Characterization and Utilization Of Waste From Ocean Quahog And Surf Clam Processing Plants

Food Science and Technology Section

Mid-Atlantic Fisheries
Development Foundation, Inc.

Sea Grant
Extension Division
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A REPORT

on

CHARACTERIZATION and UTILIZATION OF WASTE FROM OCEAN QUAHOG and SURF CLAM PROCESSING PLANT

Food Science and Technology
Section

by

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INTRODUCTION

During surf and ocean quahog clams processing, large amounts of liquid (wash water from various processing stages) and solid (clam bellies and shells) waste effluents are produced. The liquid effluents contain soluble and suspended solids which are composed of appreciable quantities of protein and small pieces of clam meat. Traditionally, these liquid and solid waste effluents are discarded in landfills or into natural waterways. Alternative disposal of these materials presents a serious problem to clam processors as most of them have neither their own sewage treatment facilities nor access to municipal sewage systems.

Proposed waste discharge guidelines for Virginia call for significant reductions in Biological Oxygen Demand (BOD) and suspended solids in liquid waste effluents. If these regulations are enacted all clam processors will be affected and will have to make major facility changes to meet the newly recommended minimum waste effluent standards.

Some of the liquid effluents (wash waters) from clam processing plants could be converted to marketable by-products and/or food ingredients (Zall and Cho, 1977). This approach may generate additional revenue to clam processors while, simultaneously, reducing BOD and suspended solids in the wash waters at the same time. Wash water obtained from surf clam processing plants has been successfully converted into a marketable clam juice (Hood et al. 1976), a dehydrated clam flavor ingredient (Joh and Hood, 1979), and protein concentrate (Hang et al. 1980).

Clams used in the above studies were subjected to a heat shock procedure in which the clams were immersed in 88°C water for 1 minute prior to being manually shucked. Recently Burnette et al. (1983) prepared a natural clam flavoring agent from ocean quahog juice obtained from a mechanical shucking operation. Although there is some information about production of a clam flavoring agent from clam juice and wash water, very little is known about the clam wash water composition, flavor profiles and their utilization as food products.

The clam belly, which constitutes from 7 to 25% of the total meat, is currently underutilized and poses a disposal problem to clam processors (Chen and Zall 1986a). The solid waste portions (bellies) of the clams which include the stomach, liver and other organs, are discarded, . Chen and Zall (1985, 1986a) found clam bellies to be good source of different proteases (D-like and B-like acid proteases). They isolated and purified acid proteases from clam bellies and studied some of their characteristics. In a separate study, Chen and Zall (1986b) isolated and characterized clam rennet (which is a crude preparation of cathepsin B-like protease) from clam bellies and compared the preparation to porcine pepsin and calf rennet for its suitability as a milk coagulant in cheese-making. They reported that clam rennet was more proteolytic and produced a softer curd than the other two coagulants. However, chedder cheese made from clam rennet was inferior to the chedder cheese made from calf rennet.

The crystalline style, which is part of the clam belly region, contains an assemblage of carbohydrate digestive enzymes which catalyze algal carbohydrate degradation (Shallenberger et al. 1974; Lindley et al. 1976). Four different carbohydrases (laminarinase, amylase, cellulase, and alginase) have been characterized from the crystalline style of surf clam bellies (Jacober et al. 1980; Jacober and Rand, 1980). The crystalline style functions by rotating against the gastric shield to grind diatomaceous and algal food while initiating enzyme hydrolysis of carbohydrate polymers (Shallenberger and Herbert, 1974; Lindley and Shallenberger, 1974). Laminarinase was found to be the major carbohydrase in the crystalline style of surf clam.

Utilization of clam bellies and its associated parts producing commercial enzymes, for use as a component of livestock or poultry feed and in the production of pet foods, may also provide an increased revenue source to clam processors while reducing wastage and meeting minimum waste effluent standards. A study of clam bellies for their proximate composition and mineral content may further enhance their use as a protein ingredient in livestock or poultry feeds and pet foods.

The objective of this study was to characterize all clam processing liquid and solid waste effluents and evaluate their potential as marketable by-products and/or food ingredients.

MATERIALS AND METHODS

Collection of washwater and belly samples:

Flow diagrams of mechanized processes used by three different clam processing plants (plants A, B, and C) in Virginia are shown in Figures 1, 2, and 3. Plant A processes surf clams (*Spisula solidissima*), while plants B and C process mostly ocean quahogs (*Arctica islandica*). Both surf clams and ocean quahogs receive a preliminary water wash to remove sand and debris from the outside of the shells and are then subjected to a short (50-100 sec.) heat treatment using either a shucking furnace or pressurized cooker. The shucking furnace, a large propane furnace, reaches temperatures from 625-815°C. A heavy metal chain belt transports the clams through the furnace to facilitate the shucking process.

The process opens the shells and the resultant clam meats and shells are subjected to further separation using various operational, sorting and washing steps. Wash water samples were collected at different stages in the processing procedures. Wash water samples (4, 3, and 4) were collected from plants A, B, and C respectively along the processing line and are indicated by numbers 1, 2, 3 and/or 4. All wash water samples were held at -17°C until used.

The clam bellies were collected from plants A, B, and C and held at -17°C until chemical analysis.

Freeze and Spray-Drying:

All wash water samples were divided into two fractions. The first wash water sample was frozen and dried without shelf heat in a Virtis Freeze-Drier (The Virtis Company, Gardner, NY). The remaining wash water sample was spray-dried in a Buchi 190 Mini Spray-Drier (Brinkmann Instruments Company, Westburg, NY). The inlet and outlet air temperatures were 130°C and 82°C respectively. All the freeze-dried and spray-dried wash water samples were stored in plastic containers in a desiccator over Drierite.

Clam Flavor Extract from Bellies:

About 750 g of frozen bellies were mixed with 1500 ml of distilled water, boiled for 25 min. and simmered for 15 min. to produce a flavored extract. The cooked mixture was filtered through a sieve (140 mesh size) to remove viscera contents and clam particles. The filtrate (clam flavor extract) was frozen and dried in the Virtis freeze-drier as previously described. The residue (mostly meat pieces) was air dried at 100°C for 24 hrs in a forced air oven. The dried clam flavor extracts and belly meat were ground to a fine powder and stored in glass bottles in a desiccator over Drierite.

Chemical Analyses:

The salt content in all wash water samples was determined by the indicating strip method (AOAC, 1984). Total solids and moisture content in the wash water samples and clam bellies was determined by drying sample aliquots in crucibles in a forced air oven overnight at 100°C. Total protein (Kjeldahl N X 6.25) and crude fat contents of freeze-dried wash water samples, clam flavor extracts of bellies, belly meat (residue), and whole bellies were determined by AOAC (1984) methods. Selected freeze-dried wash water samples, clam flavor extracts of bellies, belly meat, and whole clam bellies were dry ashed according to the AOAC (1984) method for mineral analyses. The ashed samples were dissolved in 1.2N HCl and made to known volume with 1.2N HCl. Calcium, magnesium, potassium, sodium, phosphorus, iron, copper, and zinc were determined by inductively coupled plasma emission spectroscopy (AOAC, 1984).

Selected freeze-dried wash water samples were hydrolyzed according to the method of Bittner et al. (1980) for Total Carbohydrate determination. Total sugars in the hydrolyzates were estimated by the method of Dubois et al. (1956) using glucose as the standard. Gas chromatography was employed to identify and quantitate individual sugars in the hydrolyzates of selected wash water samples. A 0.2 ml hydrolyzed sample was derivatized to aldononitrile acetates and analyzed for individual sugar analysis (McGinnis, 1982).

