growing rats, has been used in many countries because it is believed to be the best predictor of clinical tests.

The shortcomings of the PER test, including lack of precision, poor reproducibility and high cost, are well known. The PER and other methods were reviewed at the Airlie Conference in 1980, where it was agreed that the PER should be replaced by a more appropriate and precise method (FAO/WHO, 1990). Therefore, more rapid and less expensive *in vitro* assays have been developed. The *in vitro* methods for assaying digestibility all rely on the use of proteolytic enzymes to correlate with the digestion of protein *in vivo*. One of the best known *in vitro* methods was developed by Satterlee and co-workers (Hsu *et al.*, 1977; Satterlee *et al.*, 1979). The rate of enzymatic digestion is calculated from the pH drop following a 10-min incubation with trypsin and chymotrypsin, and intestinal peptidase at 37°C (Hsu *et al.*, 1977), or after an additional 10-min incubation with microbial protease at 55°C (Satterlee *et al.*, 1979). Pedersen and Eggum (1983) developed a pH-stat assay in which initial rate of alkali consumption is used to calculate a rate of hydrolysis of peptide bonds. In general the pH stat method was found to be more accurate than the pH-drop method in predicting protein digestibility of foods (Eggum *et al.*, 1989). McDonough *et al.* (1990) standardized a pH-stat method determined by six laboratories with 17 protein sources.

The Codex Committee on Vegetable Proteins (CCVP) suggested that amino acid score (based on the amount of the single most limiting amino acid) including correction for true digestibility of protein (as determined by the rat balance method) was considered to be the most suitable routine method for assessing protein quality of foods. The Committee also noted that further research should be encouraged to perfect and evaluate the most promising *invitro* procedures such as those of Satterlee *et al.* (1979) and Pedersen and Eggum (1983) for estimating protein digestibility. The purpose of this study was to compare the digestibility of protein by using *in vitro* methods (three-enzyme pH-drop, four-enzyme pH-drop, three-enzyme pH-stat) and to assess quality of protein by using *in vitro* protein digestibility-corrected amino acid score (PDCAAS) in smoked and broiled rainbow trout (*Salmo irideus*), a food item which is exported extensively from Turkey to Scandinavian countries.

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Table 1. Essential amino acid content of samples (g/16 g N) and suggested pattern

Essential amino acids	FAO/WHO/UNU Pre-school child (2–5 years)	Rainbow trout		
		raw	broiled	smoked
His	1.9	3.32	3.09	3.12
Lys	5.8	7.03	6.76	6.78
Met + Cys	2.5	4.04	3.24	3.18
Thr	3.4	4.16	3.95	3.58
Iso	2.8	4.75	4.13	4.65
Leu	6.6	6.90	6.82	6.80
Val	3.5	5.85	4.69	4.75
Phe + Tyr	6.3	7.46	7.10	6.50
Trp	1.1	1.01	0.89	0.91

MATERIALS AND METHODS

Raw and smoked rainbow trout (*Salmo irideus*) were obtained from Ege Sea Products Company, Izmir. One half of the raw fish samples were broiled at 170°C for 20 min in a preheated electric oven. All samples (raw, broiled and smoked fish) were filleted, skinned and ground twice through a plate with 5 mm holes before being divided into portions for further analyses.

Total nitrogen was determined by the Kjeldahl method using a Kjeltec 1002 Analyser (Tecator, Inc.). Protein was calculated by using a nitrogen-to-protein conversion factor of 6.25. All samples were hydrolysed in duplicate with 6 M HCl for the determination of amino acids except tryptophan. Tryptophan analysis was performed using basic hydrolysis (Schuster, 1980). Amino acids in each hydrolysate were determined by high-pressure liquid chromatography using a Shimadzu LC 3 system. The *in vitro* protein digestibility of samples and reference protein casein were measured using the three-enzyme pH-drop method described by Hsu *et al.* (1977), four-enzyme pH-drop method described in AOAC (1990) and three-enzyme pH-stat method described by McDonough *et al.* (1990).

Amino acid ratios (mg of an essential amino acid in 1.0 g of test protein/mg of the same amino acid in 1.0 g of reference pattern for 9 essential amino acids plus tyrosine and cystine) were calculated by using the 1985 FAO/WHO/UNU (FAO/WHO, 1990) suggested pattern of amino acid requirements for preschool children (2–5 years) (Table 1). The lowest amino acid ratio (%) was termed amino acid score. Protein digestibility-corrected amino acid scores (PDCAAS) of the samples were calculated by multiplying the lowest amino acid ratio multiplied by the *in vitro* protein digestibility (three-enzyme pH-stat method).The scores (PDCAAS) were expressed in percentage terms; PDCAAS above 1.00 was considered as 100% (Sarwar & McDonough, 1990).

