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## Composition, Flavor Extract, Protease, and Glycosidases of Clam Bellies Collected from Clam Processing Plants

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Clam bellies were collected from clam processing plants (A and B) and analyzed for proximate composition, mineral content, and protease and glycosidase activities. The whole clam bellies contained 49.6-52.4% of protein and large amounts of major minerals, phosphorus, calcium, magnesium, potassium, and sodium. The major minerals represented 51% of ash in whole bellies. The flavor extract of clam bellies had unacceptable flavor and was found to be not useful in incorporation of it into human food products. The crude extracts of clam bellies from two plants contained both protease and glycosidases. However,  $\beta$ -glycosidase activity was present in higher levels in the extract of clam bellies than the  $\alpha$ -glycosidase activity. Laminarinase,  $\beta$ -1,6-glucosidase, and  $\beta$ -1,4-glucosidase activities were detected in the extracts of clam bellies collected from two clam processing plants.

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 are discarded, which include the stomach, liver, and other organs. Chen and Zall (1985, 1986a) have 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 collected from a clam processing plant 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, cheddar cheese made from clam rennet was inferior to the cheddar cheese made from calf rennet.

The crystalline style is part of the clam belly region and contains an assemblage of carbohydrate digestive enzymes that catalyze algal carbohydrate degradation (Shallenberger et al., 1974; Lindley et al., 1976). Four different carbohydrases (laminarinase, amylase, cellulase, alginase) have been characterized from the crystalline style of surf clam bellies (Jacobson et al., 1980; Jacobson 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 in the production of commercial enzymes, as a component of livestock or poultry feed and in the production of pet foods, may 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 analyze clam bellies for proximate composition, mineral content, and protease and glycosidases activities. A flavor extract was also extracted from clam bellies and evaluated for its possible use in human food products.

### MATERIALS AND METHODS

**Clam Bellies.** Flow diagrams of mechanized processes used by three different clam processing plants (plants A-C) in Virginia are shown in Figures 1-3. 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 heat treatment using either a shucking furnace (50-100 s) or pressurized cooker. The shucking furnace is a large propane furnace reaching temperatures from 625 to 815 °C. A heavy metal chain belt transports the clams through the furnace to

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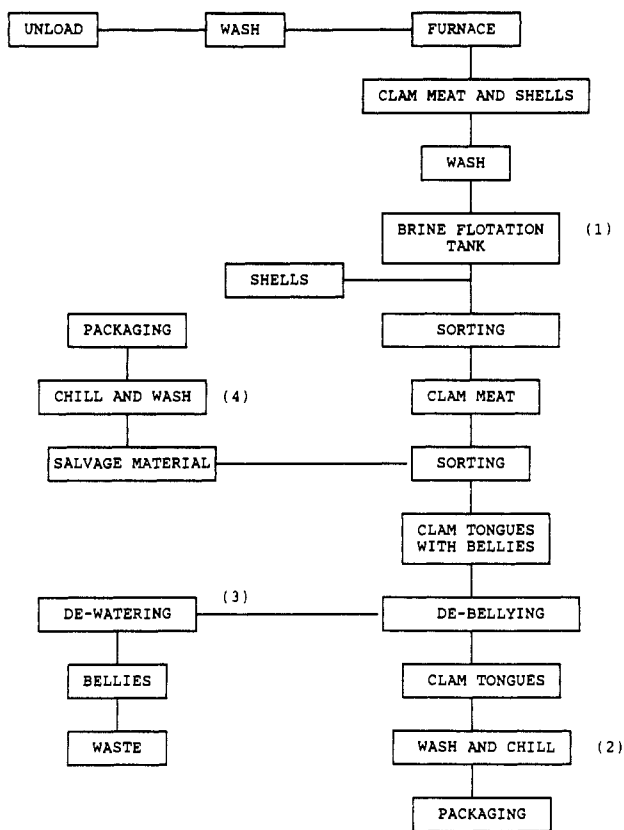


Figure 1. Flow diagram of mechanized clam process for plant A.

