

ARTIFICIAL RIPENING OF MAATJES-CURED HERRING WITH THE AID OF PROTEOLYTIC ENZYME PREPARATIONS

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ABSTRACT

For manufacturing maatjes-cured herring, the herring caught in the North Sea or Irish Sea is suitable only during months when the proteolytic activity of the appendices pyloricae is sufficiently high. For instance, only North Sea herring caught from May to July has sufficient proteolytic activity for the manufacture of a well-ripened product. For herring caught in other areas, this period may differ considerably. The present work shows that it is possible to use herring caught in other seasons if protease preparations are added to the fish together with salt. The herrings were examined organoleptically, whereas the brines were examined by chemical analysis and by chromatography on Sephadex G-25. No significant differences were found between naturally ripened herring and herring cured by the aid of enzyme preparations. The lipase content of the preparation should be low enough in order to avoid the formation of a fatty acid taste in the cured fish.

Maatjes-type cured herring, the fish product that has been made for centuries aboard Dutch fishing vessels, is usually made from North Sea herring (*Clupea harengus* L.), and in latter years from Irish Sea herring as well.

After being caught, the herring is gibbed and salted promptly. "Gibbing" means the removal of some of the intestines through an incision below the left gill. It is essential that, at this procedure, the appendices pyloricae are left in the fish; according to Luijpen (1959) this organ plays an important role in the formation of the characteristic organoleptic properties of the product. The particular taste and the soft consistency are due to the action of proteolytic enzymes from the appendices pyloricae on the fish flesh.

Since the content of proteolytic enzymes in the fish varies with the seasons, the fish is suitable for the manufacture of maatjes-cured herring only for a few months. Other variables like age and size of the fish or its source may also influence the protease content and in this way restrict the suitability of herring for the maatjes-curing process. For these reasons, there is a demand for a manufacturing method

which is less dependent upon the protease activity of the appendices pyloricae.

The aim of our investigation was, therefore, to find the conditions for obtaining an acceptable maatjes-cured herring by the addition of protease preparations. In this way a product with the desired organoleptic properties might be manufactured from herring which have relatively inactive appendices pyloricae and which consequently cannot ripen in a natural way.

MATERIALS AND METHODS

Artificially ripened herring was made by adding protease preparations and salt to gutted fresh or frozen herring. The enzyme preparations were mixed with the salt before salting the herring in the usual way, viz. mixing the herring with a certain quantity of dry salt. Shortly after the addition of salt, a brine is formed which covers the fish entirely. After storage from 7 to 31 days the fish was examined organoleptically and the brines chemically. In most cases the enzyme-treated herring was compared with naturally ripened herring and with eviscerated ("gutted") herring to which no enzyme preparations were added.

The purpose of the chemical analyses was to gather information about possible differences

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between the natural and the artificial ripening process. These analyses included: assay of total soluble nitrogen by the Kjeldahl method, protein determination by the biuret method, and determination of amino nitrogen (as a rough measure for the amino acid content). Assuming that the soluble matter is evenly distributed between the fish and the brine formed, most of the analyses were carried out in the latter.

Some of our experiments are described below. Other experiments gave similar results and are omitted here for ease of survey.

NATURAL RIPENING PROCESS

Frozen maatjes herring that had been caught in the North Sea at the end of May were used. The protein content was 16.4% and the fat content 13.8%. Herring in this stage is normally used for the preparation of maatjes-cured herring.

The protein content was determined by the Kjeldahl method; for the fat determination, the Bligh and Dyer (1959) method was used in a modification according to Ederzeel and Ritskes (1966).

Part of the herring was gibbed and part was gutted. One part of salt was added to 20 parts of herring (light salting). The fish was kept at 3° C for 1 week.

In order to remove proteins which are assumed to be of less importance in the study of the ripening process, the brines were heated for about 15 min at 80° C and then filtered warm over a fluted filter paper. All analyses were carried out with these clarified brines. In this experiment, these analyses included a Kjeldahl nitrogen determination, a measurement of the biuret value according to Strickland, Freeman, and Gurule

(1961) and a determination of the amino nitrogen content according to the method of the Dutch Food Law (1963). This latter method is based upon two alkalimetric titrations to different end points. The salt content in the brines was determined by the Volhard method.

