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HERRING STICKWATER VISCOSITY

Identity of the Gel Factor in Herring Solubles and Means of Overcoming Its Effect

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Herring solubles, prepared by concentrating herring stickwater after acid treatment, varied in viscosity and capacity to gel depending on the season when the herring were caught. Solubles from herring caught in the late fall and winter had a much higher viscosity and tendency to gel than the product from fish caught in the summer and early fall. This change in viscosity correlated with an increase in the gelatin content of the solubles and paralleled the development of milt and roe in the fish. Proteolytic enzyme treatment of stickwater prior to its condensation to solubles was effective in overcoming the action of the gelatin. The capacities of various commercially available proteolytic enzyme preparations to produce solubles with desirable physical characteristics were compared.

HERRING STICKWATER is the water phase of the press juice obtained when whole herring are "reduced" to fish meal. To prepare a fluid concentrate of this by-product, referred to as solubles, suspended and dissolved proteins must first be either removed or destroyed. In one process, the proteins are coagulated by acidulation of the stickwater and then removed by centrifugation (*10*). In another, the protein molecules are reduced to smaller fragments by the action of proteolytic enzymes (*5*).

The acidulation process has been used exclusively in this area of the Pacific coast for some years. Even though the same process was applied to the stickwater throughout the year, the physical properties of the resulting solubles varied with the season. While solubles prepared from summer herring was fluid, that produced from late fall and winter herring was viscous and tended to gel upon standing.

The purpose of this study was to investigate the reasons for the seasonal change in viscosity of herring solubles and to determine how a product with satisfactory physical characteristics could be produced throughout the year.

Experimental

Special Apparatus. The centrifuge used for all pilot plant operations was a Centriwesta Model NWA 205 (Merco Centrifuge Co., San Francisco, Calif.). This centrifuge has a continuous flow arrangement and applies a force of approximately 7500 × gravity. It can be operated with either a chamber or a disk bowl.

Source of Materials. Fresh whole Pacific herring (*Clupea pallasii*) were obtained from local fishing companies and stored at 10° F. until used.

When large volumes of stickwater, representative of a particular season were required, arrangements were made to obtain primary stickwater from a local plant. These samples were held at 10° F. until needed. As a further precaution against bacterial deterioration of the stickwater, Aureomycin was added to all samples as a preservative, before freezing, at a level of 2 p.p.m. (*7*).

A pilot plant was designed to approximate commercial plant conditions as closely as possible (*3*). There whole herring were steam cooked and pressed. The expressed fluid was heated to 212° F. and maintained there for 15 minutes to achieve maximum oil separation. After

oil removal by centrifugation, the primary stickwater was treated with either acid or enzyme.

The acid treatment consisted of lowering the pH of the primary stickwater to 5.0 with concentrated sulfuric acid and heating to 170° F. for 15 minutes. The temperature of the stickwater was then raised to 212° F. and centrifuged twice, first with the chamber bowl of the Centriwesta centrifuge to remove coagulated protein and then with the disk bowl to separate oil released by the acid and heat.

For enzyme treatment the temperature and pH of the stickwater were adjusted to the optimum for the enzyme under investigation. Following the addition to the stickwater, of a slurry of enzyme in water, digestion was allowed to proceed for the required length of time. At the completion of each enzyme digestion period the stickwater substrate was boiled for five minutes at a pH of 3.0 to insure enzyme deactivation. The stickwater was then cooled and the pH was readjusted to the desired level.

The treated stickwater was concentrated in vacuo by one of two methods: If a large quantity of solubles was required, the pilot plant concentrator was

used; for a small volume of solubles, concentration was carried out using a laboratory-size glass evaporator equipped with an antifoam head. Unless otherwise indicated, samples were evaporated to the solids concentration prevailing in commercial solubles, 50 ±1%.

In Table I a comparison is made of pertinent analytical data obtained from solubles produced in the pilot plant and from a product obtained commercially. Both samples of solubles were prepared by the acidulation process from herring caught in the same month. The most pronounced differences between the two products were found in the significantly lower levels of oil and insoluble solids in the pilot plant product. This difference can be attributed principally to the greater efficiency of the pilot plant centrifuge. It was not possible to obtain a centrifuge for use in the pilot plant, scaled down directly from those used commercially. Furthermore, the pilot plant arrangement afforded a finer degree of control of the rate of flow of stickwater into the centrifuge than would be possible under commercial conditions.