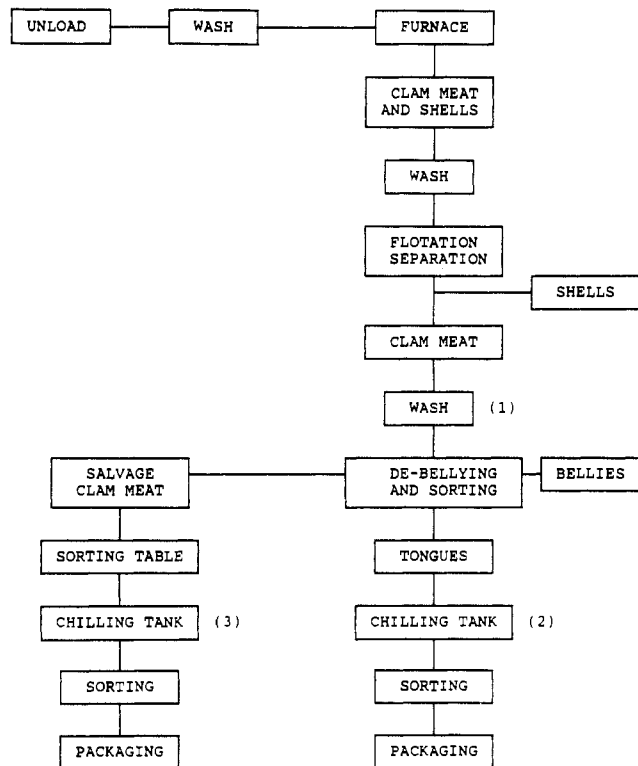


Figure 2. Flow diagram of mechanized clam process for plant B.

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. In plants A and C, the clams were machine debellied. In plant B, the clams were hand debellied.

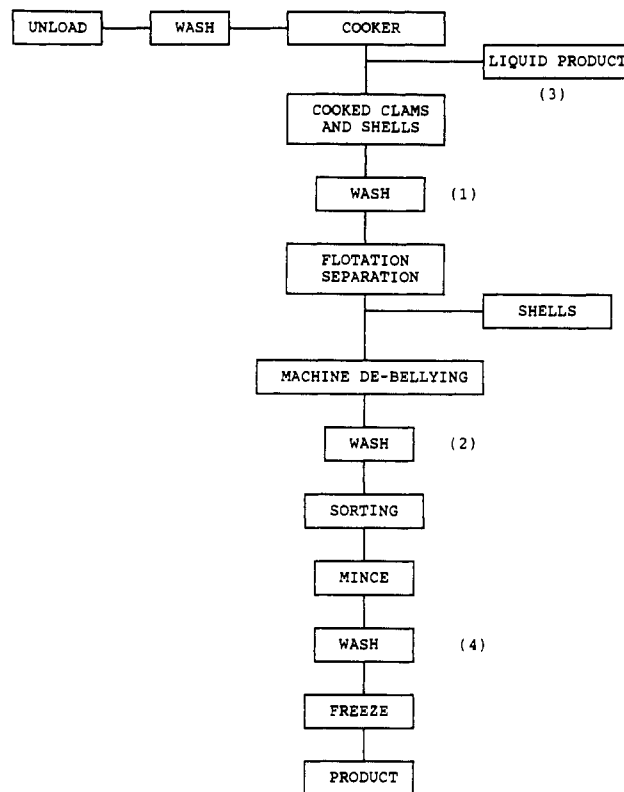


Figure 3. Flow diagram of mechanized clam process for plant C.

The clam bellies from three processing plants A–C were collected in sanitized containers and held at  $-17^{\circ}\text{C}$  until chemical and enzymatic analysis.

