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EFFECTS OF HEAT TREATMENT ON COMPOSITION AND NUTRITIVE VALUE OF HERRING MEAL

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INTRODUCTION

Manufacture of fish meal involves operations carried out at elevated temperatures. It is well known that reactions between chemically active constituents of fish proteins, lipids and reducing sugars in the presence of water may take place during these operations. Thus free amino- and carbonyl groups form readily products of reaction (MAILLARD 1912) which render amino acids, particularly lysine, nutritionally less available. Previous investigations have shown that heating of protein from fish or other foods can, in fact, reduce digestibility of the protein and decrease availability of its amino acids (BISSET and TARR 1954; BOGE 1960; CARPENTER *et al.* 1962; MASON and WEIDNER 1964; MOUSTAFA 1966; ARNESEN 1969; BJARNASON and CARPENTER 1970; DUBROV and STILLINGS 1970; KNIPFEL *et al.* 1970; EGGUM and JØRGENSEN 1971).

The purpose of the present investigations was to study in more detail the effect of heating on the quality of herring meal, particularly when conditions are comparable to those prevailing in industrial drying processes, *i.e.*: Reduction of moisture from about 50% to less than 10% at a meal temperature of maximum 100 °C during a period that may range from about 10 to 60 minutes. Simulation of these conditions requires a different approach from that applied in most earlier work, dealing with quality changes resulting from much longer treatments (one to several days). It was thus necessary to bring meal temperatures rapidly up to the test levels and, after isothermal treatments, rapidly down again to the room condition. This was accomplished using a rotary autoclave with provisions for rapid heating and cooling. Levels of meal moisture were also adjusted differently from earlier procedures, which usually involved dehydration to near dryness and subsequent addition of appropriate amounts of water. Since over-drying and rehydration could well introduce extraneous effects, the herring meals tested were freeze dried to the moisture levels required. Nutritive values of heat treated meals were measured chemically and by feeding tests on rats and chicks.

EXPERIMENTAL

MATERIALS

Preparation. — The test meals were prepared from herring (*Clupea harengus*) which had been stored on ice for four days. The fish was cooked in a continuous steam cooker at a jacket pressure of about 2 kg/cm^2,

and the emulsion of oil and stickwater removed from the cake in a screw press. The press cake was ground in a high speed chopper and vacuum packed in 1 kg-portions, stored in plastic bags under nitrogen at — 30 °C until required for heat treatments and quality evaluations.

Heat treatment. — In each experiment a batch of about 2 kg of wet herring meal was dried in a New Brunswick freeze dryer to the appropriate moisture content. Drying to low moistures tended to bring meal temperatures up in the final stages, but the majority of meals stayed below 15 °C throughout the whole freeze drying process. The freeze dried samples were treated in the autoclave at various levels of heat intensity (96—132 °C, 0—2 hrs), after which low moisture meals were submitted directly for quality evaluations, whereas high moisture meals were freeze dried a second time, so that they could be properly handled.

Fig. 1 shows the autoclave, which had provisions for heating and cooling and was operated at a rotation of 22 rpm. A hot and cold reservoir of glycerol were employed as heat source and heat sink respectively, and 3-position valves provided rapid switching from one reservoir to the other.

