account for more than the total 48 residues of protein I, the 2 peptides probably overlap each other in the molecule to some extent so that the overlapping residues are counted twice. Alternatively, protein I could consist of 96 amino acids/molecule with 2 residues each of Ile, Phe, and Thr. This is not likely because such a protein, with 2 Phe and 4 Tyr residues, should give rise to 7 chymotryptic peptides instead of the 4 found.

Since purothionins yielded only two peptides when subjected to identical chymotryptic hydrolysis (Mak and Jones, 1976b) and since the purothionin chymotryptic peptides were completely different in amino acid composition from the corn protein chymotrypic peptides, corn protein I probably is not homologous with any of the purothionins.

The corn proteins we isolated are similar to purothionins and to rye thionin in amino acid composition, size, and other chemical and physical characteristics but are not toxic to hornworm larvae. Corn protein I, which was most similar to thionins, apparently is not homologous with purothionins since it yields chymotryptic peptides that are very different from those released from purothionins under similar conditions.

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Amino Acid Content of Baltic Herring and Rainbow Trout Roe

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The amino acid content of Baltic herring and rainbow trout roe at different stages of maturity has been determined. The amino acid pattern with glutamic acid, leucine, aspartic acid, lysine, and alanine as the major components was generally uniform for both roe regardless of the maturity degree. The essential amino acids averaged 35.6 and 37.5 g/100 g of protein in herring and trout roe, respectively, and were in a good balance compared with the FAO/WHO recommendations. The first limiting amino acid in trout roe was tryptophan, and that in herring roe was the total amount of sulfur-containing acids. The amino acid score values were 90.0 and 80.0 for trout and herring roe protein, respectively.

Roe is one of the most valuable food products from fishery sources. When roe is the primary product of fish industry as in the herring industry on the Pacific coast of Canada, the harvesting of roe fish is timed just prior to the spawning to give completely mature roe. If roe is processed as a byproduct, it is collected from normal fish catches during some weeks or months before spawning (e.g., Atlantic and Baltic herring and freshwater whitefish) or during the sacrificing of cultured fish (e.g., rainbow trout). Thus, the degree of maturity of processed roe may vary significantly according to fish species or even in the roe products from the same species manufactured at different times during the roe season.

The nutritive value of roe may change with its maturation. In the gross composition of roe, protein forms the major part of the dry weight (Zaitsev et al., 1969; Vuorela et al., 1979). During maturation of the roe, in addition to the increase in the total protein content, the relative amount of protein may vary, too (Medford and Mackay, 1978; Vuorela et al., 1979). Further, the relative distribution of amino acids has been found to be significantly altered by the maturity of roe of different salmon species (Seagran et al., 1954). The amino acids of mature rainbow trout roe have been reported by Satia et al. (1974) and Cantoni et al. (1975). In certain respects, however, their results differ considerably from each other. In herring roe protein, the constituent amino acids have not been reported in the literature, whereas 10 free amino acids have been identified in Atlantic herring roe (Gjessing, 1963).

The purposes of this study were to determine the amino acid composition of the roe from Baltic herring and rainbow trout, to find out whether or not any significant changes take place during the maturation, and to evaluate the quality of roe protein in relation to the amino acid requirements in human nutrition.

MATERIALS AND METHODS

Fish and Roe. Rainbow trout (*Salmo gairdneri*) from a local fish farm and Baltic herring (*Clupea harengus*) from the Southwest Coast of Finland were obtained at

Technical Research Centre of Finland, Food Research Laboratory, SF-02150 Espoo 15, Finland (J.K.K.), and University of Turku, Department of Chemistry and Biochemistry, SF-20500 Turku 50, Finland (R.L. and R.R.L.).