In order to elucidate the protein breakdown process during ripening, gel chromatography of the brine was applied; 0.8 ml of a clarified brine was chromatographed on a Sephadex G-25 column² (length 44 cm, diameter 1.44 cm; fraction volume 3.4 ml, elution rate 19.8 ml/h), distilled water being used as an eluant. In each fraction the biuret value and the absorbance at 280 nm (nanometers or millimicrons) were measured. The results of the organoleptic evaluations and the chemical analyses are shown in Table 1. The results of gel chromatography are plotted in Figures 1 and 2. As could be expected, the values found in the brine of the gibbed herring were considerably higher than in that of the gutted herring.

TESTING OF TWO ENZYME PREPARATIONS

Frozen spawning herring, caught at the end of August in the North Sea were used. The protein content was 17.6% and fat content 18.2%.

Two enzyme preparations, Pr 8 and TG 21/63, were tested. Their proteolytic activities were determined according to Anson (1938), with denatured hemoglobin as a substrate. Lipolytic activity was determined on olive oil according to Marchis-Mouren, Sarda, and Desnuelle

² Reference to trade names in this publication does not imply endorsement of commercial products by the National Marine Fisheries Service.

TABLE 1.—Results of organoleptic evaluation of naturally ripened maatjes-cured herring and of analyses of brine in which herring ripened.

Variation	Organoleptic evaluation of the herring		Analyses of the brine ¹		
	Flavor	Texture	Biuret value (Esoo × 1000 × dilution factor)	Total N (mg N/ml)	Amino N (% w/v)
Gutted	Nontypical	Moderately firm	900	4.4	0.133
Gibbed	Good maatjes-flavor	Optimal (soft)	1720	6.9	0.255

¹ Salt content of brine was 10.5 ± 0.5% w/v.

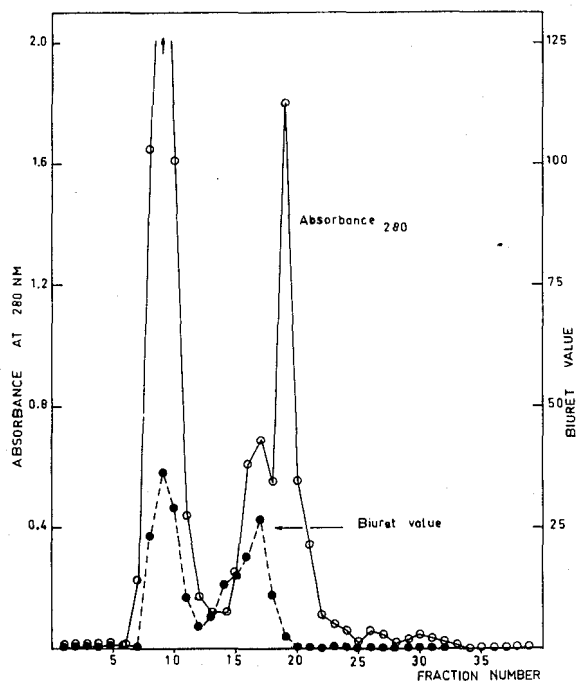


FIGURE 1.—Chromatogram of the clarified brine of gutted maatjes herring on Sephadex when herring were ripened naturally.

(1959). Pr 8 had a proteolytic activity of 5.3×10^{-4} U/mg and a lipolytic activity of 0.04 Desnuelle units/mg; for TG 21/63, these values were 9.1×10^{-4} and 18.7, respectively. These preparations, like all others used in this study, were supplied by N. V. Organon, Oss, the Netherlands. Details concerning these preparations are summarized in Table 2.

The proteolytic activity of the preparations was found by measuring the caseinolytic activity at pH 7.5 and 35° C (Ruyssen, 1969). The NF-pancreatin reference standard was used (see National Formulary XIII, 1970, p. 514). The potency of the protease preparations is expressed in terms of the minimum activity required by NF XIII. The lipase activity was measured by potentiometric titration of fatty acids hydrolyzed in an olive oil emulsion at pH 8.0 (Marchis-Mouren, Sarda, and Desnuelle, 1959). The International F.I.P. standard for pancreas lipase was used as a standard (Ruyssen, 1969). One

unit forms 1 μ mol fatty acid per minute under the conditions of the assay.

In order to eliminate the effect of the intestinal enzymes, the fish was gutted before the enzyme preparations were added.