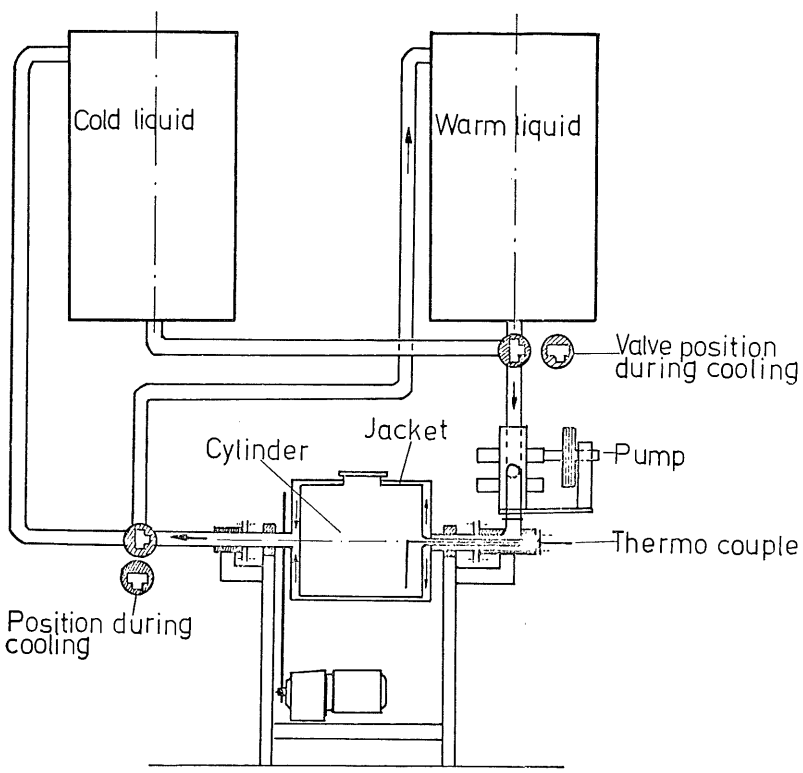

Fig. 1. Stainless steel rotary autoclave.

The autoclave had a volume of about 50 liters, and its internal wall was equipped with shelves. These enabled test meals to be lifted up and showered down in curtains during the rotation and thus provided good conditions for over-all heat transfer between the meal particles and the glycerol flowing through the jacket. Fish meal temperatures were measured by a copper-constantan thermocouple, embedded in the meal, and continuously recorded.

The thermogram in Fig. 2, showing short transient periods of heating and cooling and a very constant temperature during the test period, was typical for the majority of heat treatments.

METHODS OF ANALYSIS

Chemical. — Total nitrogen, N, was determined by the macro-Kjeldahl method (mercuric oxide catalyst) and protein calculated as Nx6.25. Pepsin digestibility was determined by the AOCS Official Method 22.025 (1960), modified by LOVERN, OLLEY and PIRIE (1964). The modification involved digestion in a dilute pepsin solution (0.0002%) and correction for solubility in acid blank (zero pepsin). Analysis for available lysine was based on Sanger's reaction with fluorodinitrobenzene adapted to animal-protein foods by CARPENTER (1960). Amino acids were analysed as described by NJAA *et al.* (1968), tryptophan according to SLUMP and SCHREUDER (1969). Fat was determined by two methods:

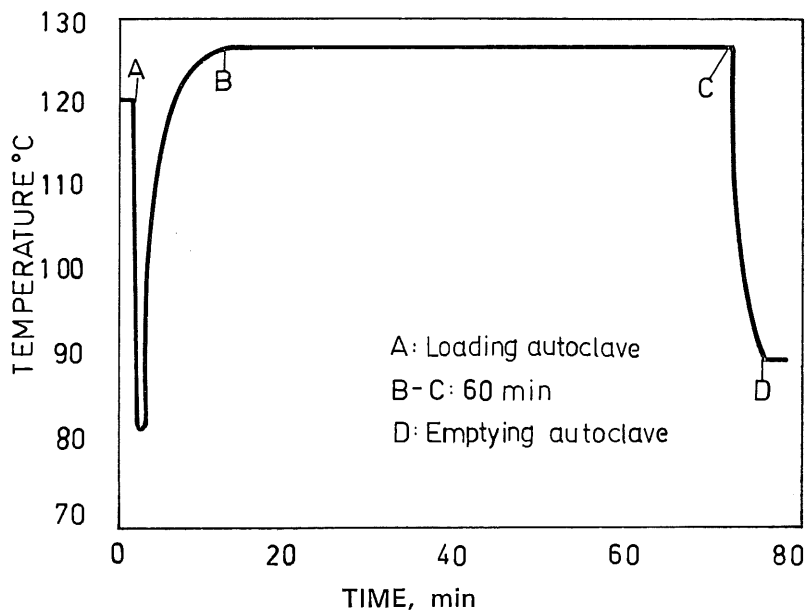

Fig. 2. Thermogram for herring meal.