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Table I. Amino Acid Content (g/100 g of Protein) of Baltic Herring (*C. harengus*) Roe at Different Stages of Maturity

amino acid	maturity index 8.4	10.0	10.5	12.8	16.4	19.3	av ± SD
alanine	5.9	5.4	5.3	6.6	5.7	5.4	5.7 ± 0.5
arginine	4.6	3.9	4.1	4.0	4.4	4.7	4.3 ± 0.3
aspartic acid	6.8	6.0	5.7	7.0	6.5	6.4	6.4 ± 0.5
cystine[a]	0.9	1.5	1.3	1.2	1.4	1.4	1.3 ± 0.2
glutamic acid	9.7	8.8	7.7	8.7	9.1	9.1	8.9 ± 0.7
glycine	2.5	2.3	2.3	3.0	2.8	2.7	2.6 ± 0.3
histidine	3.0	2.3	1.9	2.2	2.0	2.2	2.3 ± 0.4
isoleucine	4.0	3.4	3.6	4.9	4.3	4.9	4.2 ± 0.6
leucine	7.5	6.8	6.7	8.5	8.3	8.5	7.7 ± 0.8
lysine	6.8	6.4	6.3	6.0	6.1	6.4	6.3 ± 0.3
methionine	0.9	1.2	1.6	2.0	1.6	1.7	1.5 ± 0.4
phenylalanine	5.7	5.7	5.4	3.7	3.7	4.0	4.7 ± 1.0
proline	4.6	5.9	4.7	3.7	4.3	4.8	4.7 ± 0.7
serine	4.6	4.0	3.6	4.0	4.4	3.9	4.1 ± 0.4
threonine	5.1	4.4	4.1	5.0	4.6	5.2	4.7 ± 0.4
tryptophan	1.7	1.9	1.3	1.5	1.7	1.5	1.6 ± 0.2
tyrosine	4.6	6.2	5.7	4.0	4.7	5.4	5.1 ± 0.8
valine	4.4	4.3	4.1	5.9	5.2	5.5	4.9 ± 0.7
total aa[b]	83.3	80.4	75.4	81.9	80.8	83.7	80.9
essential aa	36.1	34.1	33.1	37.5	35.5	37.7	35.6
essential aa, %	43.3	42.4	43.9	45.8	43.9	45.0	44.0

[a] Determined as cysteic acid. [b] aa = amino acid.

4-week intervals starting in January 1978. The sampling was continued until spawning which was in May for trout and in June for herring.

The maturity index (MI), the roe weight as a percentage of the total body weight, was determined for each specimen. The roe was removed and handled as described earlier (Vuorela et al., 1979). Samples of rainbow trout roe were analyzed individually, but pooled samples of Baltic herring roe comprising the roe from ~6 kg of fish were prepared each sampling time.

Protein Hydrolysis. The proteins were hydrolyzed essentially according to the method of Davies and Thomas (1973). Roe samples were homogenized in 6 M HCl, and the samples were transferred to screw-cap glass bottles. The acidity of homogenized roe samples was adjusted in such a way that there was 1 mL of 6 M HCl per mg of protein, and the dissolved oxygen in the mixture was removed by bubbling a stream of nitrogen. Hydrolysis was carried out for 24 h at 110 °C. The hydrolysate was filtered and then evaporated to dryness in a rotary evaporator.

Amino Acid Determinations. Amino acids were quantified with a Hitachi Model KLA-5 amino acid analyzer with norleucine as an internal standard. The peak areas were measured by the triangulation method, and a Wang Model 2200 computer was used for calculations. The resolution and quantitation of the analyzer were regularly controlled with a commercial mixture of amino acids (Sigma Chemical Co.) as a standard.

Tryptophan, cystine, and cysteine, which are not stable during the acid hydrolysis and the succeeding ion-exchange chromatography (Moore and Stein, 1963), were analyzed separately. Cystine and cysteine were converted to cysteic acid (Schram et al., 1954) which was determined with the amino acid analyzer. Tryptophan was determined by the colorimetric method of Graham et al. (1947) based on the reaction of *p*-(dimethylamino)benzaldehyde with tryptophan in the presence of sodium nitrate and hydrochloric acid.

Nitrogen was determined titrimetrically from H_2SO_4-ashed samples after the micro-Parnas–Wagner distillation.