One part of salt was added to seven parts of herring (w/w). The fish was kept at 15° C for 3 weeks. Analyses were taken after 1, 2, and 3 weeks; organoleptic evaluation took place after 2 weeks.

Four variations were tested: (a) gutted; (b) gibbed; (c) gutted and 2.0 g of Pr 8 per kg herring added; (d) gutted and 1.2 g of TG 21/63 per kg herring added.

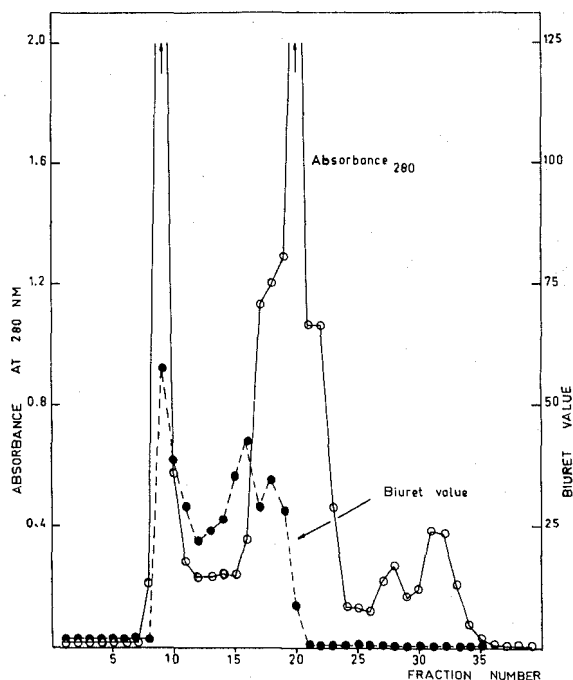


FIGURE 2.—Chromatogram of the clarified brine of gibbed maatjes herring on Sephadex when herring were ripened naturally.

TABLE 2.—Enzyme preparations used in the experiments.

Code No.	Origin and description
TG 21/63	Porcine pancreas powder
Pr 8	Porcine pancreas protease mixture with low lipase activity
Pr 34	Pancreas protease from sheep
Pr 35	Beef pancreas protease mixture
Pr 11/66	Same quality as Pr 8
W	Beef pancreas protease concentrate
CH 32/67 A	Same quality as Pr 8

TABLE 3.—Results of organoleptic evaluations and chemical analyses from experiment using two enzyme preparations.

Variation	Organoleptic evaluation		Analyses of the brine ¹					
			Biuret value			Total nitrogen		
	Flavor	Texture	After 1 week	2 weeks	3 weeks	After 1 week	2 weeks	3 weeks
Gutted	Salty; not ripened	firm	856	1,105	1,305	5.1	6.0	6.0
Gibbed	Not fully ripened	firm	--	1,320	--	--	8.1	--
Gutted + Pr 8	Ripened flavor	soft	2,620	3,670	4,320	10.0	11.8	13.4
Gutted + TG 21/63	Ripened flavor but with an unpleasant fatty acid flavor	soft	2,740	3,950	4,680	10.4	13.1	15.0

¹ The salt content in the brine was $20 \pm 2\%$ w/v.

The brine was analyzed as described earlier, but with the omission of the amino-N determination.

The results of the organoleptic evaluations and the chemical analyses are shown in Table 3. They show that, by the addition of enzyme preparations to gutted herring, it is possible to obtain a product with "maatjes-cured" organoleptic properties. A high lipolytic activity, however, seems to be undesirable.

Both the biuret value and the total nitrogen content in the brine increase during the ripening period. There seems to be a relation between the values found and the degree of ripening.

ARTIFICIAL RIPENING OF FRESH SPENT HERRING WITH ENZYME PREPARATIONS LOW IN LIPASE ACTIVITY

After the herring has spawned, the uptake of food stops and the fat content decreases gradually to relatively low values, e.g., 5 to 8%. Since the proteolytic activity of the appendices pycnoicae is low in this period, the fish as such is unsuitable for the manufacture of maatjes-cured herring.

Fresh, spent herring, caught in February in the Irish Sea were used. The protein content was 17.1% and the fat content 5.2%.

Activity of the enzyme preparations tested is shown in Table 4.

In view of the results obtained in the experiment on spawning herring, the proteolytic activity in the artificial maatjes-cured herring was reduced in this experiment.