1) Extraction with ethyl ether by the Soxhlet method and 2) extraction with chloroform + methanol + water according to Bligh and Dyer modified for fish meal by HANSON and OLLEY (1963). Carbonyl number was measured by the method of HENICK *et al.* (1954) modified by AURE *et al.* (1966). Iodine number was measured by the procedure of Wijs (AOCS Official Method CD 1—25, 1950). Total volatile nitrogen was determined by the magnesium oxide method (AOCS Official Method 1.040, 1960). Ash was determined by over-night heating at incandescence (500 °C), and moisture by dehydration at 103 °C for 4 hours.

Feeding trials. — The nutritive values of herring meals were tested on chicks essentially as described by OPSTVEDT *et al.* (1970) and on rats essentially as described by NJAA *et al.* (1966). Net protein utilizations (NPU) were calculated from nitrogen intakes and excretions. In the experiments with chicks the rations contained about 10% protein and were gived *ad lib.* The rat rations contained about 8% protein and each rat was given 10 g daily. Special rat trials were performed in an attempt to determine indigestibility of the amino acids: Two meals were fed at the 20% protein level to two groups of 6 rats for 5 days. Feed and faeces were analysed for protein, amino acids and titanium oxide, an indigestible indicator admixed to the feed (NJAA 1961). Indigestibility coefficients for individual amino acids were calculated as the indigestibility coefficients for protein in feed multiplied by ratios between amounts of each amino acid in protein of faeces and feed.

RESULTS AND DISCUSSION

PROXIMATE ANALYSES

Analytical data for all heat treated herring meals are listed in Table 1. Protein contents varied in the range of 82.5%—85.0% and tended to decrease very slightly with increasing intensity of heating. Fat contents varied between 4.9 and 6.2% (ether extractable) and 10.2 and 12.6% (BLIGH and DYER). Total volatile nitrogen varied between 0.10 and 0.15%.

FAT QUALITY

Effects of heating on the lipid phase of herring meals were studied by measuring its carbonyl- and iodine numbers. The most intensively heat treated meals were examined four weeks after treatment. The results (Table 2) demonstrated a curious effect: A carbonyl number of 323 millimol CO/kg fat in freeze dried reference meal No. 14 compared with

74—91 in heat treated meals (Nos. 15—18) would indicate a preservation of double carbon-carbon bonds as a result of heating. This was confirmed in the measurements of corresponding iodine numbers, which were 98 g I_2/100 g fat for freeze dried and 109—128 for heat treated herring

Table 1. Composition of heat treated herring meal.

Meal		Treatment		Protein %[1]	Fat %		Ash %	TVN[2] %
No.	% Moist.	Min.	°C		Ether	Bl. & Dyer		
1	7.0	0	*)	84.0	5.5	11.1	9.3	0.13
2	7.7	30	96	84.4	5.9	10.9	10.0	0.14
3	8.8	60	96	84.8	5.9	10.9	10.0	0.15
4	10.8	120	96	85.0	5.6	10.2	10.0	0.15
5	3.1	0	*)	84.2	4.9	12.1	9.8	0.10
6	36.0	60	96	84.2	6.0	11.6	9.4	0.10
7	41.0	120	96	84.2	6.1	11.5	8.6	0.11
8	27.0	60	115	84.2	5.8	12.6	9.2	0.15
9	27.0	120	115	83.6	5.9	12.2	9.7	0.15
10	9.3	0	*)	85.0	5.9	10.9	9.7	0.10
11	8.4	30	116	84.8	5.7	10.6	10.4	0.11
12	7.5	60	116	83.9	5.8	11.9	10.8	0.12
13	6.4	120	116	84.0	5.8	10.7	10.4	0.13
14	3.3	0	*)	83.5	5.9	11.7	9.3	0.10
15	1.1	60	127	83.1	5.4	11.0	9.4	0.10
16	2.5	120	132	83.0	5.5	10.9	9.6	0.12
17	30.0	60	126	83.0	6.2	10.7	10.3	0.14
18	32.0	120	124	82.5	6.1	11.1	10.3	0.12

[1]) All percentages except moisture, are given on a dry weight basis.
[2]) Total volatile nitrogen.
*) Freeze dried reference meal (not heat treated).

Table 2. Carbonyl- and iodine numbers in heat treated herring meal.