RESULTS

The maturity indexes for Baltic herring increased from 8.4 in January to 19.3 in June when almost one-fifth of the total weight of fish consisted of roe. The comparable MI values for rainbow trout were 8.8 and 17.2.

The amino acid contents of herring and trout roe are presented in Tables I and II, respectively. All amino acid values are expressed, if not otherwise noted, as percent of protein, calculated to 16% nitrogen (grams of amino acid per 16 g of nitrogen).

The major amino acid, glutamic acid, averaged ~10% of protein in both roe followed by leucine, aspartic acid, lysine, and alanine in order of decreasing amount. Cystine, methionine, and tryptophan showed the smallest values ranging from 1.3 to 1.6% of protein in herring roe whereas in trout roe tryptophan alone had the lowest level, 0.9%.

The essential amino acids composed 42.4–45.8 and 39.2–46.3% of the total amount of amino acids in herring and trout roe samples, respectively.

DISCUSSION

Amino Acid Content. Using microbiological methods, Seagran et al. (1954) determined 10 amino acids in the roe of 5 salmon species all belonging to the genus *Oncorhynchus*. Their study included all the essential amino acids as well as arginine and histidine. All roe had leucine and lysine as the major components whereas tryptophan had the lowest level. Further, Seagran et al. (1954) reported changes in the amounts of most amino acids with the maturation of roe. With king salmon (*Oncorhynchus tshawytscha*) and sockeye salmon (*Oncorhynchus nerka*), which were studied in most detail, the total amount of the essential amino acids increased from 40.9 to 47.3% and from 41.5 to 49.2% of protein, respectively.

The maturation of rainbow trout or Baltic herring roe does not seem to be accompanied by changes in the amino acid content of the roe protein (Tables I and II). The standard deviation for the basic method used in this study was found to range from 5 to 10% for the different amino acids (except arginine, 14%). For individual amino acids in the trout roe samples the standard deviation of the average values (Table II) remained within the accuracy of the method except for glutamic acid which, however, did not show any systematic changes. Slightly higher deviations in herring roe (Table I) can be explained at least in part by the sampling which was not necessarily made from the same herring population each time.

Table II. Amino Acid Content (g/100 g of Protein) of Rainbow Trout (*S. gairdneri*) Roe at Different Stages of Maturity

amino acid	maturity index 8.8	10.0	12.3	16.6	17.2	av ± SD
alanine	5.6	6.1	6.8	7.0	6.0	6.3 ± 0.6
arginine	6.8	5.1	5.1	5.2	6.0	5.6 ± 0.7
aspartic acid	6.6	6.9	7.0	7.4	6.8	6.9 ± 0.3
cystine[a]	3.7	3.6	3.5	4.2	4.2	3.8 ± 0.3
glutamic acid	8.7	11.1	11.2	11.6	8.6	10.2 ± 1.5
glycine	2.6	2.3	2.2	2.3	2.2	2.3 ± 0.2
histidine	3.1	2.7	2.8	2.5	2.7	2.8 ± 0.2
isoleucine	5.0	4.6	4.9	4.1	5.2	4.8 ± 0.4
leucine	7.7	7.8	8.1	7.6	8.5	7.9 ± 0.4
lysine	6.4	6.7	6.1	6.0	6.7	6.4 ± 0.3
methionine	2.7	2.3	2.4	2.3	2.2	2.4 ± 0.2
phenylalanine	5.3	4.6	4.9	4.9	5.8	5.1 ± 0.5
proline	4.3	4.5	4.6	4.4	4.9	4.5 ± 0.2
serine	4.6	4.5	4.5	5.2	4.9	4.7 ± 0.3
threonine	4.1	4.1	4.2	4.0	4.7	4.2 ± 0.3
tryptophan	0.9	0.9	1.0	1.0	0.9	0.9 ± 0.1
tyrosine	4.4	4.9	5.2	5.2	4.6	4.9 ± 0.4
valine	5.4	6.0	6.5	5.6	5.5	5.8 ± 0.4
total aa[b]	87.9	88.7	90.5	90.5	85.4	88.6
essential aa	37.5	37.0	38.1	35.5	39.5	37.5
essential aa, %	42.7	41.7	42.1	39.2	46.3	42.4

[a] Determined as cysteic acid. [b] aa = amino acid.