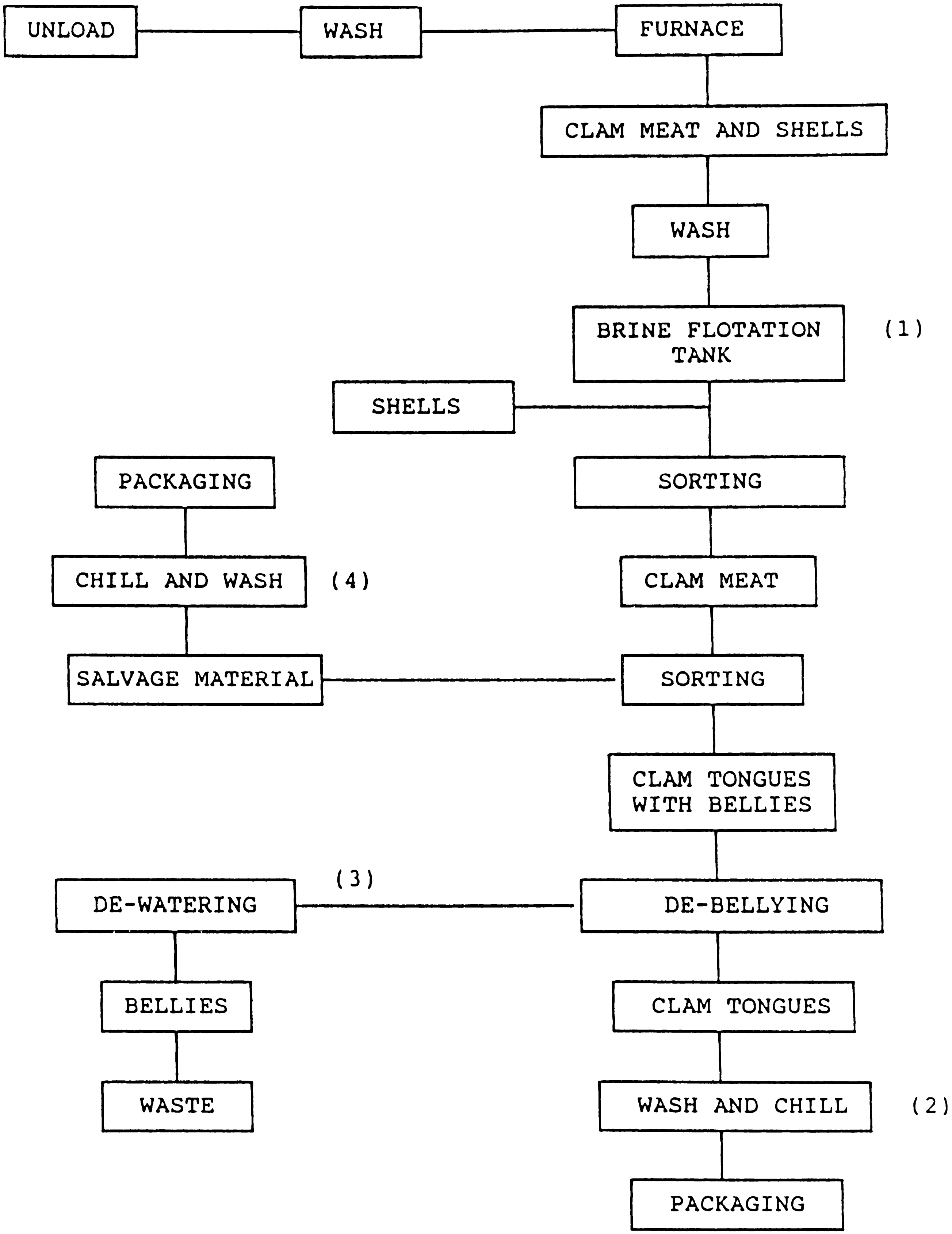

Figure 1. Flow diagram of mechanized clam process for Plant A

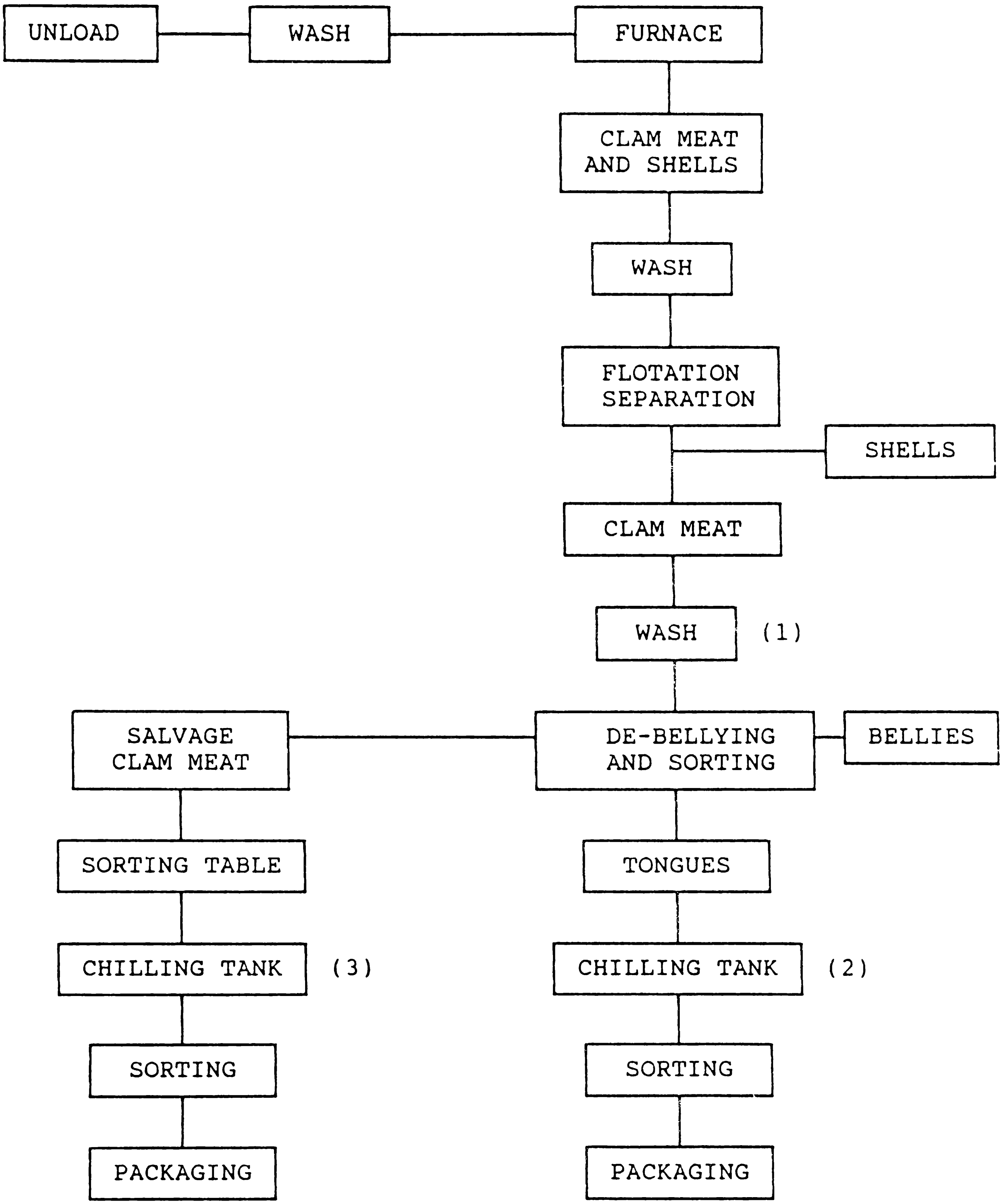

Figure 2. Flow diagram of mechanized clam process for Plant B

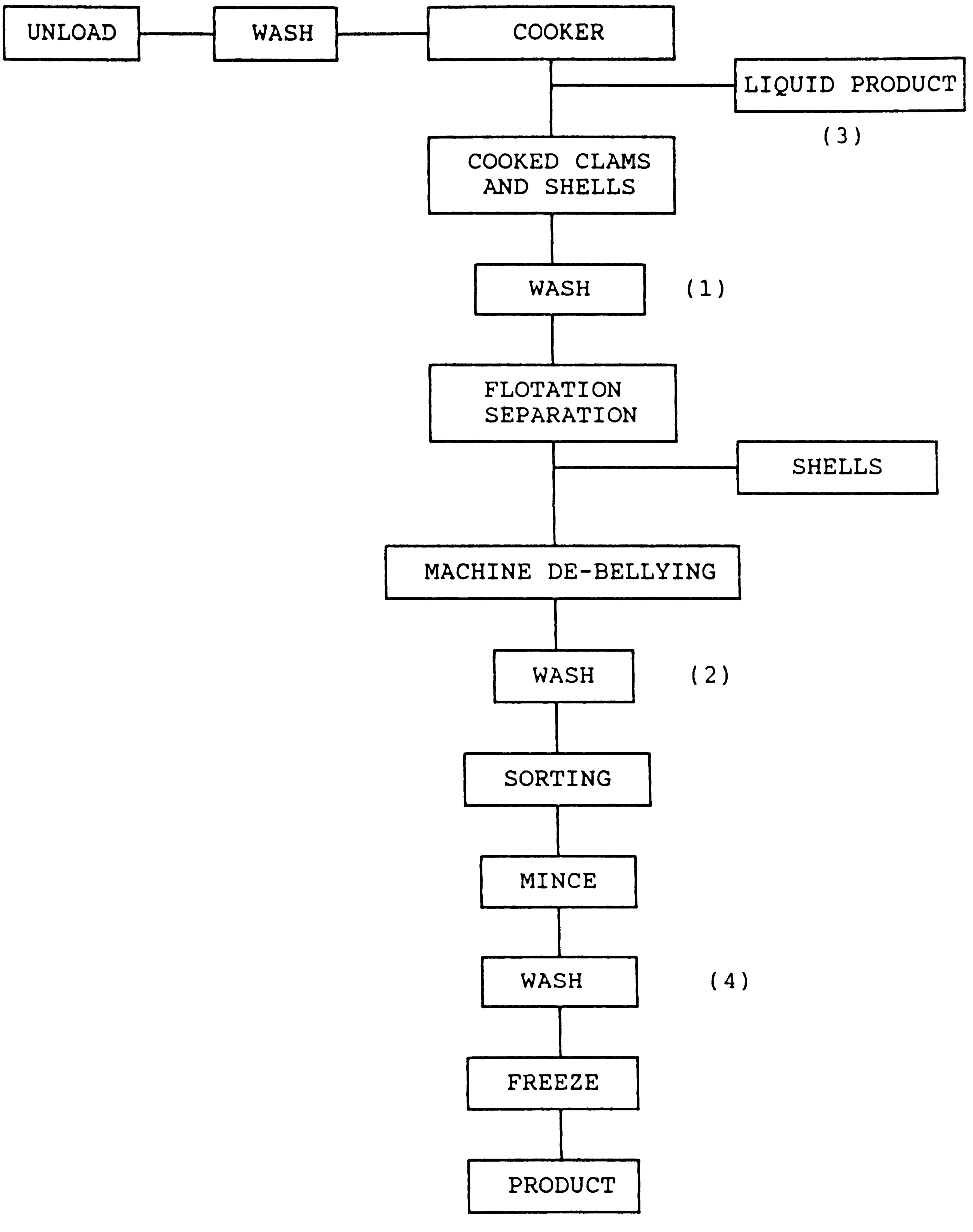

Figure 3. Flow Diagram of mechanized clam process for Plant C

Amino acids were determined by hydrolyzing 10 mg of selected wash water samples with 0.2 ml of 6.6N HCl for 40 hr at 110°C in reactivials. The hydrolyzates were dried at 100°C with nitrogen flush and dissolved in a dilution buffer (Hare, 1977). The individual amino acids were identified and quantified by a Beckman 344 HPLC Amino Acid Analyzer using Norleucine as an internal standard.