Meal		Treatment		Carbonyl number mmol CO/kg fat	Iodine number g I_2/100 g fat
No.	% Moist.	Min.	°C		
14	3.3	0	*)	323	98
15	1.1	60	127	91	120
16	2.5	120	132	74	120
17	30.0	60	126	91	109
18	32.0	120	124	80	128

*) Freeze dried reference meal.

meals. These findings point to some form of heat stabilization against oxidation of the lipid phase, a phenomenon which has also been observed by others (LEA, PARR and CARPENTER 1960). It has further been reported that short heat treatments of herring meal improve nutritive value as measured by chick growth (TARR, BIELY and MARCH 1954).

CHEMICAL EVALUATION OF PROTEIN

Amino acids. — Contents of essential, semi essential and non essential amino acids in heat treated herring meals are listed in Tables 3a and 3b. Treatment at 115/116 °C resulted generally in slightly lower amino acid concentrations in high moisture meals (nos. 8 and 9) than in low moisture meals (Nos. 12 and 13) respectively. This tendency was not apparent for treatments at the higher temperatures of 124—132 °C. On the other hand, for the high temperature treatments the time factor appeared to be of greater significance, the longer treatments generally resulted in the lower levels of amino acids (meals 16 and 18). Also, recoveries of N as amino acid-N were lowest for these two meals.

Available lysine. — Analytical data for available lysine in heat treated

Table 3a. Essential and semi essential amino acids in heat treated herring meal.

Meal		Treatment		Contents of amino acids, % of protein									
No.	% Moist.	Min.	°C	Isoleu	Leu	Lys	Met	Cys	Phe	Tyr	Thr	Try	Val
5	3.1	0	*)	4.8	8.2	8.6	3.1	0.9	3.9	3.6	3.5	1.2	4.7
6	36.0	60	96	4.6	8.1	7.9	3.0	0.8	3.8	3.5	4.6	1.2	5.1
7	41.0	120	96	4.8	8.2	8.6	3.1	0.9	3.9	3.6	3.5	1.2	5.8
8	27.0	60	115	4.5	7.9	8.1	3.0	0.6	3.8	3.6	4.6	1.1	5.3
9	27.0	120	115	4.6	8.0	6.9	2.9	0.6	4.1	3.6	4.3	1.1	5.3
10	9.3	0	*)	4.4	7.6	8.8	2.7	0.7	3.6	3.3	4.5	1.2	5.2
11	8.4	30	116	4.4	7.6	8.8	2.7	0.7	3.6	3.3	4.2	1.2	5.2
12	7.5	60	116	4.9	8.4	8.6	2.9	0.7	3.8	3.7	4.5	1.2	5.7
13	6.4	120	116	4.8	8.4	7.9	2.5	0.8	4.1	3.8	4.8	1.2	5.3
14	3.3	0	*)	4.5	8.1	8.4	2.9	0.6	4.2	4.0	4.4	1.2	5.5
15	1.1	60	127	4.7	8.0	9.0	3.1	0.9	3.9	3.7	4.6	1.2	5.3
16	2.5	120	132	4.7	6.7	6.9	2.8	0.6	3.8	3.1	3.5	1.2	4.8
17	30.0	60	126	4.9	8.4	8.2	3.2	0.6	4.3	3.8	4.6	1.2	5.6
18	32.0	120	124	4.8	7.2	7.3	2.8	0.5	3.6	3.7	4.1	1.2	4.7

*) Freeze dried reference meal.

Table 3b. Non essential amino acids in heat treated herring meal.