**Clam Flavor Extract from Bellies.** About 750 g of frozen bellies was 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 without shelf heat in a Virtis freeze-drier (The Virtis Co., Gardner, NY). The residue (mostly meat pieces) was air-dried at  $100^{\circ}\text{C}$  for 24 h 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.** Total solids and moisture content in clam bellies were determined by drying sample aliquots in crucibles in a forced-air oven overnight at  $100^{\circ}\text{C}$ . Total protein (Kjeldahl  $\text{N} \times 6.25$ ) and crude fat contents of clam flavor extracts of bellies, belly meat (residue), and whole bellies were determined by AOAC (1984) methods. 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 1.2 N HCl and made to known volume with 1.2 N HCl. Calcium, magnesium, potassium, sodium, phosphorus, iron, copper, and zinc were determined by inductively coupled plasma emission spectroscopy (AOAC, 1984).

Amino acids were determined by hydrolyzing 10 mg of clam belly flavor extract samples with 0.2 mL of 6.6 N HCl for 40 h at  $110^{\circ}\text{C}$  in reactivials. The hydrolyzates were dried at  $100^{\circ}\text{C}$  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.

**Protease and Glycosidase Activities in Clam Bellies.** Approximately 200 g of frozen clam bellies was 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 13000g for 30 min at 4 °C. The supernatant was again centrifuged at 38000g for 25 min at 4 °C and filtered through four layers of cheesecloth. 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 (1986a) using bovine hemoglobin as a substrate. The protease activity in the crude enzyme extract was estimated over wide pH (2.0–9.0) and temperature (4, 21, 30, 37, 45, 55 °C) ranges. The protease activity was expressed in hemoglobin units (HU). One hemoglobin unit was arbitrarily defined as a 0.001/min increase in A280 under assay conditions employing hemoglobin as the substrate.

Enzyme activities ( $\alpha$ - and  $\beta$ -glycosidase) in the crude enzyme extract were measured by the method of Reddy et al. (1984a,b). *p*-nitrophenyl  $\alpha$ -D-glycopyranoside and *p*-nitrophenyl  $\beta$ -D-glycopyranoside were used as substrates. A 300- $\mu$ L portion of a 1 mM solution of *p*-nitrophenyl  $\alpha$ - or  $\beta$ -D-glycopyranoside in 0.2 M 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  $\mu$ L 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 0.2 M 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  $\mu$  mol of *p*-nitrophenol/min at 37 °C. The protein content in the crude enzyme extract was determined by the method of Peterson (1977). Specific activities of  $\alpha$ - and  $\beta$ -glycosidase were expressed as micromoles of *p*-nitrophenol released per minute per milligram of protein at 37 °C.

#### Individual Glycosidase Activity in Clam Bellies.

About 200 g of frozen clam bellies was mixed with 400 mL of 0.05 M citrate-phosphate buffer (pH 6.60) and homogenized in a Waring Blendor at high speed for 2 min. The homogenate was centrifuged at 10400g for 20 min at 4 °C. The supernatant was again centrifuged at 20000g for 20 min at 4 °C. The supernatant obtained at 20000g was used as a crude enzyme extract for assaying individual glycosidase activities.

Laminarin, dextran, maltose, gentiobiose, cellobiose, xylobiose, and xylan were used as substrates, respectively, for measuring laminarinase,  $\alpha$ -1,6-glucosidase,  $\alpha$ -1,4-glucosidase,  $\beta$ -1,6-glucosidase,  $\beta$ -1,4-glucosidase,  $\beta$ -1,4-xylosidase, and xylanase activities in the crude enzyme extract of clam bellies (Reddy et al., 1984a). One milliliter of substrate solution (1 mg/mL) of laminarin, dextran, maltose, gentiobiose, cellobiose, xylobiose, or xylan in 0.05 M citrate-phosphate buffer (pH 6.60) was mixed with 2 mL of the same buffer and equilibrated to 37 °C. The crude enzyme extract (1 mL) was added to the substrate solution and mixed, and the mixture was incubated at 37 °C for 60 min. The reaction was terminated by boiling the enzyme-substrate mixture for 20 min. Two separate controls were prepared for each enzyme. One containing substrate, buffer, and enzyme extract was prepared identically except that it was boiled immediately after addition of the enzyme extract. Other controls were prepared to contain buffer and enzyme extract and were incubated at 37 °C for 60 min and subsequently boiled. After boiling and cooling, the samples were centrifuged at 35000g for 10 min at 4 °C and the supernatant fluid was collected in