RESULTS AND DISCUSSION

The amino acid composition, shown in Table 1, indicates that the content of essential amino acids is generally much higher in raw samples than in processed samples. This is especially the case for lysine which, in overheated fish, was drastically reduced compared to untreated fish (El & Kavas, 1993).

In vitro protein digestibilities of fish samples determined by three different methods are shown in Table 2. A similar trend was observed for the results obtained by three different methods in all samples and a significant correlation was found between methods (Table 3). Bodwell *et al.* (1980) reported similar results in a study on protein digestibilities obtained by three- and four-enzyme pH-drop methods ($r = 0.88$). Bodwell *et al.* (1980) and Eggum *et al.* (1989) found good agreement between the *in vitro* and *in vivo* values of protein digestibilities of various protein sources, with the exception of legumes, which had *in vitro* values higher than *in vivo* values. Rich *et al.* (1980) and Marletta *et al.* (1992) found significant correlations between results of the four-enzyme pH-drop *in vitro* method and the *in vivo* method. Various researchers studying protein digestibility with

Table 2. Protein digestibility, AAS and PDCAAS values of samples

	Rainbow trout		
	raw	broiled	smoked
In vitro protein digestibility (%)			
Three-enzyme pH-drop	87.1	84.0	83.4
Four-enzyme pH-drop	84.7	81.4	80.8
Three-enzyme pH-stat	95.5	94.0	91.2
AAS (%)	100.0	100.0	100.0
PDCAAS (%)	99.8	97.0	93.9

Table 3. Correlations between estimates of digestibility in all *in vitro* methods

Method Regression coefficient	Regression equation	Regression coefficient
x = three-enzyme pH-drop y = four-enzyme pH- drop	$y = -11.686437 + 1.109988x$	$r = 0.989$
x = three-enzyme pH-drop y = three-enzyme pH-stat	$y = 10.357538 + 0.980099x$	$r = 0.876$
x = four-enzyme pH-drop y = three-enzyme pH-stat	$y = 20.857694 + 0.880746x$	$r = 0.883$

pH-drop (three and four enzyme) and pH-stat methods suggested that the use of the pH-stat could be considered the most appropriate for a good prediction of protein digestibility (Pedersen & Eggum, 1983; Mozersky & Panettieri, 1983; Eggum *et al.*, 1989; McDonough *et al.*, 1990; Swaisgood & Catignani, 1991; Boisen & Eggum, 1991). In general, *in vivo* (rat) protein digestibility for raw fish ranging from 90.6 to 96.6% was reported (McDonough *et al.*, 1990; FAO/WHO, 1990). In our study, protein digestibility values which are determined by the pH-stat method for raw rainbow trout are in agreement with these reported values. Compared with raw rainbow trout, broiling reduced the digestibility of protein by 3.5, 3.9 and 1.63% using three-enzyme pH-drop, four-enzyme pH-drop and three-enzyme pH-stat methods, respectively. Also, smoking reduced the protein digestibility by 4.21, 4.21 and 4.51% using the respective methods. Smoked trout had higher protein digestibility than broiled trout. The white-fleshed fishes, like rainbow trout, were reported to have higher *in vitro* digestibilities than dark-fleshed ones (Lee & Ryu, 1986). This might suggest a faster rate of enzymatic tissue degradation in white-fleshed fish than in dark-fleshed varieties owing to the weaker muscle structure of the white-fleshed fish. Tissue degradation may enhance the digestibility of white-fleshed fishes. (Lee & Ryu, 1986).

Opstvedt *et al.* (1984) found a linear decrease in the content of -SH (sulfhydryl) groups and a concomitant increase in the content of S–S bonds when rainbow trout was heated at increasing temperatures from 50 to 115°C. The impact of disulphide bond formation on protein utilization is not fully known, but some experimental data indicate that it may reduce protein digestibility (Opstvedt *et al.*, 1984). Mauron (1984) reported that protein digestibility was reduced as a result of complex chemical (crosslinking) reactions such as protein interactions or protein–fat interactions when food was broiled at high temperatures. Also, Opstvedt (1988) reported that smoking conditions (time, temperature, compounds of wood smoke) reduced protein digestibility.

Amino acid scores (AAS) and protein digestibility corrected amino acid scores (PDCAAS) of samples are shown Table 2. In animal protein, AAS and PDCAAS were reported as 100 and 97–100%, respectively (Sarwar *et al.*, 1989; Sarwar & McDonough, 1990). Our values are in agreement with the reported values. PDCAAS of raw trout was reduced 5.88% with the smoking process and 2.77% with the broiling process.

In conclusion, the *in vitro* protein digestibility values of fish samples which are determined by pH-stat method are in agreement with reported values. Therefore the pH-stat method can be used for protein digestibility instead of the *in vivo* method estimation of PDCAAS method.

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