Meal		Treatment		Contents of amino acids, % of protein							
No.	% Moist.	Min.	°C	Ala	Arg	Asp	Glu	Gly	His	Ser	Pro
5	3.1	0	*)	6.5	6.8	9.1	12.8	5.1	2.4	4.4	5.0
6	36.0	60	96	6.1	6.6	8.9	12.7	4.9	2.3	4.6	4.6
7	41.0	120	96	6.5	7.0	9.1	13.4	5.0	2.4	4.5	5.3
8	27.0	60	115	6.4	6.8	9.3	12.4	5.1	2.2	4.4	3.9
9	27.0	120	115	6.3	6.0	8.7	14.8	4.9	2.0	3.9	4.3
10	9.3	0	*)	6.2	6.6	9.1	12.9	4.6	2.2	4.0	4.1
11	8.4	30	116	5.9	6.4	9.7	12.6	4.3	2.1	3.8	3.7
12	7.5	60	116	5.7	7.3	9.3	12.1	4.7	2.4	4.2	4.6
13	6.4	120	116	6.5	6.7	9.9	14.4	5.0	2.3	4.3	4.1
14	3.3	0	*)	6.4	7.2	7.6	12.8	5.3	2.8	4.1	4.8
15	1.1	60	127	6.2	7.4	9.0	12.8	5.0	2.6	4.2	5.0
16	2.5	120	132	5.8	6.8	7.0	10.3	4.2	2.3	3.7	4.3
17	30.0	60	126	6.6	7.4	9.1	11.9	5.1	2.4	4.2	4.0
18	32.0	120	124	6.1	6.3	8.6	11.6	4.6	2.3	3.7	3.6

*) Freeze dried reference meal.

herring meal (Table 4) were not systematically related to conditions of treatment. However, during prolonged heat treatments (2 hours), there was a tendency for available lysine to decrease with increasing moisture. Available lysine was also poorly related to the other nutritive criteria examined in our experiments. CARPENTER et al (1962) found that extensive heating of herring meal (27 hrs at 130 °C) led to a consistent decrease in the availability of several essential amino acids.

Pepsin digestibility. — Table 4 includes the results of analyses for pepsin digestibility of the herring meals. In the course of two hours treatment at 96 °C, there was a gradual decrease in pepsin digestibility for both dry meals Nos. 2—4 and wet meals Nos. 6 and 7. In relation to the freeze dried reference meals, the lowest value attained at each moisture level was 76.0% (No. 4) and 66.4% (No. 7) respectively. After two hours heating at 115 °C of wet meal No. 9, digestibility dropped to 43.7% of the value for the reference meal. The digestibility of dry meals decreased to about the same extent whether they were heated for two hours at 96 °C (No. 4) or at 116 °C (No. 13). However, short treatments (30 min) affected digestibility less at the low than at the high temperature, cf. meals Nos. 2 and 11. The most intensively heated meals (Nos. 15—18) were also most seriously affected: After two hours of heating at 132 °C

Table 4. Available lysine and pepsin digestibility in heat treated herring meal.

Meal		Treatment		Available lysine		Pepsin digestibility	
No.	% Moist.	Min.	°C	lysine/g 16 g N[1]	%[2]	%[1]	%[2]
1	7.0	0	*)	7.1	100	81.1	100.0
2	7.7	30	96	6.7	94	71.5	88.0
3	8.8	60	96	6.8	96	68.0	83.8
4	10.8	120	96	6.2	87	61.7	76.0
5	3.1	0	*)	7.0	100	84.6	100.0
6	36.0	60	96	6.1	87	59.9	70.8
7	41.0	120	96	7.0	100	56.2	66.4
8	27.0	60	115	6.9	98	40.5	47.8
9	27.0	120	115	5.9	84	37.0	43.7
10	9.3	0	*)	7.0	100	85.5	100.0
11	8.4	30	116	6.7	96	68.4	80.0
12	7.5	60	116	7.0	100	66.9	78.2
13	6.4	120	116	6.6	94	66.8	78.1
14	3.3	0	*)	6.6	100	87.6	100.0
15	1.1	60	127	6.8	103	72.4	82.6
16	2.5	120	132	6.4	97	58.4	66.6
17	30.0	60	126	6.0	91	39.6	45.2
18	32.0	120	124	6.1	92	29.3	33.4

[1]) Measured data.
[2]) Calculated data referred to values for freeze dried meals.
*) Freeze dried reference meal.

the digestibility of dry meal No. 16 had dropped to 66.6% of the value for the freeze dried reference meal, while the corresponding figure for wet meal No. 18 was 33.4%.