**Table I. Proximate Composition and Mineral Content of Clam Bellies Collected from Clam Processing Plants A and B**

component <sup>a</sup>	clam bellies					
	whole bellies		belly flavor extract		residue	
	plant A	plant B	plant A	plant B	plant A	plant B
moisture, %	74.1	85.9				
protein, %	52.4	49.6	47.0	45.0	56.6	51.4
ash, %	6.8	8.2	10.5	8.2	5.1	3.5
crude fat, %	20.8	12.2	3.1	1.5	24.3	14.8
minerals						
phosphorus, mg/g	9.7	7.8	12.3	10.8	8.6	4.2
calcium, mg/g	2.7	2.7	1.9	1.1	3.9	3.6
magnesium, mg/g	1.7	1.6	2.5	1.7	1.2	1.2
potassium, mg/g	10.1	7.6	17.2	10.8	4.4	1.7
sodium, mg/g	10.4	12.9	16.1	17.7	5.6	4.4
iron, $\mu$ g/g	202.0	150.0	130.0	166.0	250.0	387.0
copper, $\mu$ g/g	34.0	25.0	61.0	90.0	43.5	36.0
zinc, $\mu$ g/g	69.0	72.5	33.0	41.5	116.5	111.5

<sup>a</sup> Protein, ash, crude fat, and minerals are expressed on dry-weight basis.

a vial for analysis of glucose or xylose by high-pressure liquid chromatography (Reddy et al., 1984b). Activities of laminarinase,  $\alpha$ -1,6-glucosidase,  $\alpha$ -1,4-glucosidase,  $\beta$ -1,6-glucosidase,  $\beta$ -1,4-glucosidase,  $\beta$ -1,4-xylosidase, and xylanase were expressed as micromoles of glucose or xylose released/60 min per milligram of protein at 37 °C.

## RESULTS AND DISCUSSION

**Proximate Composition, Mineral, and Amino Acid Content.** The clam bellies collected from plant C were not analyzed for proximate composition and mineral content because they were completely ruptured and compressed with part of shell particles during processing stages. Further they appeared to be unfit for animal or human use.

The proximate composition and mineral content of whole clam bellies, belly flavor extract, and belly meat (residue) are presented in Table I. 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 debellied, 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 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, sodium) accounted for 40%, 51%, and 43%, respectively, of ash in whole bellies, belly flavor extract, and residue (Table I). 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 not nutritionally 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 II). These four amino acids accounted for about 32% of total crude protein in belly flavor extracts. The belly flavor

**Table II. Amino Acid Content (mg/g) of Belly Flavor Extracts from Clam Bellies Collected from Clam Processing Plants A and B**

amino acid	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 III. Glycosidase Activity in the Crude Enzyme Extract of Clam Bellies Collected from Clam Processing Plants A-C**

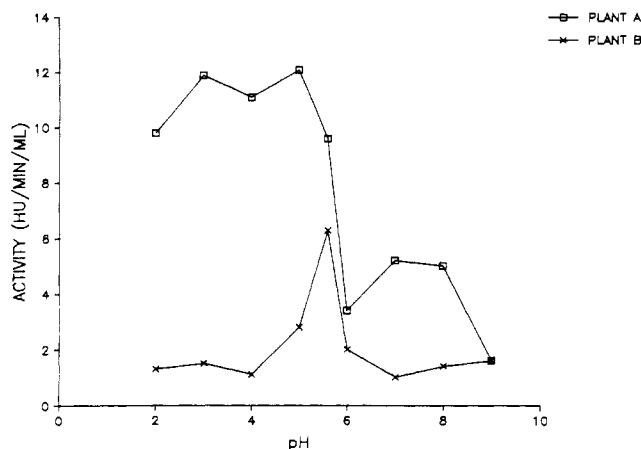
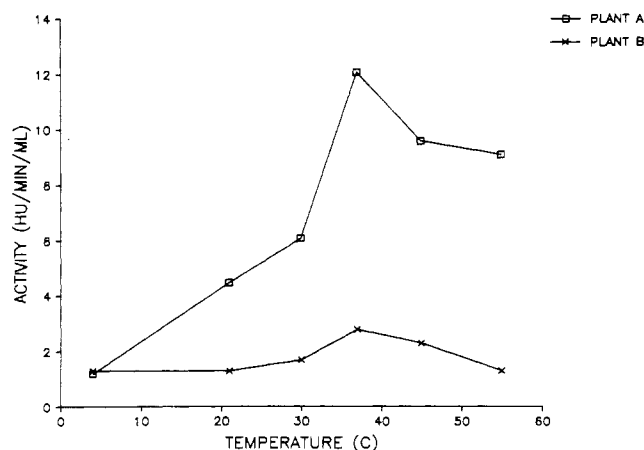
plant	glycosidase activity <sup>a</sup>	
	$\alpha$	$\beta$
A	2.1	9.0
B	1.9	10.3
C	ND <sup>b</sup>	ND