Direct Gas Chromatographic Analysis of Flavor Volatiles:

Analyses of volatile flavor profiles of selected freeze-dried and spray-dried wash water samples from plants A, B and C were performed by the rapid, direct gas chromatographic method of Dupuy et al. (1987).

Protease and Glycosidase Activities in Clam Bellies:

Approximately 200 g of frozen clam bellies were mixed with 400 ml of cold distilled, deionized water and homogenized in a Waring Blendor at high speed for 2 min. The homogenate was centrifuged at 13,000 x g for 30 min. at 4°C. The supernatant was again centrifuged at 38,000 x g for 25 min. at 4°C and filtered through four layers of cheese cloth. The filtrate was used as a crude enzyme extract for assaying protease and glycosidase activities.

The protease activity in the crude enzyme extract was measured by the method of Chen and Zall (1986) using bovine hemoglobin as a substrate. The protease activity in the crude enzyme extract was estimated over a wide pH range (2.0 to 9.0) and temperatures (4, 21, 30, 37, 45, and 55°C). The protease activity was expressed in hemoglobin units (HU). One HU was arbitarily defined as a 0.001/min increase in A280 under assay conditions employing hemoglobin as the substrate.

Enzyme activities (α - and β - glycosidase) in the crude enzyme extract were measured by the method of Reddy et al. (1984). p-nitrophenyl-α-D-glycopyranoside and p-nitrophenyl β-D-glycopryanoside were used as substrates. A 300- 1 portion of a 1mm solution of p-nitrophenyl α- or β-D-glycopyranoside in 0.2M citrate-phosphate buffer (pH 6.60) was mixed with 1600 ml of the identical buffer and equilibrated to 37°C. After the addition of 100 μl of a crude enzyme extract, the mixture was mixed well, and incubated for 10 min. at 37°C. The reaction was terminated by the addition of 5.0 ml of 0.2M sodium carbonate. The yellow product (p-nitrophenol) was determined by absorption measurement at 405 nm in a Perkin-Elmer double-beam spectrophotometer. p-nitrophenol was used as a standard and 1 unit of glycosidase was defined as the release of 1 μmol of p-nitrophenol per min at 37°C. The protein content in the crude enzyme extract was determined by the method of Peterson (1977). Specific activities of α- and β-glycosidase were expressed as micromoles of p-nitrophenol released per minute per milligram of protein at 37°C.

Sensory Evaluation of Clam Dips made with Selected Wash Water Samples:

Selected freeze-dried wash water samples from plant A, B, and C at 1.75% (w/w) were added to a clam dip formulation in place of clam meat and juice. The basic clam dip formulation consisted of mayonnaise (227 g), sour cream (227 g), and clam meat (170 g). Salt, pepper, and chopped parsley were added for taste. The dips, prepared with and without wash water samples, were served with unsalted crackers to experienced panelists to determine various sensory characteristics. The taste panelists were asked to evaluate the clam dips using a nine point hedonic scale, where 9 = extremely like, 5 = neither like or dislike and 1 = extremely dislike.

In a separate test, selected clam dips prepared with wash water samples from Plants A, B, and C were evaluated for acceptability and other sensory characteristics with a commercial clam dip using a nine point hedonic scale. Sensory evaluation data was analyzed for significance using an analysis of variance and Duncun's multiple range test.

RESULTS AND DISCUSSION

WASH WATER SAMPLES:

Proximate Composition, Carbohydrate, and Mineral Content:

The proximate composition, carbohydrate, and mineral content of freeze-dried wash water samples collected from plants A, B and C are presented in Tables 1, 2, and 3.

The brine water of plant A contained higher solids, ash, and salt than the other wash waters (tongue, belly, and salvage) (Table 1) and was not considered for further carbohydrate, mineral and volatile profile analysis and possible incorporation into foods. The total solids content ranged from 2.3-3.7% in tongue, belly, and salvage wash waters. The belly wash water contained a higher protein and crude fat than the tongue and salvage wash waters. Glucose was the major sugar in tongue, belly, and salvage wash waters and accounted for about 87% of the total carbohydrates in tongue wash water. The ash content in tongue, belly, and salvage wash waters was comprised largely of phosphorus, calcium, magnesium, potassium, and sodium (Table 1). Iron, copper, and zinc was present in small amounts in tongue, belly, and salvage wash waters.

The salvage wash water from plant B had higher ash, salt, and solids than clam meat and tongue wash waters (Table 2). The tongue wash water contained more protein, solids, and total carbohydrates and lower ash and crude fat than clam meat wash water. Total carbohydrates consisted mainly of fucose, mannose, and glucose in clam meat and tongue wash waters. Glucose represented 73.3%, 95.7% of the total carbohydrate respectively in clam meat and tongue wash waters. Phosphorus, calcium, magnesium, potassium and sodium were the major minerals in clam meat and tongue wash waters (Table 2). Iron, copper, and zinc were present in small amounts in clam meat and tongue wash waters.

The brine and clam meat wash waters of plant C contained low amounts of protein and high amounts of ash and salt (Table 3). These two wash waters were not analyzed for carbohydrate and mineral contents. The cooker liquid water had higher protein, solids, salt, and ash than mince wash water. The mince wash water contained about 50% total carbohydrates. Glucose was the major sugar and accounted for 75.7% and 90.0% of the total carbohydrates in the cooker liquid and mince wash water respectively (Table 3). The major minerals, phosphorus, calcium, magnesium, potassium, and sodium, represented 38% of ash in both the cooker liquid and mince wash water with sodium and potassium being the major minerals. The cooker liquid and mince wash water contained small amounts of iron, copper, and zinc. Burnette et al. (1983) found large amounts of sodium and potassium in concentrate ocean quahog and freshly pressed ocean quahog clam juices. They concluded that these two minerals may be present as chloride salts.

Amino Acids:

Glutamic acid, glycine, alanine, arginine, and aspartic acid were the major amino acids in tongue, belly, and salvage wash waters of plant A (Table 4) and accounted for more than 35% of the total crude protein. Glycine alone represented more than 9% of total crude protein in tongue, belly, and salvage wash waters. The presence of substantial amounts of ammonia indicate decomposition of amino acids during clam processing.

Lysine, in addition to aspartic acid, glutamic acid, glycine, alanine, and arginine, was present in large amounts in the clam meat and tongue wash waters of plant B (Table 5). These six amino acids comprised 42% and 33% respectively of the total crude protein in clam meat and tongue wash waters with Glycine accounting for more than 10%. It appears that decomposition in both fractions of some amino acids also occurred in the clam meat and tongue wash waters of plant B due to the presence of appreciable amounts of ammonia.

Aspartic acid, glutamic acid, glycine and alanine were the major amino acids in the cooker liquid and mince wash waters of plant C (Table 5). These four amino acids amounted to 27% and 25.8% respectively of the total crude protein in the cooker liquid and mince wash waters with Glycine representing about 5% of total crude protein in cooker liquid and mince wash waters.

Overall, spray-drying of wash water samples from plants A, B, and C resulted in decreased concentrations of individual amino acids and free ammonia (See Appendix Tables). Spray-dried wash water samples from plants A, B, and C lacked flavor intensity and were too salty for use in foods. Methionine in some of the wash water samples was destroyed during spray-drying. Amino acids (glycine, valine, alanine, proline, methionine and especially glutamic acid) are thought to be involved in the overall flavor of seafoods (Hashimoto, 1965). Omission or removal of some of these amino acids from seafoods may result in a much weaker taste and complete disappearance of characteristic flavor (Hayashi et al. 1981; Konosu and Hashimoto, 1965; Konosu and Yamaguchi, 1982).

Table 1. Proximate composition, salt, and mineral content of wash water samples collected from a clam processing plant (plant A).

Component	Wash Water Samples			
	Brine Water	Tongue Wash Water	Belly Wash Water	Salvage Wash Water
Salt (%)	14.7	0.2	0.2	1.3
Water (%)	65.0	96.3	97.1	97.7
Solids (%)	35.0	3.7	2.9	2.3
Protein (%)	7.6	43.4	46.6	30.6
Crude Fat (%)	8.5	3.4	19.2	1.7
Ash (%)	86.6	13.5	12.6	51.3
Total Carbohydrate(%)	-	25.1	9.0	11.0
Arabinose (%)	-	0.2	0.5	0.1
Mannose (%)	-	0.3	0.5	-
Glucose (%)	-	21.8	5.4	8.5
Minerals:				
Phosphorus (mg/g)	-	10.1	11.1	9.3
Calcium (mg/g)	-	3.5	5.0	5.9
Magnesium (mg/g)	-	2.7	2.7	3.5
Potassium (mg/g)	-	18.7	13.3	22.7
Sodium (mg/g)	-	49.4	66.7	13.8
Iron (μg/g)	-	294.0	337.5	179.0
Copper (μg/g)	-	26.0	27.0	6.5
Zinc (μg/g)	-	46.0	62.0	22.5

Protein, crude fat, ash, total carbohydrate and mineral contents were presented on a dry weight basis.

Table 2. Proximate composition, salt, and mineral content of wash water samples collected from a clam processing plant (Plant B).