Six variations were tested: (a) gutted; (b)

gibbed; (c, d, e, f) gutted and added respectively: 1.0 g Pr 34, 1.0 g Pr 35, 1.0 g Pr 11/66 and 55 mg W per kg of fish. The fish was kept at 3° C for 3 weeks. One part of salt was added to 10 parts of herring.

The brine was analyzed as described earlier. In analyses of the herring, 30 g of ground herring fillets were homogenized with 100 ml of water in an Ultra-Turrax mixer. The mixture was heated to 80° C and, after cooling to room temperature, filtered through fluted filter paper. In the extract thus obtained, the same analyses as earlier described for the brine were carried out. Gel chromatography was performed as described earlier.

The results of the organoleptic evaluations and the chemical analyses are shown in Table 5.

The brines of the variations "gutted" and "gutted + Pr 35" were chromatographed. The results are plotted in Figures 3 and 4.

With the preparations Pr 35 and Pr 11/66, an acceptable product was obtained. W gave rise to a good texture but developed less flavor. Pr 34 caused some off-flavor, probably because of the comparatively high lipolytic activity. It seems to us that a lipolytic activity lower than 1 U/mg is desirable for a preparation which has a protease activity equivalent to the minimum activity required by NF XIII.

TABLE 4.—Proteolytic and lipase activities of enzyme preparations.

Enzyme preparation	Proteolytic activity according to NF XIII	Lipase activity: Desnuelle U/mg
Pr 34	1.6 × NF	1.8
Pr 35	1.0 × NF	0.2
Pr 11/66	3.0 × NF	0.2
W	20 × NF	0.6

TABLE 5.—Results of organoleptic evaluations and chemical analyses from experiment with the artificial ripening of fresh spent herring with enzyme preparations low in lipase activity.

Variation	Organoleptic evaluation		Chemical analyses					
	Flavor	Texture	In the brines ¹			In the extracts		
			Biuret value	Total N	Amino N	Biuret value	Total N	Amino N
Gutted	Nontypical	Moderately firm	1,345	5.6	0.138	192	0.55	0.017
Gibbed	Nontypical	Moderately firm	1,665	7.8	0.178	--	--	--
Gutted + Pr 34	Fair, but with some off-flavor	Soft	2,690	8.8	0.255	--	--	--
Gutted + Pr 35	Fair	Too soft	2,900	9.1	0.252	--	--	--
Gutted + Pr 11/66	Fair	Soft	2,355	7.3	0.210	487	1.29	0.036
Gutted + W	Nontypical	Soft	2,460	8.2	0.224	--	--	--

¹ The salt content in the brines was $12 \pm 1\%$ w/v.

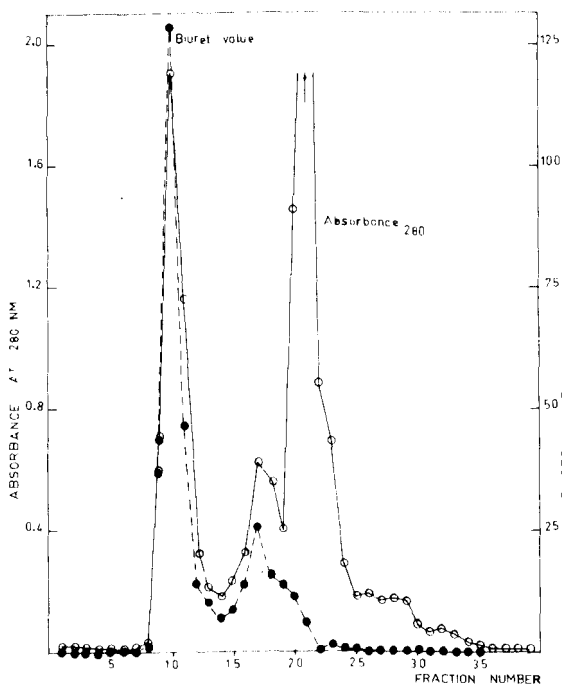


FIGURE 3.—Chromatogram of the clarified brine of gutted herring, without the addition of enzymes on Sephadex G-25, in experiment on artificial ripening of fresh spent herring with enzyme preparations low in lipase activity.

There exists a relation between the texture of the herring and the biuret value found in the brine. Between texture and total amount of nitrogen in the brine no apparent relation was shown. The ratio total N to amino N is about the same in the brine and in the herring extract, indicating that analysis of the brine give information about the ripening process in the herring.