<sup>a</sup>Units of *p*-nitrophenol released from prototype substrates (*p*-nitrophenyl  $\alpha$ -D-glucopyranoside and *p*-nitrophenyl  $\beta$ -D-glucopyranoside) per minute per milligram of protein at 37 °C. <sup>b</sup>ND = not detected.

extracts were considered for possible use in human food products; however, a preliminary evaluation indicated that the belly flavor extract had a distinct algallike odor (other than clam flavor). Consequently, its use in human food products may be doubtful because of this unacceptable flavor.

**Glycosidase and Protease Activities.**  $\beta$ -Glycosidase activity was present in higher levels in the crude enzyme extract of clam bellies collected from plants A and B than the  $\alpha$ -glycosidase activity (Table III). Neither  $\alpha$ - nor  $\beta$ -glycosidase activity was detected in the belly waste of plant C. Presence of laminarinase ( $\beta$ -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 nonspecific proteases. Plant C bellies extract did not contain proteases since they were denatured during thermal processing operations (i.e., mechanical shucking procedure) (Figure 3). Irrespective of pH and temperature, protease activity was lower in belly extracts from plant B as compared to plant A (Figures 4 and 5). The relationship between pH and protease activity of crude belly extracts from plants A and B is shown in Figure 4. The optimum protease pH activity was found to be pH 3.0 and 5.0 for crude belly extracts from plant A and pH 5.60 for crude belly extracts from plant B when hemoglobin used as the substrate. The optimum temperature was 37 °C for protease activity of crude bellies extract from plants A and B (Figure 5). Protease activity decreased as the incubation temperature increased.

**Figure 4.** Effect of pH on the protease activity in crude enzyme extract of clam bellies collected from clam processing plants A and B.**Figure 5.** Effect of temperature on protease activity in crude enzyme extracts of clam bellies collected from clam processing plants A and B.**Table IV. Glycosidase Activity in the Crude Enzyme Extract of Clam Bellies Collected from Clam Processing Plants A and B**

enzyme	activity, <sup>a</sup> mol/mg protein	
	plant A	plant B
laminarinase	0.12	0.35
$\alpha$ -1,6-glucosidase	ND <sup>b</sup>	0.06
$\alpha$ -1,4-glucosidase	ND	0.22
$\beta$ -1,6-glucosidase	2.18	0.16
$\beta$ -1,4-glucosidase	0.17	0.73
$\beta$ -1,4-xylosidase	ND	ND
xylanase	ND	ND

<sup>a</sup>Micromoles of glucose or xylose released from respective substrates per 60 min per milligram of protein at 37 °C. <sup>b</sup>ND = not detected.

**Individual Glycosidase Activities.** Xylanase and  $\beta$ -1,4-xylosidase activities were not detected in the clam belly enzyme extracts collected from plants A and B (Table IV). The enzyme extract of clam bellies collected from plant A had high  $\beta$ -1,6-glucosidase activity. Laminarinase,  $\alpha$ -1,4-glucosidase, and  $\beta$ -1,4-glucosidase appeared to be the predominant enzymes in the clam belly extract collected from plant B. The extract from clam bellies of plant A did not contain  $\alpha$ -1,4-glucosidase and  $\alpha$ -1,6-glucosidase activities. This may be due to the process in which the bellies are removed from clams. In plant A, the bellies were mechanically removed and dewatered, whereas the bellies

were manually removed from the clams in plant B.