Viscosity Measurements. Viscosity was measured by two methods. In the first, a Stormer viscometer (15) equipped with a paddle-type rotor driven by a 200-gram load was used. This instrument is used in the local industry for product control work. A soluble is not considered suitable for commerce in this area if the Stormer reading on it exceeds 30 seconds. The instrument, however, can give erroneous information when the product tends to gel (17). To overcome this disadvantage a supplementary measurement, the pour residue test, was devised. In this test a 50-ml. beaker containing a weighed amount of solubles was placed in a water bath, at a fixed temperature, for a stated period of time. The beaker containing the sample was then placed at an angle of 45° and allowed to drain for 30 minutes and the percentage by weight remaining in the beaker was determined. The viscosity was expressed as the per cent retained in the beaker under the given conditions.

The viscosity of certain samples did not reach a maximum until some time had elapsed after their preparation. To obtain a measure of this thixotropic effect, both Stormer and pour residue tests were conducted at 2-hour and 24-hour intervals after the solubles had been prepared. Because the viscosity increases as the temperature decreases and it is desirable that solubles remain fluid even in winter, viscosities were measured at 50° F. as well as at room temperature.

Determination of Total Solids. The total solids content of the herring stickwater and solubles was determined by means of a Cenco moisture balance (Central Scientific Co., Chicago, Ill.).

Determination of Insoluble Solids. A Gooch crucible with an acid-washed asbestos mat was prepared and weighed after drying. An 0.5-gram sample of solubles was mixed with 2.0 grams of Celite filter aid suspended in 100 ml. of water and filtered through the crucible with the aid of suction. The filter was then thoroughly washed with successive 30-ml. aliquots of ethyl alcohol, diethyl ether, and acetone. Following this, the crucible was dried and weighed. The amount of solids retained in the crucible, corrected for the amount of Celite added, was referred to as the insoluble solids and expressed as per cent by weight of the original solubles.

Determination of α-Amino Nitrogen. Free α-amino nitrogen was determined by Sahyun's (16) modified Sorensen formol titration method.

Extraction and Determination of Gelatin. Approximately 2-gram samples of herring solubles, accurately weighed, were acetone dried, and the gelatin was extracted by the method of Neuman and Logan (14). Trichloroacetic acid was added to the extracts to a concentration of 5% (w./v.) and the nongelatin proteins which precipitated were removed by filtration (8). The precipitate was washed with 5% trichloroacetic acid, and the filtrate and washings were then combined, evaporated to dryness, dissolved in water, and diluted to 10 ml. Aliquots of this gelatin extract were hydrolyzed in sealed combustion tubes at 230° F. for 6 hours with 6N hydrochloric acid, decolorized with charcoal, and filtered. After dilution to volume, hydroxyproline was determined on the filtrates, by the modified method of Martin and Axelrod (12), which was further modified to include a heating period of 15 minutes at 80° C. after the addition of ferrous sulfate to ensure the removal of peroxide.

To establish whether hydroxyproline could be determined directly in the hydrolyzates obtained, a known volume of the hydrolyzate was chromatographed on Whatman No. 1 filter paper using

water saturated 1-butanol–acetic acid as a solvent (1). The spot corresponding to hydroxyproline was eluted and the method described above for the colorimetric determination of the amino acid was applied to the eluate. The results obtained with three samples of solubles showed that the hydroxyproline values obtained by the chromatographic technique differed from those obtained by direct determination on the hydrolyzate by less than 1%.

To obtain a value for hydroxyproline in herring gelatin, gelatin was extracted from fresh herring scales by the method of Burley and Solomons (2) and analyzed for hydroxyproline. The value of 8.55% obtained was in good agreement with other values reported in the literature for fish gelatins (2, 4). Using this value the gelatin content of each extract from herring solubles was calculated from its hydroxyproline content.

Results and Discussion

To establish the degree of change in the properties of solubles prepared at different seasons of the year, solubles were produced in the pilot plant at approximately monthly intervals using the acidulation process. Viscosity determinations were carried out on each sample by both the Stormer and pour residue tests. The results of this study,

Table I. Comparison of Solubles Prepared in Pilot Plant and Commercially by Using Acidification Process

	Pilot Plant Product, %	Commercial Product, %
Water	51.0	53.48
Total solids	49.0	46.52
Insoluble solids	4.93	7.18
Protein	39.16	35.37
Oil	0.95	2.20
Ash	9.87	8.94

Table II. Comparison of Physical Properties and Analytical Data on Samples of Herring Solubles Prepared at Approximately Monthly Intervals