The chromatograms show that, in the brine of the ripened herring, the amount of biuret-positive material with larger retention times has increased. This indicates that the amount of small protein fragments increased.

The chromatographic pattern of the brine of an artificially ripened herring (Figure 4) does

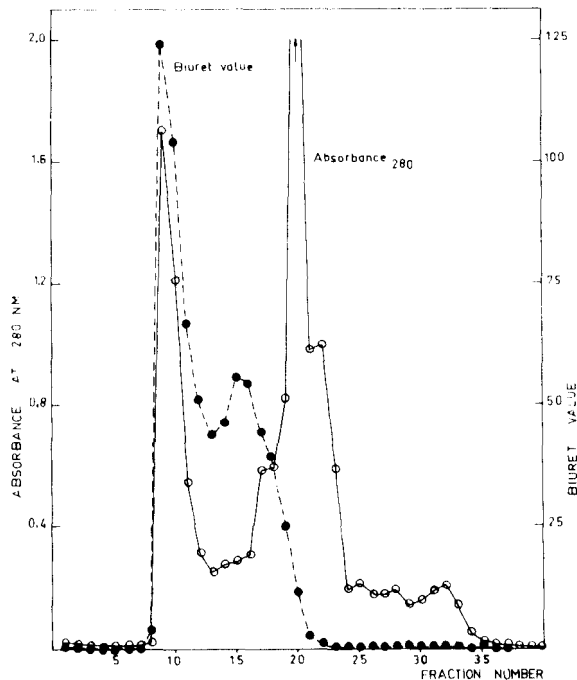


FIGURE 4.—Chromatogram of the clarified brine of gutted herring, with the addition of the enzyme Pr 35, on Sephadex G-25, in experiment on artificial ripening of fresh spent herring with enzyme preparations low in lipase activity.

not differ essentially from that of the gibbed herring brine (Figure 2). With the methods used, no obvious difference was found between the natural and the artificial ripening process.

THE ARTIFICIAL RIPENING OF LEAN HERRING AND ITS RELATION TO CHANGES IN THE RESULTS OF BRINE ANALYSIS

Fresh herring caught in the North Sea at the beginning of April were used. The protein content was 16.7%; fat content, 10.6%.

Two variations were studied: (a) gutted; (b) gutted and with 1.0 g of Pr 35 per kg of herring added. One part of salt was added to 10 parts of herring. The fish was kept at 3° C. Samples for analysis were taken after 11, 17, 24, and 31 days. Organoleptic evaluation was carried out after 24 days.

The brine was analyzed as described earlier.

After 24 days at 3° C, organoleptic evaluation showed: variation (b) had a soft texture and a well-ripened flavor, whereas variation (a) was still firm and had less flavor.

Results of chemical analysis of the brine are plotted in Figure 5 and summarized in Table 6.

The salt content in the brines was $16 \pm 2\%$ w/v.

During the ripening process there is a steady increase in biuret value, total N, and amino N in the brine. This finding demonstrates again the relation between these values and the degree of ripening.

THE ARTIFICIAL RIPENING OF FRESH LEAN HERRING WITH DIFFERENT AMOUNTS OF A PROTEASE PREPARATION

Fresh spent herring caught near the Hebrides in August was used. This herring contained 17.4% of protein and 8.2% of fat.

In this experiment an enzyme preparation CH 32/67 A was tested, with a proteolytic activity of 1.93 times NF XIII units and a lipolytic activity of 0.3 Desnuelle units per mg.

One part of salt was added to 10 parts of fish. Six variations were tested: (a) gutted; (b) gibbed; (c, d, e, f) gutted and 0.5, 1.0, 2.0, and 5.0 g of CH 32/67 A added, respectively. The fish was kept at 3° C for a month, then evaluated and analyzed.

The brine was analyzed as discussed earlier. Results are shown in Table 7.