Component	Wash Waters Samples		
	Clam Meat Wash Water	Tongue Wash Water	Salvage Wash Water
Salt (%)	0.05	0.1	4.5
Water (%)	99.4	98.8	95.0
Solids (%)	0.6	1.2	5.0
Protein (%)	47.4	55.2	9.7
Crude Fat (%)	4.5	2.8	4.1
Ash (%)	27.2	17.6	86.9
Total Carbohydrate (%)	13.1	16.1	-
Fucose (%)	0.3	0.4	-
Mannose (%)	0.2	-	-
Glucose (%)	9.6	15.4	-
Minerals:			
Phosphorus (mg/g)	10.8	15.0	-
Calcium (mg/g)	20.0	5.0	-
Magnesium (mg/g)	6.6	4.6	-
Potassium (mg/g)	28.1	36.8	-
Sodium (mg/g)	44.3	30.6	-
Iron (μg/g)	341.5	90.5	-
Copper (μg/g)	24.5	10.0	-
Zinc (μg/g)	65.0	35.0	-

Protein, crude fat, ash, total carbohydrate, and mineral contents were presented on a dry weight basis.

Table 3. Proximate composition, salt, and mineral content of wash water samples collected from a clam processing plant (Plant C).

Component	Wash water samples			
	Brine Water	Clam Meat Wash Water	Cooker Liquid Product	Mince Wash Water
Salt (%)	21.8	0.9	0.5	0.1
Water (%)	78.3	98.5	98.0	98.6
Solids (%)	21.7	1.5	2.0	1.4
Protein (%)	1.5	11.2	31.8	19.4
Crude fat (%)	0.2	1.6	0.1	3.7
Ash (%)	97.4	74.2	44.1	20.2
Total Carbohydrate (%)	-	-	11.1	50.3
Xylose	-	-	-	0.2
Mannose	-	-	0.2	-
Glucose	-	-	8.4	45.0
Minerals:				
Phosphorus (mg/g)	-	-	2.5	4.3
Calcium (mg/g)	-	-	20.1	2.6
Magnesium (mg/g)	-	-	13.6	2.0
Potassium (mg/g)	-	-	21.2	12.4
Sodium (mg/g)	-	-	110.2	57.1
Iron (μg/g)	-	-	67.5	85.0
Copper (μg/g)	-	-	6.0	6.5
Zinc (μg/g)	-	-	110.0	26.5

Protein, crude fat, ash, total carbohydrate, and mineral contents were presented on a dry weight basis

Table 4. Amino acid content (mg/g) of wash water samples collected from a clam processing plant (Plant A).

Amino Acid	Wash Water Samples		
	Tongue Wash Water	Belly Wash Water	Salvage Wash Water
Aspartic acid	19.6	42.1	11.2
Threonine	9.3	20.1	5.4
Serine	9.5	20.4	5.5
Glutamic Acid	23.0	50.6	17.6
Glycine	44.3	42.2	33.9
Alanine	41.4	33.7	36.0
Valine	9.1	21.5	5.0
Methionine	3.5	6.0	1.3
Isoleucine	8.3	18.7	5.7
Leucine	12.0	28.5	7.5
Tyrosine	1.6	4.0	2.0
Phenylalanine	8.5	16.3	4.6
Lysine	14.7	35.9	8.3
Histidine	6.9	14.3	6.3
Arginine	24.7	36.2	19.4
Ammonia	5.7	12.5	5.0
Total Crude Protein (%)	43.4	46.6	30.6

Volatile Flavor Profiles:

Direct sample injection gas chromatography can determine flavor volatiles in foods and food products and be used for evaluating their quality (St. Angelo et al. 1987). Volatile profiles of selected wash water samples (spray-dried and freeze-dried) collected from plants A, B and C and freeze-dried clam juice are presented in Figures 4, 5, 6, and 7. Figure 4 represents a very low volatile profile of freeze-dried clam juice since most of the volatiles eluted between 30-45 min.

Figure 5 indicates that there is little difference between spray-dried and freeze-dried wash water samples in plant A. Spray-drying caused a decrease in some volatiles, however, the difference was very small. Tongue and salvage wash water samples have more clam volatiles than freeze-dried clam juice (compared to retention peaks between 30-45 min in Figure 5 with Figure 4).

In plant B, the clam meat wash water had a stronger clam volatile (based on the retention times of peaks between 30-45 min with clam juice in Figure 4) as compared to tongue wash water (Figure 6). The tongue wash water contained smaller concentrations of clam volatiles.

Volatile profiles of cooker liquid and minced wash waters exhibited a similar pattern as that observed in the freeze-dried clam juice (Figure 7). Most of the volatiles were lost from the cooker liquid and minced wash waters during spray-drying. Overall, spray-drying of wash water samples from plants A, B and C resulted in decreased concentrations of volatiles (see Fig. 5, 6, and 7).

Table 5. Amino acid content (mg/g) of wash water samples collected from clam processing plants (Plant B and Plant C)

Amino Acid	Wash Water Samples of Plant B		Wash Water Samples of Plant C	
	Clam Meat Wash Water	Tongue Wash Water	Cooker Liquid Product	Mince Wash Water
Aspartic acid	28.0	15.8	24.2	9.8
Threonine	11.6	7.4	9.1	3.3
Serine	13.4	8.5	9.6	4.4
Glutamic Acid	24.8	27.2	28.6	14.5
Glycine	48.3	58.5	14.7	10.5
Alanine	48.9	55.4	18.3	14.9
Valine	11.5	7.5	7.3	2.8
Methionine	-	2.0	2.4	2.6
Isoleucine	10.0	6.7	6.8	4.1
Leucine	16.2	10.1	9.8	4.5
Tyrosine	2.7	-	3.1	1.3
Phenylalanine	9.8	5.6	6.6	2.5
Lysine	20.5	12.2	10.8	4.4
Histidine	15.4	7.2	10.6	4.0
Arginine	29.2	14.2	10.5	6.7
Ammonia	9.2	5.9	4.0	2.5
Total Crude Protein (%)	47.4	55.2	31.8	19.3

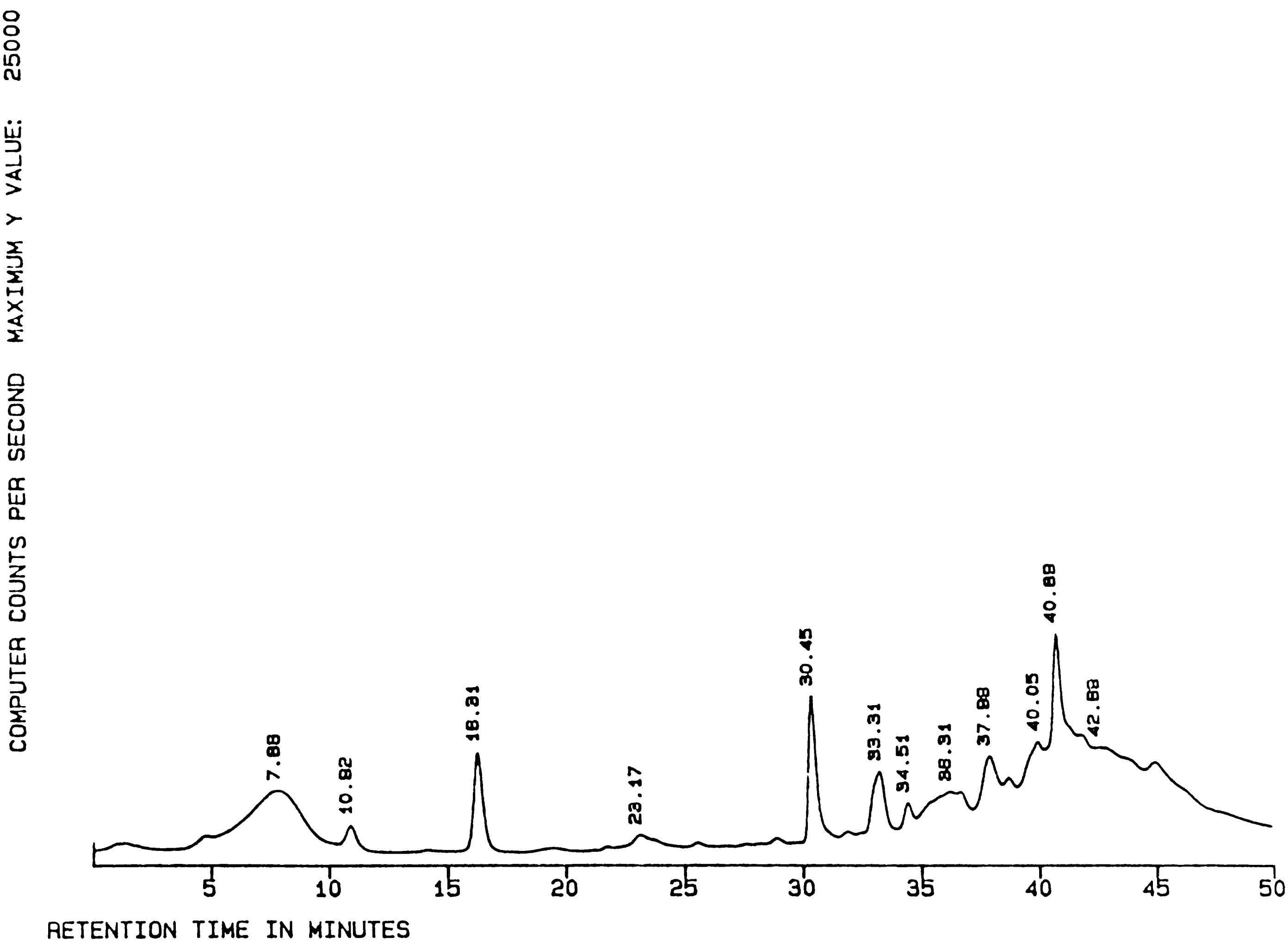

Figure 4. Volatile profile of freeze-dried clam juice.