Table III. Essential Amino Acid Content (g/100 g of Protein) and Amino Acid Score of Different Fish Roe, Hen Egg, and the FAO/WHO Provisional Scoring Pattern

amino acid	Baltic herring[a]	rainbow trout[a]	rainbow trout[b]	salmon[c]	hen egg[d]	FAO/WHO pattern[e]
isoleucine	4.2	4.8	4.7	7.2	5.8	4.0
leucine	7.7	7.9	7.6	9.9	8.9	7.0
lysine	6.3	6.4	8.3	8.8	6.7	5.5
total sulfur aa[f]	2.8	6.2			5.3	3.5
cystine	1.3[g]	3.8[g]	nd[h]	nd	3.0	
methionine	1.5	2.4	2.1	2.9	2.3	
total aromatic aa	9.8	10.0			10.3	6.0
phenylalanine	4.7	5.1	5.2	4.8	6.7	
tyrosine	5.1	4.9	nd	nd	3.6	
threonine	4.7	4.2	6.0	5.9	5.1	4.0
tryptophan	1.6	0.9	nd	0.9	1.5	1.0
valine	4.9	5.8	8.9	7.2	7.5	5.0
amino acid score	80.0	90.0				

[a] This study. [b] Satia et al. (1974). [c] Seagran et al. (1954). [d] Sikka and Johari (1979). [e] FAO/WHO (1973). [f] aa = amino acid. [g] Determined as cysteic acid. [h] nd = not determined.

Cantoni et al. (1975) reported for rainbow trout roe that glutamic acid formed 13.4% and the other major components, leucine, aspartic acid, lysine, and alanine, ranged from 9.4 to 7.5% of the total amino acids. These values agree with our results: glutamic acid as the major component (11.5% as calculated from the total amino acids) followed by leucine, aspartic acid, lysine, and alanine (8.9–7.1% each). In the rainbow trout roe analyzed by Satia et al. (1974), valine and lysine were the major components (10.3 and 9.6% of the total amino acids, respectively) whereas glutamic acid and leucine both reached the level of 8.8%. Tryptophan formed only 0.9% of protein in rainbow trout roe analyzed in this study. Tryptophan was not analyzed by Satia et al. (1974) and Cantoni et al. (1975).

The amino acid content of Baltic herring roe was very similar to that of rainbow trout roe (Tables I and II). Glutamic acid was the major component, although its proportion was slightly smaller, 8.9%, in herring roe compared to 10.2% of protein in trout roe. Lower values can also be found in the amounts of arginine (4.3 and 5.6% of protein in herring and trout roe, respectively). The most striking difference, however, was found in the sulfur-containing amino acids, methionine and cystine, which averaged 1.5 and 1.3% of protein in herring roe but 2.4 and 3.8% in trout roe, respectively.

Quality of Roe Protein. The proportion of total amino acids that must be supplied as essential amino acids (the E/T ratio) is suggested to be 36.0% of protein (FAO/WHO, 1973). In Baltic herring roe the essential amino acids averaged 35.6% (Table I) and in rainbow trout roe 37.5% of protein (Table II), showing thus a very good E/T ratio.

The average content of the essential amino acids as well as cystine and tyrosine in Baltic herring roe, rainbow trout roe, salmon roe, and hen egg is compared (Table III) with the suggested FAO/WHO (1973) amino acid scoring pattern. Rainbow trout roe, like roe generally, is well balanced with the essential amino acids, and with a good E/T ratio it may be considered as a high-quality protein source. In Baltic herring roe the content of sulfur-containing amino acids does not reach the respective level in the FAO/WHO scoring pattern and this is the main difference if Baltic herring roe protein is compared to other roe proteins or to egg protein.