In these experiments, then, pepsin digestibility was the most sensitive criterion for distinguishing between herring meals exposed to heat at various levels of intensity. The significance of moisture during heating was clearly brought out: All moist meals (27% moisture or more) had pepsin digestibilities below 60%, whereas dry meals (except No. 16) had digestibilities above 60%. Further, digestibilities of moist meals appeared to be more affected by temperature than by duration of heating. Pepsin digestibility was well correlated with nutritive data obtained in animal feeding trials, more so with apparent digestibility determined on rats than with NPU determined on rats and chicks (Table 7).

EVALUATION OF PROTEIN QUALITY BY FEEDING TRIALS

The results of feeding the experimental meals to rats and chicks are listed in Table 5. The rat trials brought out only slight effects of the heat treatment on the nutritive values of *dry* meals: Two hours heating at 116 °C and 132 °C (meals Nos. 13 and 16) resulted in apparent digestibility drops to only 97.2 and 95.2% respectively with reference to the freeze dried meals. The values of NPU for these two meals were 95.3 and 91.8% respectively. The effects of heating *wet* meals under nearly the same conditions were larger: Two hours heating at 115 and 124 °C (meals Nos. 9 and 18) reduced the apparent digestibility to 87.7

Table 5. Nutritive values (rat and chick trials) for heat treated herring meal.

Meal		Treatment		RATS				CHICKS	
				Apparent digest. %		NPU %		NPU %	
No.	% Moist.	Min.	°C	4)	5)	4)	5)	4)	5)
1	7.0	0	*)					70.0	100.0[1]
2	7.7	30	96					69.0	98.6[1]
3	8.8	60	96					71.5	102.1[1]
4	10.8	120	96					68.7	98.1[1]
5	3.1	0	*)	82.1 a	100.0	61.2 a	100.0	67.5	100.0[1]
6	36.0	60	96	79.8 b	97.2	59.8 a	97.7	65.2	96.6[1]
7	41.0	120	96	78.7 b	95.9	55.9 b	91.3	60.2	89.2[2]
8	27.0	60	115					54.4	80.6[2 3]
9	27.0	120	115	72.0 c	87.7	54.5 b	89.1	57.6	85.3[2 3]
10	9.3	0	*)	86.9 a	100.0	85.7 a	100.0	66.1	100.0[1]
11	8.4	30	116	84.7 b	97.5	83.5 b	97.4	65.9	99.7[1]
12	7.5	60	116	84.3 b	97.0	83.1 b	97.0	65.3	98.8[1]
13	6.4	120	116	84.5 b	97.2	81.7 b	95.3	64.0	96.8[1]
14	3.3	0	*)	85.8 a	100.0	79.0 a	100.0	65.7 a	100.0[1]
15	1.1	60	127					65.9 a	100.3[1]
16	2.5	120	132	81.7 b	95.2	72.5 b	91.8	63.8 a	97.1[1]
17	30.0	60	126	74.5 c	86.8	64.7 c	81.9	59.6 b	90.7[1]
18	32.0	120	124	73.9 c	86.1	64.1 c	81.1	50.4 c	76.7[3]

a, b, c Values with different signs are significantly different, calculated within group.

[1], [2], [3] Values with different signs are significantly different, calculated on the whole material.

[4]) Measured data.

[5]) Calculated data referred to values for freeze dried meals.

*) Freeze dried reference meal.

and 86.1% respectively compared with the values for the freeze dried meals. The corresponding values for NPU were 89.1 and 81.1% respectively.

In chicks all values of NPU for heat treated *dry* meals were only slightly reduced with reference to the freeze dried meals. On the other hand, NPU for the most intensely heated *wet* meal (No. 18) was reduced to the value of 76.7%.

Heat treatments of herring meal did not affect apparent digestibility in rats to the same extent as it affected pepsin digestibility *in vitro*. This finding was not unexpected: First, the *in vitro* analyses include only the acid insoluble fractions of the meal. Second, the animal has a more versatile digestive system than that represented by the single enzyme pepsin, contained in a dilute solution of hydrochloric acid.

Apparent indigestibilities of individual amino acids in two selected herring meals — one unheated reference meal (No. 14) and one meal containing 30% moisture, heated for one hour at 126 °C (No. 17) —

Table 6. Indigestibility of heat treated herring meal, tested on rats.