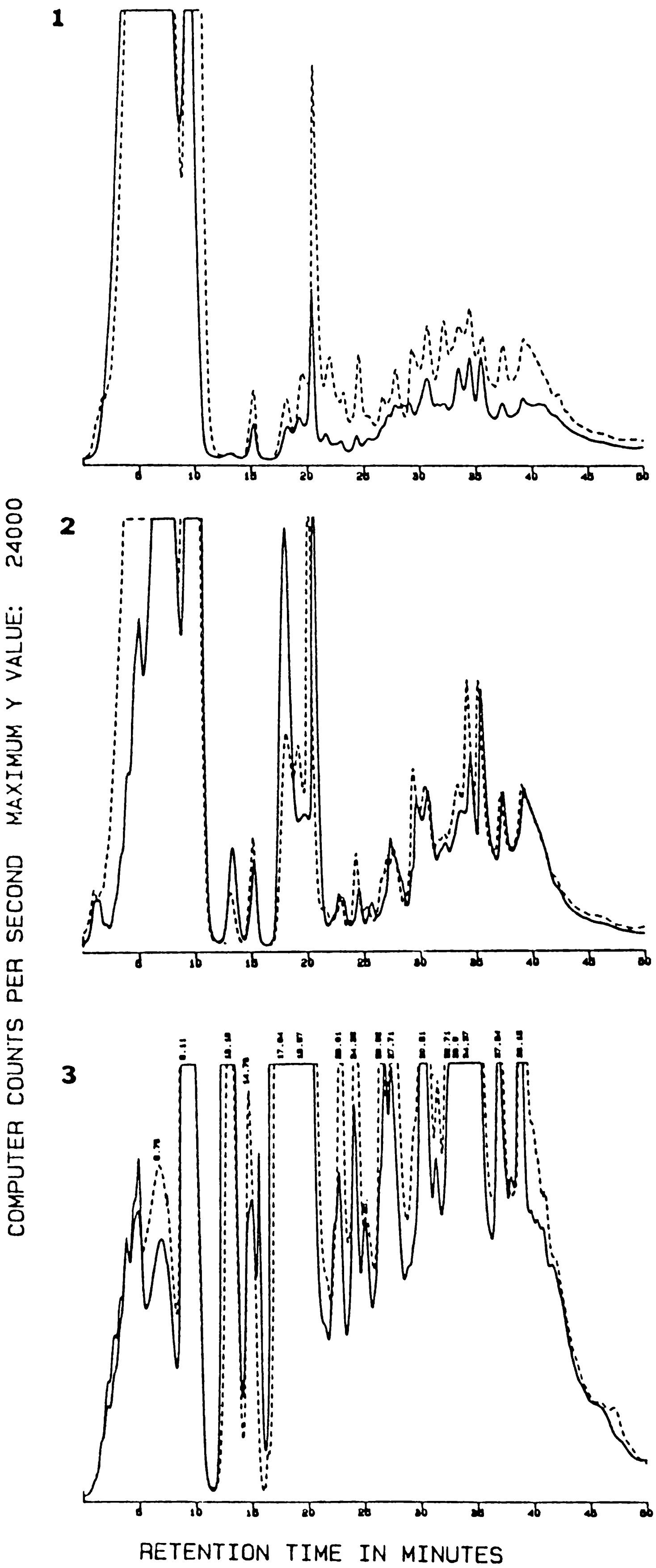

Figure 5. Volatile profiles of salvage wash water (1), tongue wash water (2), and belly wash water (3) of plant A. The solid line represents spray-dried and the broken line represents freeze-dried sample.

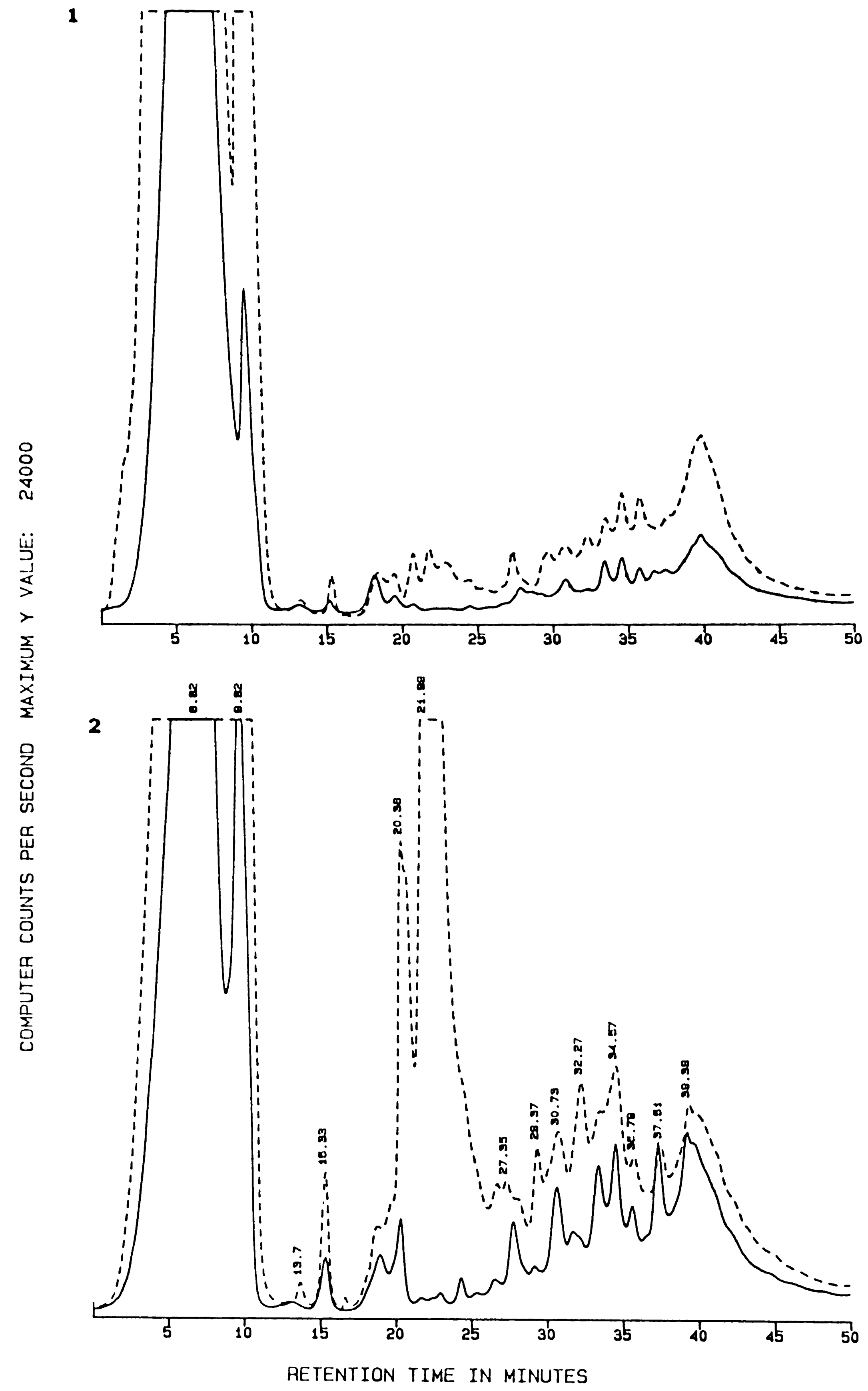

Figure 6. Volatile profiles of tongue wash water (1), and clam met wash water (2) of plant B. The solid line represents spray-dried and the broken line represents freeze-dried.