The amino acid score (chemical score) based on the first limiting amino acid was calculated by using the FAO/WHO provisional amino acid scoring pattern as the reference. In Baltic herring roe protein the sulfur-containing amino acids gave the lowest score, 80.0. Valine, which was

revealed as the second limiting amino acid, had the value 98.0 whereas all the other amino acids exceeded the respective levels in the reference pattern. For rainbow trout roe only tryptophan, which was the first limiting amino acid with the score 90.0, did not exceed the recommended level.

In most foods and diets lysine, total sulfur-containing amino acids, or tryptophan is found to be the first limiting component (FAO/WHO, 1973). In roe lysine seems to exist in relatively high proportions as in other fish protein. On the contrary, valine shows a low score in Baltic herring roe and in this aspect the results are comparable to those of mullet (*Mugil cephalus*) roe reported by Lu et al. (1979). The overall quality of the roe protein studied is comparable to the FAO/WHO amino acid pattern and to the egg protein which is often used as the reference.

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Protein Solubility Characteristics of an Ultrafiltered Full-Fat Soybean Product

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Protein dispersibility (PDI) as a function of pH and concentration of various salts was studied for a full-fat soy protein product produced by ultrafiltration (UF). For the acidic and neutral pH regions, PDI was higher than that of the raw material (ground whole soybeans) and a commercial soy isolate. A significant difference in salting out at pH 6.7 was observed depending on the order of mixing of ingredients; protein dispersed after NaCl was dispersed showed much larger salting out effects than if the protein was dispersed in water prior to NaCl solution. At pH 6.7, PDI of UF soy was 6–20% between 0.01 and 0.2 M $CaCl_2$. When tricalcium phosphate was used at 0.01–0.15 mol of calcium/L, PDI was 81–89%. Phytic acid had a significant effect on protein solubility in the acidic pH region, and its presence may also mask the true effects of low levels of Ca^{2+} on solubility characteristics.

Much attention has been focused on nontraditional protein sources such as alfalfa, cottonseed, algae, blood, and, of course, soybeans in order to augment the limited supply of protein in the world. A substantial amount of processing is usually necessary to convert these materials into more readily utilizable forms, and as a result the product may have less than desirable functional properties. Recently, ultrafiltration (UF) has been shown in our laboratory to be a viable means of producing purified protein–fat products from whole soybeans (Omosaiye et al., 1978; Omosaiye and Cheryan, 1979a,b). By selecting the appropriate membrane pore size and operating conditions, it is possible to simultaneously fractionate and concentrate water extracts of soybeans under mild operating conditions, using much less energy than that required by conventional processes that require heating and cooling. The functional properties of such a UF soy product warrant attention not only because of the novelty of the process and relatively mild processing conditions but also because the final product is greatly reduced in undesirable components such as oligosaccharides, phytic acid, and trypsin inhibitor compared to the original soybeans and has no lipoxygenase-induced "painty" off-flavors. A product with such a desirable combination of physical properties produced by a relatively simple process is uncommon, especially in the full-fat form.

The objective of this study was to evaluate protein solubility characteristics of a full-fat soy protein product produced by ultrafiltration. Solubility is a critical functional property, since a protein generally has to be in solution in order to exert its other desirable functional characteristics (Kinsella, 1976). Nitrogen Solubility Index and Protein Dispersibility Index (PDI) are the two most common methods of evaluating solubility characteristics. They differ chiefly in that the former is a low-shear, long-time method, while the latter is done at high shear for a short time. Because most food products are generally prepared commercially under high-shear, short-time conditions for production efficiency, PDI is considered a better indication of solubility behavior in such systems (Pour-El, 1976) and hence this test was used in our studies. The effect of pH, sodium chloride, calcium chloride, and calcium phosphate tribasic on PDI was studied. In addition, phytic acid, a common constituent of many vegetable protein products, has been shown to complex with proteins, resulting in lowered solubility and possible shifts in the pH–solubility profile (Smith and Rackis, 1957; Shen, 1976; Cheryan, 1979). Since many commercial soy products

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