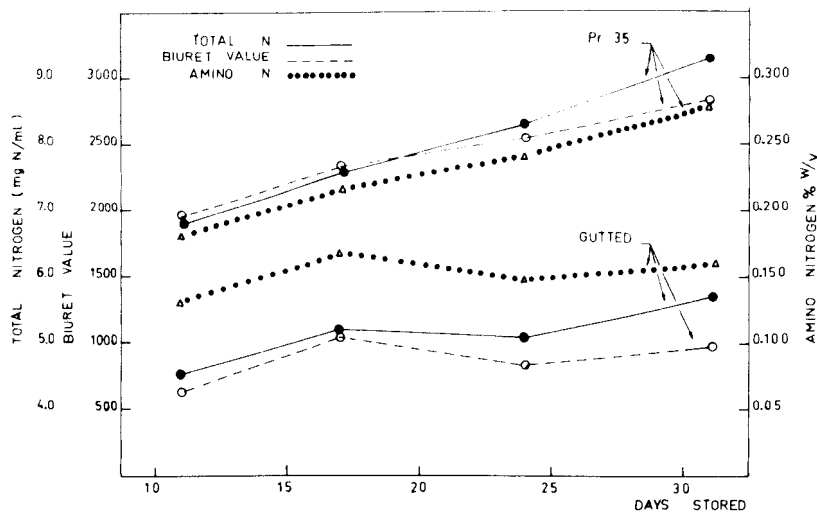


FIGURE 5.—Results of analyses of clarified brines of gutted herring with and without addition of Pr 35, in experiment on the artificial ripening of lean herring and its relation to changes in the result of brine analysis.

TABLE 6.—Chemical analyses of the brines¹ in experiment on artificial ripening of lean herring.

Brines	Gutted	Gutted + Pr 35
Biuret value:		
after 11 days	625	1,915
after 17 days	1,010	2,320
after 24 days	840	2,520
after 31 days	960	2,820
Total N:		
after 11 days	4.5	6.8
after 17 days	5.2	7.6
after 24 days	5.1	8.3
after 31 days	5.7	9.3
Amino N:		
after 11 days	0.131	0.183
after 17 days	0.165	0.215
after 24 days	0.146	0.238
after 31 days	0.160	0.282

¹ The salt content in the brines was $16 \pm 2\%$ w/v.

An addition of 0.5 g of CH 32/67 A per kg of herring is too low to obtain a well-ripened product under these conditions. An addition of 1.0 g per kg, however, seems somewhat too high. A suitable dose is probably the amount of protease that corresponds with a proteolytic activity of 2000 mg-eq NF XIII powder per kg of herring.

In the brines (b) and (d) the same amino-N contents were found, but the biuret value in (d) was considerably higher than in (b). This suggests that the action of the enzyme preparation is particularly the breakdown of muscle protein to larger fragments, whereas the endogenous enzymes show more peptidase activity. This is in accordance with the observation that an overdose of the enzyme preparation results in a very soft rather than in a strong-tasting herring.

SUMMARY

The addition of a certain quantity of a protease preparation to herring which is unsuitable for maatjes curing has a favorable effect on both flavor and texture of the herring.

An addition of 2000 mg-eq NF XIII powder per kg of herring is proposed. Doubling or halving this amount had a pronounced effect on the organoleptic properties of the cured herring. The lipase activity should be low.

In the brines the biuret value, the total N content and the amount of amino N increase gradually. There appears to be a distinct relation between the biuret value in the clarified brine and the consistency of the herring; the same is true for the amino N content in the brine and the flavor of the herring.

The ratio biuret value: amino N content found in the brine was higher for artificially ripened herring than for the naturally ripened product. This finding indicates that the enzyme preparations are poorer in peptidase activity than the appendices pyloricae from the herring.

The protein breakdown in the artificially ripened herring, however, does not seem to be essentially different from that in the naturally ripened herring. The patterns obtained by chromatography of the brines over Sephadex G-25 did not show any essential difference.

ACKNOWLEDGMENTS

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TABLE 7.—Results of organoleptic evaluations and chemical analyses from experiment on artificial ripening of fresh lean herring with different amounts of a protease preparation.

Variation	Organoleptic evaluation		Chemical analyses of brines ¹		
	Flavor	Texture	Biuret value	Total N	Amino N
Gutted	Salty; no ripened flavor	Firm	1,300	5.4	0.195
Gibbed	Nontypical	Moderately firm	1,640	9.0	0.328
Gutted + 0.05% CH 32/67 A	Fair	Moderately soft	2,320	8.0	0.274
Gutted + 0.10% "	Fair	Optimal (soft)	2,875	9.2	0.334
Gutted + 0.20% "	Strongly ripened	Too soft	3,210	10.7	0.398
Gutted + 0.50% "	Not tasted	Too soft	3,650	11.9	0.465

¹ The salt content in the brines was $12 \pm 1\%$ w/v.

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