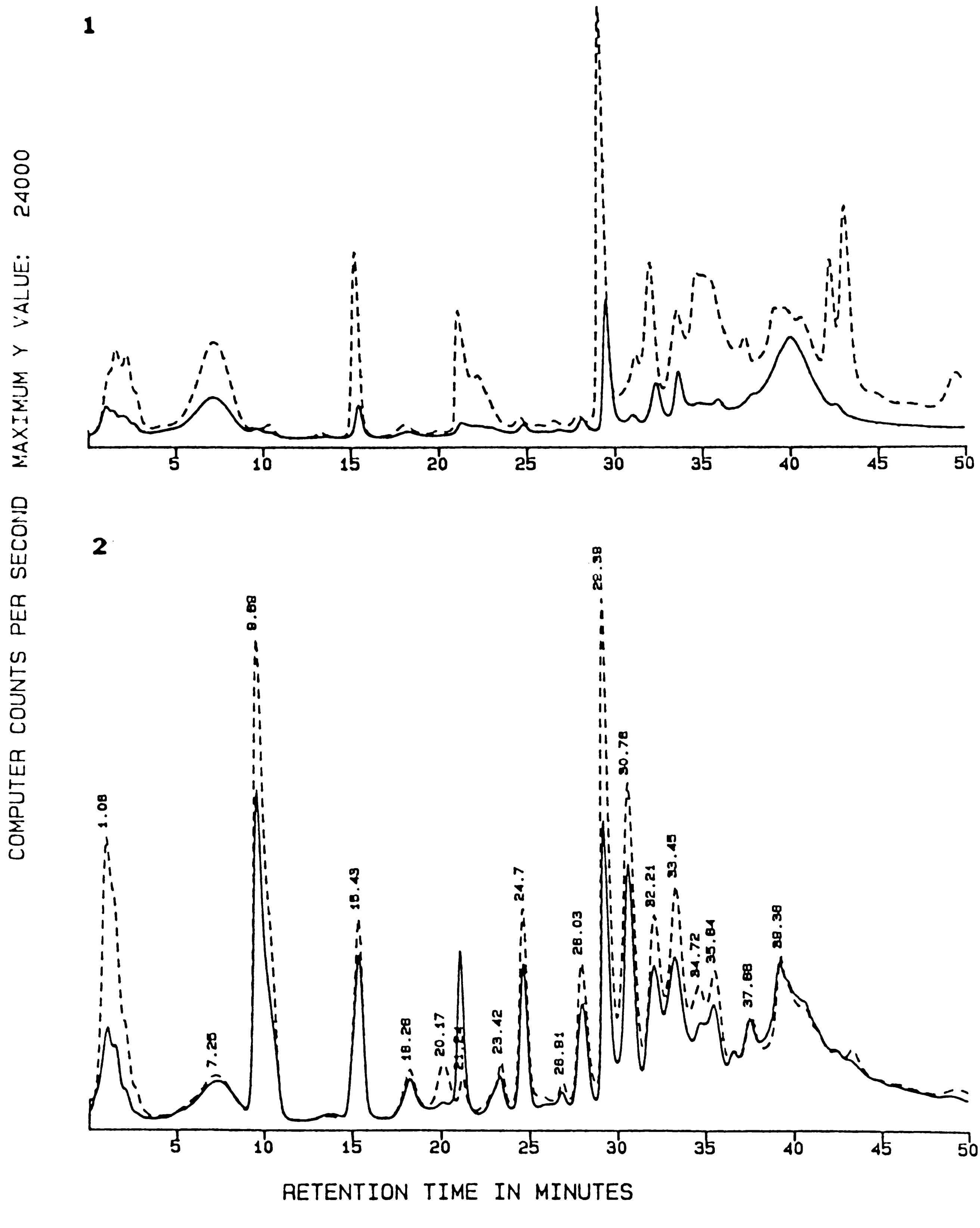

Figure 7. Volatile profiles of cooker liquid product (1), and mince wash water (2) of plant C. The solid line represents spray-dried and the broken line represents freeze-dried.

Sensory Evaluation of Clam Dips made with Selected Wash Water Samples from Plant A, B, and C:

Addition of selected freeze-dried wash water samples (tongue, belly, and salvage wash waters of plant A, tongue wash water of plant B, and cooker liquid and mince wash water of plant C) at 1.75% (w/w) to a sour cream-based clam dip did not significantly change appearance, odor, and texture (Table 6) when evaluated by a sensory panel using a nine point Hedonic scale.. The panelists found no significant ($P \leq .05$) changes in overall acceptability, taste, odor, texture, and appearance of clam dips containing 1.75% (w/w) freeze-dried wash water samples (tongue and salvage wash waters of plant A; tongue wash water of plant B and mince wash water of plant C). However, the panelists found differences in taste (saltiness) and overall acceptability of some clam dips containing 1.75% (W/W) of some wash water samples. The panelists consistently gave lower scores to control clam dips containing no clam juice or clam meat.

Clam dips made with 1.75% (w/w) of freeze-dried wash water samples (belly and salvage wash waters of plant A; tongue wash water of plant B, and mince wash water of plant C) were evaluated along with a commercial clam dip for appearance, odor, taste, texture, and overall acceptability. The panelists did not detect any significant ($P < .05$) differences in appearance, odor, taste, texture, and overall acceptability of clam dips containing wash water samples (salvage wash water of plant A; tongue wash water of plant B, and mince wash water of plant C) when compared to a commercial clam dip (Table 7). Most taste panel members indicated a preference for clam dips containing various wash water samples over a commercial clam dip. Some of the wash water samples from clam processing plants may be used as a food ingredient in clam dips and as a soup-base in the production of clam-flavored soups and foods.

CLAM BELLIES

Proximate composition, mineral, and amino acid content:

The proximate composition and mineral content of whole clam bellies, belly flavor extract and belly meat (residue) is presented in Table 8. The whole bellies collected from plant B had higher water and ash and lower crude fat and protein contents than those from plant A. This variation may be due to the different processing steps used in plants A and B. In plant A, the clams were machine de-bellied which results in rupturing the bellies and a concomitant loss of water. The residue of clam bellies from plants A and B contained high amounts of protein and crude fat when compared to the belly flavor extract. However, belly flavor extracts had appreciable amounts of protein, crude fat and ash. Phosphorus, calcium, magnesium, potassium, and sodium were the major minerals in whole clam bellies, belly flavor extracts and residue of plants A and B. These minerals represented

Table 6. Sensory evaluation of clam dips made with selected wash water samples collected from three clam processing plants (Plants A, B, and C).

Plant	Sample	Appearance	Odor	Taste	Texture	Overall Acceptability
	Control	6.4 a,b	4.9 b	3.9 c	6.5 a,b	4.3 d
Plant A	Tongue Wash Water	6.9 a,b	6.8 a	7.0 a	7.4 a	7.1 a
	Belly Wash Water	7.3 a,b	6.4 a	5.4 b	7.1 a,b	6.1 b,c
	Salvage Wash Water	7.2 a,b	7.0 a	6.8 a	7.4 a	7.2 a
Plant B	Tongue Wash Water	7.3 a,b	7.0 a	7.5 a	7.3 a	7.4 a
Plant C	Cooker Liquid Water	7.4 a,b	6.0 a	5.1 b	6.9 a,b	5.6 c
	Mince Wash Water	7.7 a	6.8 a	6.8 a	7.2 a,b	6.8 a,b

Means in columns with different letters are significantly different ($P \leq 0.05$).

TABLE 7. Sensory evaluation of a commercial clam dip and clam dips made with selected wash water samples collected from three clam processing plants (Plants A, B, and C).

Plant	Sample	Appearance	Odor	Taste	Texture	Overall Acceptability
Commercial clam dip		6.5 a	6.1 a	6.4 a,b	6.5 a	6.5 a
Plant A	Belly Wash Water	6.6 a	6.6 a	5.7 b	7.3 a	6.4 a
	Salvage Wash Water	7.3 a	6.3 a	6.0 a,b	7.4 a	6.3 a
Plant B	Tongue Wash Water	7.5 a	6.7 a	7.1 a	6.9 a	7.1 a
Plant C	Mince Wash Water	7.5 a	6.7 a	7.2 a	7.5 a	7.2 a

Means in columns with different letters are significantly different ($P \leq 0.05$).

Table 8. Proximate composition and mineral content of clam bellies collected from two processing plants (Plant A and B).

Component	Clam Bellies		
	Whole bellies	Belly flavor extract	Residue
		Plant A	
Moisture (%)	74.1	-	-
Protein (%)	52.4	47.0	56.6
Ash (%)	6.8	10.5	5.1
Crude fat (%)	20.8	3.1	24.3
Minerals:			
Phosphorus (mg/g)	9.7	12.3	8.6
Calcium (mg/g)	2.7	1.9	3.9
Magnesium (mg/g)	1.7	2.5	1.2
Potassium (mg/g)	10.1	17.2	4.4
Sodium (mg/g)	10.4	16.1	5.6
Iron (μg/g)	202.0	130.0	250.0
Copper (μg/g)	34.0	61.0	43.5
Zinc (μg/g)	69.0	33.0	116.5
		Plant B	
Moisture (%)	85.9	-	-
Protein (%)	49.6	45.0	51.4
Ash (%)	8.2	8.2	3.5
Crude fat (%)	12.2	1.5	14.8
Minerals:			
Phosphorus (mg/g)	7.8	10.8	4.2
Calcium (mg/g)	2.7	1.1	3.6
Magnesium (mg/g)	1.6	1.7	1.2
Potassium (mg/g)	7.6	10.8	1.7
Sodium (mg/g)	12.9	17.7	4.4
Iron (μg/g)	150.0	166.0	387.0
Copper (μg/g)	25.0	90.0	36.0
Zinc (μg/g)	72.5	41.5	111.5

Protein, ash, crude fat, and minerals are expressed on dry weight basis.

51%, 48% and 47% respectively of ash in whole bellies, belly flavor extract, and residue of plant A. In Plant B, five minerals (phosphorus, calcium, magnesium, potassium, and sodium) accounted for 40%, 51% and 43% respectively of ash in whole bellies, belly flavor extract, and residue (Table 8). The whole bellies from plants A and B contained small amounts of copper and zinc. Clam bellies have been reported to contain large amounts of silica, however, in this study no attempts were made to measure silica because it is nutritionally not important.

Belly flavor extracts from plants A and B contained large concentrations of amino acids namely, aspartic acid, glutamic acid, glycine, alanine, and ammonia (Table 9). These four amino acids accounted for about 32% of total crude protein in belly flavor extracts. The belly flavor extracts were considered for possible use in human food products, however, a preliminary evaluation indicated that the belly flavor extract had a distinct algal-like odor (other than clam flavor). Consequently, its use in human food products may be doubtful because of this unacceptable flavor.

Glycosidase and protease activities:

β -glycosidase activity was present in higher levels in the crude enzyme extract of clam bellies collected from plants A and B than the α-glycosidase activity (Table 10). Neither α- nor β-glycosidase activity was detected in the belly waste of plant C. Presence of laminarinase (β-1,3-glucanase) has been reported in the stomach and intestine extracts of clam (Shallenberger and Herbert, 1974). The crystalline style has been reported to contain a large variety of carbohydrate digestive enzymes, however, in this study, no attempt was made to identify individual clam belly carbohydrases.