It can be concluded that clam bellies may be used in the fermentation of agricultural wastes (as wheat straw and corn cobs) and shellfish wastes (crabs, shrimp, crawfish) for the production of ruminant feeds. They may also be used as a source for the production of various specific and nonspecific industrial carbohydrases. Since clam bellies have high protein and low ash contents, they may be further studied for the production of clam-flavored pet foods. Maximum recovery of clam bellies can be achieved by installing self-cleaning screens on the debellies process flume. The use of a subsequent compacting (pressing) operation to remove excess water and concentrate belly material may be considered. The solid materials should be rapidly chilled or frozen to prevent degradation in enzyme activity. Installation of the equipment and operational procedures can be achieved with relatively minor equipment cost and plant modification.

**Registry No.** Phosphorus, 7723-14-0; calcium, 7440-70-2; magnesium, 7439-95-4; potassium, 7440-09-7; sodium, 7440-23-5; iron, 7439-89-6; copper, 7440-50-8; zinc, 7440-66-6;  $\alpha$ -glycosidase, 74315-95-0;  $\beta$ -glycosidase, 39346-29-7;  $\alpha$ -1,6-glucosidase, 37288-48-5;  $\alpha$ -1,4-glucosidase, 9001-42-7;  $\beta$ -1,6-glucosidase, 55326-47-1;  $\beta$ -1,4-glucosidase, 37288-52-1; laminarinase, 9025-37-0.

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## Soluble Phenolic Monomers in Forage Crops

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The proportion of alkali-labile phenolic monomers soluble in neutral detergent, methanol, water, or rumen buffer was determined in mature alfalfa and corn stem material by HPLC. The determination of soluble proportion was influenced by treatment, with neutral detergent generally extracting the highest proportion of phenolics. Phenolic monomers were quantified in stems and buffer-treated stem residues of a wide variety of forage crop species. A large range in the concentrations of the major alkali-labile phenolics, *p*-coumaric acid (PCA) and ferulic acid (FA), was found with much higher concentrations in grasses than legumes. The rumen buffer soluble proportion of alkali-labile PCA and FA in grasses (9 and 9%, respectively) was much lower than in legumes (84 and 90%, respectively). The high solubility of PCA and FA indicated that most of the alkali-labile PCA and FA in legumes was not bound to hemicellulose or lignin. Caffeic acid, not previously reported to be a major alkali-labile component of forages, was detected and confirmed to comprise approximately 6 g kg<sup>-1</sup> of the dry weight of immature limpogress stems.

Phenolic monomers have been implicated in inhibition of structural carbohydrate digestion. Ferulic acid (FA) and *p*-coumaric acid (PCA) were toxic to cellulolytic bacteria (Chesson et al., 1982). Herald and Davidson (1983) observed bacterial inhibition due to hydroxycinnamic acids, with PCA being the most effective inhibitor tested. In

another study, PCA and *p*-hydroxybenzaldehyde (PHBAL) were toxic to cellulolytic bacteria but syringic acid (SYA), *p*-hydroxybenzoic acid (PHBA), and hydrocinnamic acid stimulated growth of these bacteria (Borneman et al., 1986). Vanillin (VAN) depressed both cellulose and xylan digestion (Varel and Jung, 1986), while benzoic, cinnamic, and caffeic acids depressed digestion of cellulose (Jung, 1985).

The presence of free or nonesterified phenolic monomers in plant tissues is not well documented. Jung et al. (1983a) detected no free, ether-soluble phenolic compounds in alfalfa hay, soybean stover, smooth bromegrass hay, or corn stalklage samples. *p*-Hydroxybenzoic, vanillic, *p*-coumaric, and ferulic acids were found in water extracts of alfalfa

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