Month Caught	Insoluble solids, %	Stormer Values[a] 2 hr.	Stormer Values[a] 24 hr.	Pour Residue Test[b] 77° F. 2 hr.	Pour Residue Test[b] 77° F. 24 hr.	Pour Residue Test[b] 50° F. 24 hr.
July	1.42	15.7	16.1	0.5	0.7	1.5
August	7.64	29.6	80.4	9.1	14.5	28.3
September	6.72	107.0	268.0	19.8	32.0	45.6
November	8.83	c	c	54.9	34.1	100.0
December	6.47	279.0	c	36.1	41.5	100.0
January	7.36	236.0	350.0	14.1	27.7	100.0

[a] Seconds/100 revolutions/200 grams at 77° F. Values determined at indicated times after preparation of solubles.

[b] Per cent of solubles retained in beaker at indicated temperatures and times after preparation of solubles.

c Too strong a gel for Stormer test to be applied.

recorded in Table II, show clearly the increased viscosity and gelling in solubles from winter-caught herring. Although the results suggest that some lowering in viscosity occurred after preparation of the November sample, it is unlikely that the differences shown are significant. Not only can viscosity values in these samples not be determined with great accuracy when the viscosities are so high, but also differences in the amount of heat applied could account for variations in the strength of the gel in these samples of solubles.

As insoluble solids are known to increase the viscosity of solubles (13), the rise in this component between July and August could perhaps have accounted for the viscosity differences noted in the samples from these two months. Because only minor changes in the insoluble solids levels occurred between August and January, however, this factor cannot explain the considerable differences in viscosity values obtained after August.

The extent of gel formation was somewhat greater in the samples prepared experimentally than was normally observed in those obtained commercially, probably because of a more efficient evaporator in the pilot plant system, resulting in the application of less heat to the stickwater than would normally be applied under industrial conditions.

The progressive increase in gel formation in solubles as the season progressed from summer to winter appeared to parallel the development of the gonads in herring. Stickwater was prepared from whole late season herring and from late season herring of the same batch from which the milt and roe had been removed. Results showed that the removal of milt and roe did not decrease the viscosity, but actually appreciably increased the Stormer values of the solubles.

In a further attempt to elucidate the factors involved in the viscosity changes of solubles, a pool of primary stickwater prepared from herring caught in February was divided into five parts and subjected to various treatments to evaluate their effect on the physical properties of the resulting solubles (Table III). As would be expected, the untreated stickwater produced solubles of high viscosity. The effect of removing insoluble solids completely by high speed centrifugation was determined. Untreated primary stickwater was centrifuged in a Servall SS-1 angle head centrifuge (Ivan Sorvall Inc., Norwalk, Conn.) at 20,000 X gravity for 20 minutes. The clear, supernatant liquid obtained was concentrated without further treatment. The product formed (sample 2, Table III) did not have properties appreciably different from those of the product from untreated stickwater. Lowering the pH of the stickwater to 5.0 followed by centri-

Table III. Effect of Treating Stickwater in Various Ways on Physical Properties of the Solubles Produced

Sample No.	Treatment before Centrifugation	Stormer Values[a]		Pour Residue Test[a]		
				70° F.		50° F.
		2 hr.	24 hr.	2 hr.	24 hr.	24 hr.
1	Untreated	198.7	352.3	11	19	100
2	Centrifugation	136.3	551.1	4.8	9.3	100
3	pH to 5.0, centrifugation	30.2	53.2	0.8	1.3	100
4	pH to 3.9, centrifugation	36.5	48.5	0.2	0.3	75.7
5	Enzyme digested[b]	17.4	17.9	0.8	1.4	3.0

[a] See Table II.
[b] Rhozyme B-6, 0.5% level, pH 6.0, 140° F. Digestion period, 2 hours.

fugation resulted in a very significant decrease in viscosity as determined by the Stormer test. This latter treatment is essentially that used in the acidulation process (10). The product still gelled, however, at 50° F., as indicated by the 100% retention obtained in the pour residue test at that temperature. Perhaps not all the protein had coagulated at pH 5.0, but a further lowering of the pH to 3.9 followed by centrifugation did not produce any significant changes in either the Stormer or pour residue values of the solubles obtained. Digestion with a commercial proteolytic enzyme, however, reduced the viscosity to very low levels as determined by the Stormer test and completely eliminated gel formation—i.e., a protein was responsible for the viscosity effects. If the gelling agent was a protein it would have to be one which was soluble in water and dilute salt solution and was not coagulated by heat or acid. Gelatin has such properties, therefore it seemed not unlikely that this substance might be responsible for the gelling effect.

