PROTEIN DIGESTIBILITY - CORRECTED AMINO ACID SCORE. METHOD FOR ASSESSING PROTEIN QUALITY OF RAINBOW TROUT

EL S.N. and KAVAS A.

Ege University, Engineering Faculty, Food Engineering Department, Bornova - Ýzmir - TURKEY.

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SUMMARY

Protein digestibility and protein quality of rawrainbow 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 digestibility of samples were determined using in vitro, three enzyme method in a pH - stat and three and four enzyme pH - drop method.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 / UNUfor pre -school children. Protein digestibility of raw , broiled and smoked rainbow troutwere found to be 87.07 % , 84.00 % , 83.40 % using 3 enzyme pH - drop method and 84.73 % , 81.43 % , 80.82 % using 4 enzyme pH - drop method and 95.51 % , 93.95 % , 91.20 % using 3 enzymepH --stat method, respectively . When the Amino Acid Score was corrected for in vitro (3 enzyme pH - stat method) protein digestibility , the resulting values of99.81 % , 97.05 % , and 93.94 % were obtained . Amino Acid Score corrected for protein digestibility seems to predict accuratelythe 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 was 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 minute incubation with trypsin, chymotrypsin, and intestinal peptidase at 37 C (Hsu et al.,1977) or after an additional 10 minutes 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 pH - stat method determined by 6 laboratories with 17 protein sources. 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 in vitro 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 (3 enzyme pH - drop , 4 enzyme pH - drop , 3 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.

Materials and Methods :

Raw and smoked rainbow trout (Salmo irideus) were obtained from Ege Sea
Products Company , Ýzmir . One half of the raw fish samples were broiled at 170C for 20 minutes in a preheated electrical 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 Kjeltec 1002 Analyser

(Tecator , Inc.) Protein was calculated by using a nitrogen - to - protein conversion factor of 6.25 . All samples were hydrolyzed in duplicates with 6 N HCL for the determination of amino acids except tryptophan. Tryptophan analysis was performed by using basic hydrolysis (Schuster, 1980). Amino acids in each hydrolysate were determined by High Pressure Liquid Chromatography using Shimadzu LC 3 .
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 p H - 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 score (PDCAAS) of the samples were calculated by multiplying the lowest amino acid ratio x in vitro protein digestibility (3 enzyme pH-stat method). The scores (PDCAAS) were expressed in percentage terms , PDCAAS above 1.00 was considered as 100 % (Sarwar and 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 the processed samples . This is especially the case for lysine which in overheated fish was drastically reduced compared to untreated fish (El and Kavas, 1993).

In vitro protein digestibility 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 4 enzyme pH -drop and 3 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 4 enzyme pH - drop in vitro method and the in vivo method., Various researchers studying protein digestibility with pH - drop (3 and 4 enzyme) and pH-stat methods suggested that the use of pH-stat could be considered the most appropriate for a good prediction of protein digestibility (Pedersen and Eggum, 1983; Mozersky and Panettieri , 1983 ; Eggum et al , 1989 ; McDonough et al ., 1990 ; Swaisgood and Catignani , 1991 ; Boisen and Eggum , 1991). In general , in vivo (rat) protein digestibility for raw fish ranging from 90.6 to 96.6 % were reported (McDonough et al., 1990 ; FAO/WHO, 1990). In our study , protein digestibility values which are determined by 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 3 enzyme pH-drop, 4 enzyme pH-drop and 3 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. This might suggest a faster rate of enzymatic tissue degradation in white - fleshed fishes than in dark - fleshed varieties owing to the weaker muscle structure of the white - fleshed fishes. Tissue degradation may enhance the digestibility of white-fleshed fishes. (Lee and 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 temparatures from 50 C 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 and McDonough,1990). Our values are in aggreement with the reported values . PDCAAS of raw trout was reduced 5.88 % with smoking process and 2.77 % with broling process.

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

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