Constituent	Indigestibility, % of total constituent		
	Meal No.		Decreased digestibility
	14	17	
Protein	12.1	19.1	7.0
Aspartic acid	12.8	20.7	7.9
Threonine	10.6	16.9	6.3
Serine	12.0	19.1	7.1
Glutamic acid	9.0	14.7	5.7
Proline	13.9	16.7	2.8
Glycine	11.8	14.5	2.7
Alanine	9.9	14.4	4.5
Valine	9.3	16.7	7.4
1/2 Cystine	28.7	47.6	18.9
	} 14.7	} 19.3	} 4.6
Methionine	11.5	14.3	2.8
Isoleucine	9.4	16.9	7.5
Leucine	7.7	14.5	6.8
Tyrosine	9.4	16.9	7.5
Phenylalanine	9.2	15.6	6.4
Lysine	8.3	15.2	6.9
Histidine	8.3	14.3	6.0
Arginine	6.1	9.4	3.3
Tryptophan	10.0	20.8	10.8

No. 14: Freeze dried reference meal.
No. 17: 30% moist. meal treated 60 min. at 126 °C.

are listed in Table 6. Amounts of amino acids not digested ranged from about 6 to 14% in the reference sample and from 9 to 21% in the heat treated sample, representing digestibility losses of about 3—11% (third column of Table 6).

Analyses of the indigestibility of protein and amino acids revealed that 76 and 75% respectively of their indigestible nitrogen could be accounted for from indigestible amino acid N. Indigestibilities of cystin were relatively high, a finding which parallels high losses of cystin resulting from extended heating of bovine plasma albumin (26 hrs at 145 °C) by BJARNASON and CARPENTER (1970). However, the cystin concentration in herring meal is relatively low, and this particular analysis is expected to be less reliable. Otherwise, reduction in digestibility was quite evenly distributed among the amino acids tested. This also applied to lysine, which is particularly prone to reactions with other meal constituents during heating.

Correlation coefficients between selected quality indices in the heat treated herring meals were calculated. Table 7 shows that pepsin digestibility, apparent digestibility in rats, and NPU in rats and chicks were significantly correlated. Available lysine, however, did not correlate with the other indices.

Table 7. Correlation coefficients calculated between various quality indices for heat treated herring meal.

Quality indices	Correlation coefficient, r
Pepsin digestibility/available lysine	0.53
—»— /NPU chicks	0.91**
—»— /NPU rats	0.92**
—»— /Apparent digestibility in rats	0.97**
Available lysine/NPU chicks	0.37
—»— /NPU rats	0.26
—»— /Apparent digestibility in rats	0.52
NPU chicks/Apparent digestibility	0.83**
NPU rats /Apparent digestibility	0.93**
NPU rats /NPU chicks	0.82**

** Significance $P<0.01$.

SUMMARY

1. The effect of heat treatment on quality of herring meal has been investigated using a rotating autoclave operated under variable conditions (meal moisture: 1.1%—41%; meal temperature: 96° C—132 °C; heating time: 0—2 hrs).
2. In terms of proximate chemical analyses all heat treated meals, containing 82.5—85.0% protein on a dry weight basis, would be considered of a very high grade.
3. Short heat treatments at about 125 °C appeared to stabilize the lipid phase towards oxidation during storage: After four weeks an unheated reference meal had a carbonyl number of 323 mmol CO/kg fat whereas heat treated meals had values in the range of 74—91 mmol CO/kg fat. Corresponding iodine numbers were on the average 100 g I_2/100 g fat for the unheated meal and 120 g I_2/100 g fat for heat treated meals.
4. Analyses indicated some slight decrease in amino acids of the herring meals resulting from the heat treatments.
5. No clear correlation was found between available lysine and conditions of heat treatment, but there was a tendency for availability to decrease with increasing moisture content during prolonged heating of meals.
6. During the heat treatments, pepsin digestibility for *dry* meals dropped to 66% while that for *wet* meals dropped at most to about 33% with reference to freeze dried meals.
7. Pepsin digestibility was well correlated with values for nutritive criteria obtained in animal feeding tests.
8. Apparent indigestibility of individual amino acids in two selected meals showed some increase due to heat treatment.

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