The crude clam bellies extracts collected from plants A and B contained non-specific proteases. The plant C bellies extract did not contain proteases since they were denatured during the processing of operations (i.e. cooking and mechanical shucking). Irrespective of pH and temperature, protease activity was lower in the bellies extracts from plant B as compared to the extracts from plant A (Figures 8 and 9). The relationship between pH and protease activity of crude bellies extracts from plants A and B is shown in Figure 8. The optimum protease pH activity was found to be pH 3.0 and 5.0 for crude bellies extract from plant A and pH 5.60 for crude bellies extract from plant B when hemoglobin used as substrate. The temperature optimum was 37°C for protease activity of crude bellies extract from plants A and B (Figure 9). The protease activity was decreased as the incubation temperature increased.

It can be concluded that the clam bellies may be used in the fermentation of agricultural wastes (such as wheat straw and corn cobs) and shellfish wastes (crabs, shrimp, and crawfish) for production of ruminant feeds. They may also be used as a source for the production of various specific and non-specific industrial carbohydrases. Since clam bellies have high protein and low ash contents, they may be studied further for the production of clam-flavored pet foods.

Table 9. Amino acid content (mg/g) of clam belly flavor extracts (Plants A and B).

Amino Acid	Clam Belly Flavor Extract	
	Plant A	Plant B
Aspartic acid	28.3	28.2
Threonine	12.4	16.5
Serine	13.6	15.4
Glutamic Acid	42.9	36.7
Proline	10.1	14.3
Glycine	47.5	42.3
Alanine	32.5	35.5
Valine	12.3	15.1
Methionine	3.8	4.7
Isoleucine	9.8	12.0
Leucine	14.6	19.0
Tyrosine	2.1	2.0
Phenylalanine	8.3	10.3
Lysine	18.9	14.0
Histidine	6.9	7.7
Arginine	21.2	13.9
Ammonia	14.1	16.3
Total Crude Protein (%)	47.0	45.0

Table 10. Glycosidase activity in the crude clam bellies enzyme extract collected from three clam processing plants (Plants A, B, and C).

Plant	Glycosidase activity[a]	
	α	β
A	2.1	9.0
B	1.9	10.3
C	ND[b]	ND

[a] units of p-nitrophenol released from prototype substrates (p-nitrophenyl α-D-glucopyranoside and p-nitrophenyul-β-D-glucopyranoside) per minute per milligram of protein at 37°C.

[b] ND, not detected

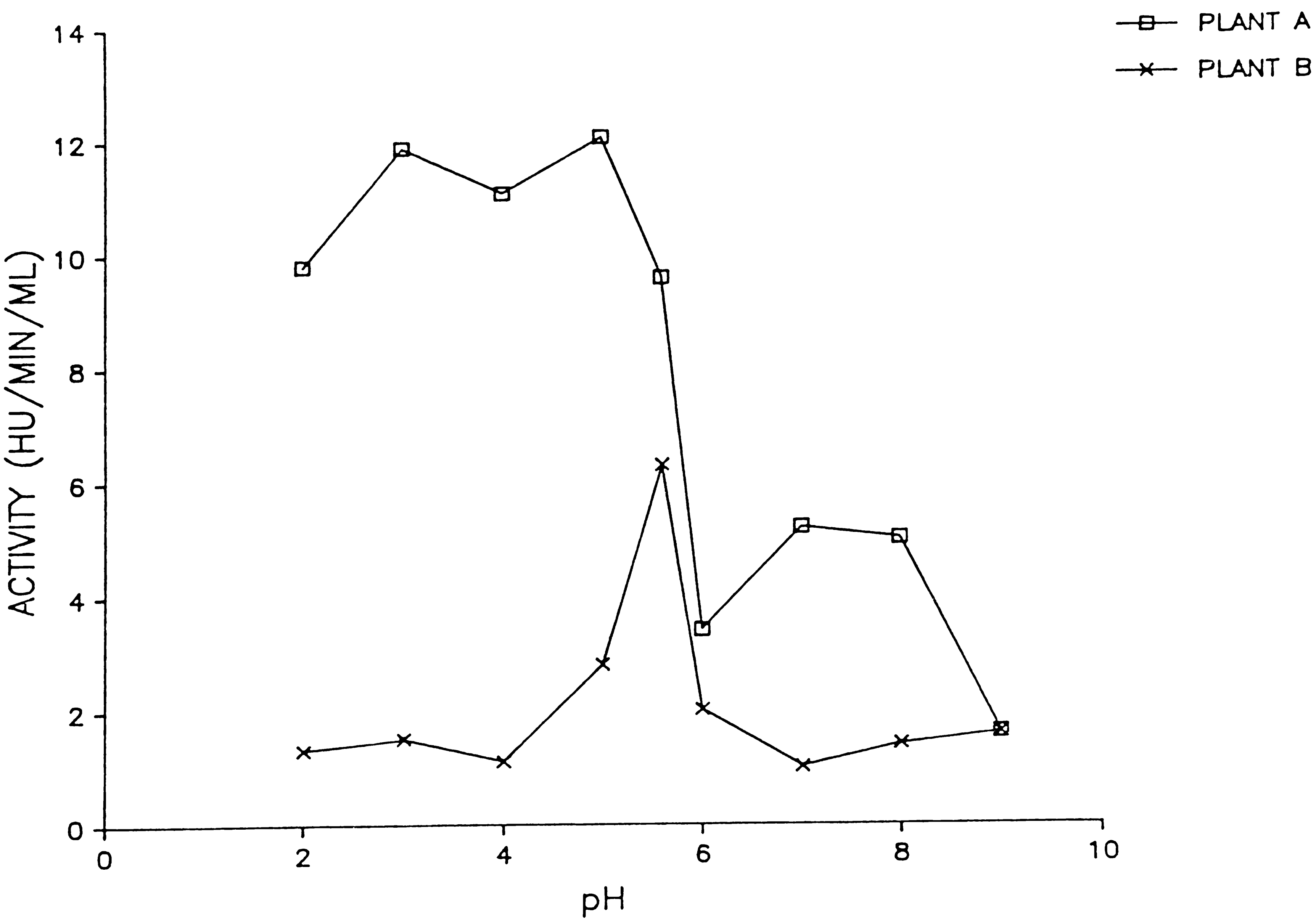

Figure 8. Effect of pH on the protease activity in crude enzyme extract of clam bellies collected from two processing plants (Plants A and B).

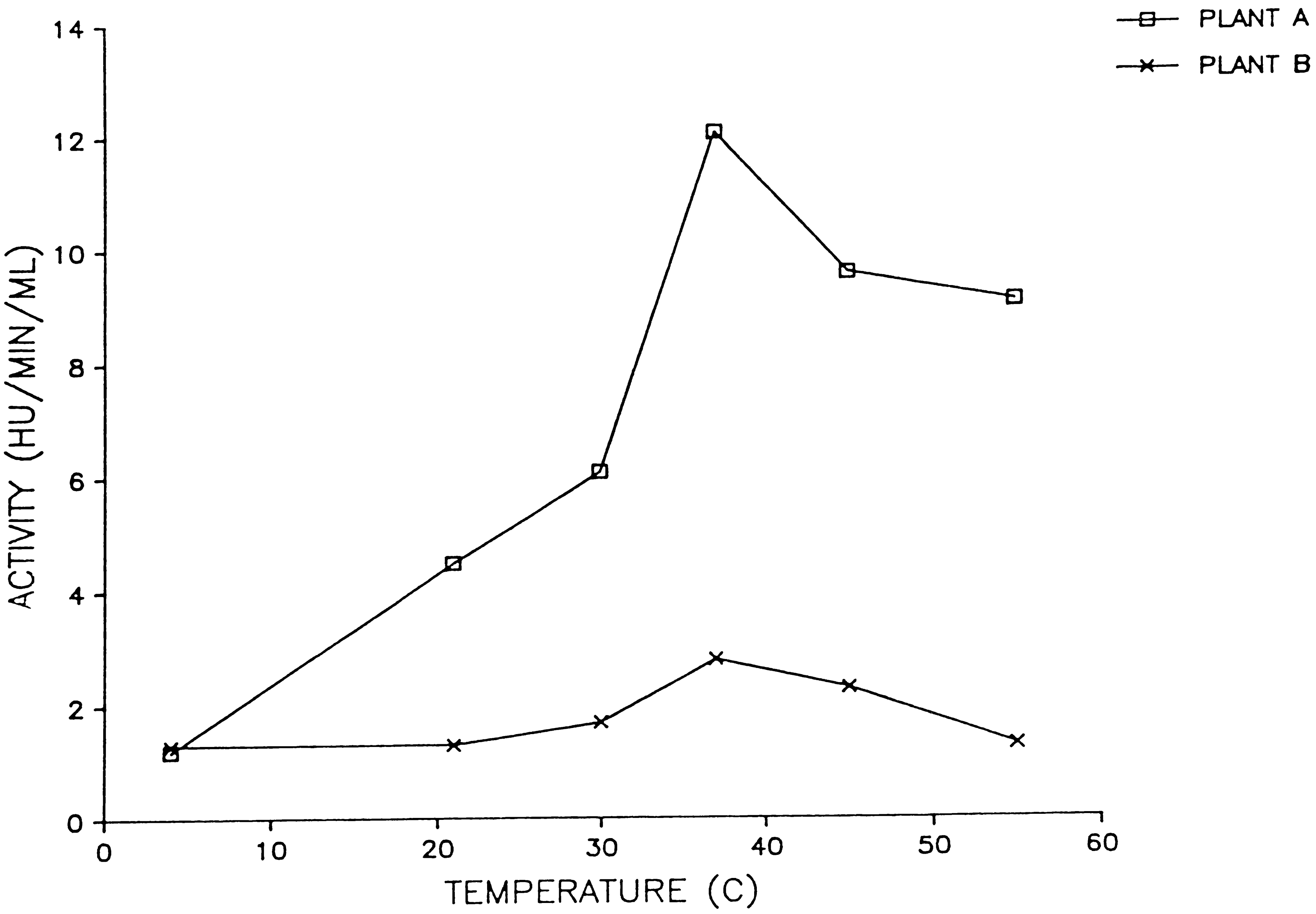

Figure 9. Effect of temperature on protease activity in crude enzyme extracts of clam bellies collected from two clam processing plants (plants A and B).