To investigate the possibility that seasonal variations in the gelatin content of solubles might be responsible for the viscosity differences observed, solubles were prepared from herring caught, as nearly as possible, at monthly intervals over a period of a year. Quantitative estimations of the gelatin content of the solubles were made and the results obtained were compared with the viscosity characteristics of the corresponding solubles (Table IV). The marked drop in the gelatin content of the solubles between March and April and a corresponding decrease in the viscosity of the solubles coincided with the spawning of the herring.

As the changes in the gelatin content of the solubles correlated so well with the seasonal changes in the viscosity of the solubles, gelatin is probably responsible for the variations in the product when the acidulation procedure is used in its preparation.

Tests were run to find which of a number of proteolytic enzymes, commercially available, would be most

Table VI. Effect of Length of Time of Enzyme Treatment of Stickwater on Viscosity of Solubles

Time of Enzyme Digestion,[a] Minutes	Viscosity of Solubles			
	Stormer Values[b]		Pour Residue Test[c]	
	2 hr.	24 hr.	77° F.	50° F.
Control	199.0	241	22.5	100.00
30	33.1	43.0	12.5	19.90
60	26.8	29.7	5.9	20.70
120	21.4	25.6	6.2	15.90

[a] Rhozyme B-6, 0.5% level, pH 6.0, 140° F.
[b] See Table II.
[c] Determined at indicated temperatures 24 hours after preparation of solubles.

Table VII. Effect of Holding Stickwater at 212° F. for Varying Periods on Viscosity of Corresponding Herring Solubles

Sample No.	Length of Heat Treatment of Stickwater, Minutes	Stormer Values of Solubles[a]
1	0	62.1
2	<1	43.3
3	30	41.8
4	60	41.3
5	120	33.2

[a] Determined at 77° F. 2 hours after preparation of solubles.

suitable for use in treating stickwater. Primary stickwater prepared from February herring was used as the substrate. In one case, this stickwater was acidified to pH 5.0 and the acid coagulable proteins were removed by centrifugation. The various proteolytic enzymes were then tested for their ability to destroy the gelatin in the supernatant solution. In another experiment the primary stickwater was used as the substrate without preliminary acidulation. This test was thus designed to demonstrate the capacity of the proteolytic enzymes, not only to attack gelatin, but also to break down proteins coagulable by acid. As the enzymes most active in attacking

the gelatin were also most active in lowering the viscosity when used on the unacidified stickwater, only the latter results, as they are of more general significance, are shown in Table V. Each enzyme selected was tested under its optimum conditions as recommended by the manufacturer. There was no correlation between the capacity of the enzymes to release α-amino groups and their ability to reduce the viscosity of the solubles.

For the most economical use of an enzyme preparation, it is desirable to know the minimum time required for a given concentration of enzyme to exert its maximum activity. The results in Table VI show that a 1-hour digestion period produced satisfactory solubles, from a viscosity standpoint, when a Rhozyme B-6 preparation was used at a 0.5% concentration.

As the presence of increased amounts of gelatin in the solubles, from late season herring, appears to be responsible for the high viscosity of solubles prepared by acid treatment, methods other than treatment of the stickwater with proteolytic enzymes might conceivably be effective in reducing the viscosity. The capacity of gelatin to produce a stable gel can be reduced by prior heat treatment of the gelatin solution (9). Consequently, the application of heat to stickwater before the preparation of solubles was investigated as a possible means of reducing the viscosity of the latter. Primary stickwater, pH 5.0, was heated to 212° F. for 30, 60, and 120 minutes (Table VII). The heated stickwater was cooled and then centrifuged, for 20 minutes, at 12,000 r.p.m. in the Servall SS-1 centrifuge. To avoid using different amounts of heat during the

concentration of the heat treated samples of stickwater, the use of heat at this step was eliminated, by concentrating the samples at room temperature. A rotary evaporator with the receiver for the distillate chilled in an acetone-dry ice mixture was used.

The most pronounced reduction in viscosity occurred during the initial few seconds; however, heating at 212° F. for 2 hours failed to bring the viscosity of the solubles to the acceptable reading of 30 seconds by the Stormer viscometer test. A similar test at 185° F. instead of 212° F. presented the same picture, except that the total decrease in the viscosity of the solubles was less. Prolonged heating at the same temperature had little, if any, additional effect on the viscosity of the product.

Conclusions

The source of the gelatin from herring and the reason that its increase in solubles should parallel the development of milt and roe in the fish have formed the subject of a separate investigation which will be reported elsewhere.