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APPENDIX

Table 1. Proximate composition, salt, and mineral content of washwater samples collected from a clam processing plant (Plant A).

Component	Brine Water		Tongue Wash Water		Belly Wash Water		Salvage Wash Water	
	Spray-Dried	Freeze-Dried	Spray-Dried	Freeze-Dried	Spray-Dried	Freeze-Dried	Spray-Dried	Freeze-Dried
Salt (%)	14.7		0.2		0.2		1.3	
Water (%)	65.0		96.3		97.1		97.7	
Protein (%)	7.4	7.6	42.4	43.4	45.8	46.6	28.6	30.6
Crude Fat (%)	8.8	8.5	3.2	3.4	12.5	19.2	9.0	1.7
Ash (%)	87.5	86.6	15.3	13.5	13.6	12.6	53.9	51.3
Total Carbohydrates (%)	-	-	-	25.1	-	9.0	-	11.0
MINERALS:								
Phosphorus (mg/g)	-	-	10.5	10.1	11.3	11.1	7.1	9.3
Calcium (mg/g)	-	-	3.0	3.5	2.9	5.0	3.8	5.9
Magnesium (mg/g)	-	-	2.5	2.7	2.7	2.7	3.1	3.5
Potassium (mg/g)	-	-	22.1	18.7	16.9	13.3	19.9	22.7
Sodium (mg/g)	-	-	36.3	49.4	33.6	66.7	15.8	13.8
Iron (μg/g)	-	-	192.0	294.0	379.5	337.5	151.5	179.0
Copper (μg/g)	-	-	20.5	26.0	41.0	27.0	9.0	6.5
Zinc (μg/g)	-	-	36.5	46.0	59.0	62.0	22.5	22.5

Protein, crude fat, ash, total carbohydrate, and mineral contents were presented on a dry weight basis.

Table 2. Proximate composition, salt, and mineral content of washwater samples collected from collected from a clam processing plant (Plant B).

Component	Clam Meat Wash Water		Tongue Wash Water		Salvage Wash Water	
	Spray-Dried	Freeze-Dried	Spray-Dried	Freeze-Dried	Spray-Dried	Freeze-Dried
Salt (%)	0.05		0.1		4.5	
Water (%)	99.4		98.8		95.0	
Protein (%)	-	47.4	-	55.2	9.0	9.7
Crude Fat (%)	-	4.5	-	2.8	5.2	4.1
Ash (%)	29.6	27.2	17.2	17.6	86.8	86.9
Total Carbohydrates (%)	-	13.1	-	16.1	-	-
MINERALS:						
Phosphorus (mg/g)	13.3	10.8	16.3	15.0	-	-
Calcium (mg/g)	22.3	20.0	5.6	5.0	-	-
Magnesium (mg/g)	7.1	6.6	4.3	4.6	-	-
Potassium (mg/g)	28.8	28.1	29.6	36.8	-	-
Sodium (mg/g)	45.9	44.3	25.2	30.6	-	-
Iron (μg/g)	33.5	341.5	141.5	90.5	-	-
Copper (μg/g)	25.5	24.5	11.0	10.0		
Zinc (μg/g)	68.0	65.0	52.5	35.0		

Protein, crude fat, ash, total carbohydrate, and mineral contents were presented on a dry weight basis.

Table 3. Proximate composition, salt, and mineral content of washwater samples collected from collected a clam processing plant (Plant C).

Component	Brine Water		Clam Meat Wash Water		Cooker Liquid Product		Mince Wash Water	
	Spray-Dried	Freeze-Dried	Spray-Dried	Freeze-Dried	Spray-Dried	Freeze-Dried	Spray-Dried	Freeze-Dried
Salt (%)	21.8		0.9		0.5		0.1	
Water (%)	78.3		98.5		98.0		98.6	
Protein (%)	1.5	1.5	11.1	11.2	31.2	31.8	-	19.4
Crude Fat (%)	0.2	0.2	0.9	1.6	0.7	0.1	-	3.7
Ash (%)	96.7	97.4	74.6	74.2	43.1	44.1	19.2	20.2
Total Carbohydrates (%)	-	-	-	-	-	11.1	-	50.3
MINERALS:								
Phosphorus (mg/g)	-	-	-	-	2.6	2.5	4.5	4.3
Calcium (mg/g)	-	-	-	-	20.2	20.1	2.6	2.6
Magnesium (mg/g)	-	-	-	-	13.6	13.6	1.9	2.0
Potassium (mg/g)	-	-	-	-	21.1	21.2	12.2	12.4
Sodium (mg/g)	-	-	-	-	110.0	110.2	56.0	57.1
Iron (μg/g)	-	-	-	-	71.0	67.5	52.5	85.0
Copper (μg/g)	-	-	-	-	6.0	6.0	6.0	6.5
Zinc (μg/g)	-	-	-	-	115.5	110.0	25.5	26.5

Protein, crude fat, ash, total carbohydrate, and mineral contents were presented on a dry weight basis.

Table 4. Amino acid content of (mg/g) of washwater samples collected from a clam processing plant (Plant A).

Amino Acid	Tongue Wash Water		Belly Wash Water		Salvage Wash Water	
	Spray-Dried	Freeze-Dried	Spray-Dried	Freeze-Dried	Spray-Dried	Freeze-Dried
Aspartic Acid	18.8	19.6	33.4	42.1	11.1	11.2
Threonine	8.9	9.3	15.7	20.1	5.1	5.4
Serine	9.7	9.5	17.1	20.4	5.6	5.5
Glutamic Acid	26.7	23.0	42.0	50.6	17.5	17.6
Proline	6.6	-	10.7	-	-	-
Glycine	44.1	44.3	36.6	42.2	37.4	33.9
Alanine	41.4	41.4	28.8	33.7	39.8	36.0
Valine	7.9	9.1	15.5	21.5	3.8	5.0
Methionine	0.8	3.5	0.5	6.0	1.0	1.3
Isoleucine	6.8	8.3	12.4	18.7	3.7	5.7
Leucine	10.8	12.0	18.3	28.5	5.8	7.5
Tyrosine	1.8	1.6	3.0	4.0	1.4	2.0
Phenylalanine	6.4	8.5	11.7	16.3	3.6	4.6
Lysine	14.0	14.7	26.2	35.9	7.1	8.3
Histidine	5.9	6.9	9.1	14.3	4.1	6.3
Arginine	22.8	24.7	26.5	36.2	20.2	19.4
Ammonia	4.9	5.7	8.4	12.5	3.3	5.0

Table 5. Amino acid content of (mg/g) of washwater samples collected from a clam processing plant (Plant B).

Amino Acid	Clam Meat Wash Water		Tongue Wash Water	
	Spray-Dried	Freeze-Dried	Spray-Dried	Freeze-Dried
Aspartic Acid	23.3	28.0	19.8	15.8
Threonine	10.9	11.6	10.3	7.4
Serine	12.3	13.4	11.6	8.5
Glutamic Acid	30.4	24.8	28.8	27.2
Proline	7.4	-	6.7	-
Glycine	46.6	48.3	54.3	58.5
Alanine	47.3	48.9	49.4	55.4
Valine	10.6	11.5	9.3	7.5
Methionine	-	-	0.6	2.0
Isoleucine	8.3	10.0	7.5	6.7
Leucine	13.5	16.2	12.1	10.1
Tyrosine	2.5	2.7	3.4	-
Phenylalanine	8.3	9.8	7.8	5.6
Lysine	18.1	20.5	17.7	12.2
Histidine	8.4	15.4	5.1	7.2
Arginine	22.1	29.2	20.8	14.2
Ammonia	7.2	9.2	7.3	5.9

Table 6. Amino acid content of (mg/g) of washwater samples collected from a clam processing plant (Plant C).

Amino Acid	Cooker Liquid Product		Mince Wash Water	
	Spray-Dried	Freeze-Dried	Spray-Dried	Freeze-Dried
Aspartic Acid	23.6	24.2	9.0	9.8
Threonine	8.9	9.1	2.6	3.3
Serine	8.7	9.6	3.9	4.4
Glutamic Acid	28.2	28.6	15.5	14.5
Proline	-	-	-	-
Glycine	14.7	14.7	11.3	10.5
Alanine	17.6	18.3	15.2	14.9
Valine	7.7	7.3	2.4	2.8
Methionine	2.5	2.4	-	2.6
Isoleucine	7.4	6.8	2.4	4.1
Leucine	9.9	9.8	3.6	4.5
Tyrosine	2.0	3.1	1.0	1.3
Phenylalanine	6.6	6.6	1.9	2.5
Lysine	9.8	10.8	3.9	4.4
Histidine	10.1	10.6	1.7	4.0
Arginine	9.7	10.5	6.1	6.7
Ammonia	4.3	4.0	1.9	2.5