Proteolytic enzyme treatment has been shown, in this study, to be capable of eliminating the capacity of solubles to gel. As might be expected from the heterogeneous nature of a substrate which consists of all the suspended and dissolved proteins extracted from the fish during cooking, there appears to be little to choose between available enzymes in so far as their capacity to carry out the digestion is concerned. The lack of correlation between α-amino nitrogen release and viscosity change in solubles can be explained either by differences in ratio of endopeptidase to exopeptidase activity in the various enzyme preparations or in their capacity to attack non-gelatin proteins as compared to gelatin. It would seem that the relative cost of the various preparations would have a considerable bearing on the selection of an enzyme for this particular process. Because enzyme preparations can vary in activity from batch to batch, any doubts concerning the relative merits of different preparations should be resolved by direct comparison of activities on the substrate to be treated.

Heat treatment of acid treated stickwater has been found to lessen the effect of the gelatin, but not to the extent desired at the temperature tested. The results obtained in this study would tend to support a report (13) that the temperature rather than the duration of heating is the important factor in reducing the gelling capacity of gelatin. Although higher temperatures than those tested here might very well eliminate the effect of the gelatin, it would be necessary to determine whether or not such temperatures also affected the heat-

Table IV. Relation between Gelatin Content and Viscosity of Herring Solubles Prepared from Herring Caught at Approximately Monthly Intervals

Month Caught	Gelatin in Solubles, %	Viscosity of Solubles		Pour Residue Test[a], 50° F., 24 hr.
		Stormer Values[a]		
		2 hr.	24 hr.	
August	1.18	20.8	21.8	6.74
September	1.62	28.0	42.1	100.00
November	2.26	63.0	94.0	100.00
December	2.31	128.0	136.0	100.00
January	2.68	>150.0	>150.0	100.00
February	2.56	138.0	>150.0	100.00
March	2.42	>150.0	>150.0	100.00
April	0.99	23.0	23.6	7.85
May	0.99	20.3	20.7	6.35
June	0.91	23.3	24.2	7.10

[a] See Table II.

Table V. Effect of Treating Primary Stickwater with Proteolytic Enzyme Preparations on Viscosity of Resulting Solubles

Enzyme Used[a]	Enzyme Concentration[b]	Pour Residue Test on Solubles[c]	α-Amino N Released in Stickwater[d]		
			0.5 hr.	1 hr.	2 hr.
Control	0	100.00	0	0	0
Rhozyme B-6	0.5	7.7	0.0470	0.0470	0.0504
	0.05	23.0			
	0.005	26.3			
Ficin	0.5	27.5	0.0924	0.1064	0.1288
	0.05	37.2			
	0.005	100.0			
Bromelin	0.5	12.0	0.1400	0.1428	0.1484
	0.05	37.4			
	0.005	25.4			
Protease	0.5	14.8	0.1456	0.1652	0.2184
	0.05	17.0			
	0.005	25.1			
Papain	0.5	26.0	0.0010	0.0212	0.0500
	0.05	39.7			
	0.005	100.0			
Collagenase	0.5	13.30	0.0200	0.0414	0.0506
	0.05	26.60			
	0.005	31.00			

[a] Stickwater treated with each enzyme for 2 hours under following conditions: rhozyme B-6, pH 6.0, 140° F.; ficin, pH 5.0, 104° F.; bromelin, pH 5.0; 140° F.; protease, pH 8.0, 104° F.; papain, pH 5.0, 98° F.; collagenase, pH 6.5, 98° F.
[b] Expressed as per cent of total solids present in stickwater.
[c] Determined at 50° F., 24 hours after preparation of solubles.
[d] Expressed as mg./ml. and determined at indicated times during digestion.

labile factors important to the nutritional value of the solubles.

As solubles are used as supplements in animal feeds, consideration should be given to the relative nutritional value of solubles prepared by acid and enzyme treatment of stickwater. When the two types of solubles were used to supplement both natural and synthetic rations for the growth of chicks, no significant differences in the nutritive value of the products could be detected (*11*).

Acknowledgment

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Fungicide Distribution and Disinfection Efficiency in Seed Treatment—Correction

On page 327 [J. Agr. Food Chem. **7**, 326 (1959)], the caption for Figure 2 should read:

Results from seed treatment tests with log Φ as a function of log dose (O, ⏺, ◖) compared to probit analysis (●)

In section III of the subcaption of Figure 2, the last symbol (●) should be O.

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