

(No figures submitted with text)

## STABILITY OF FROZEN MINCE FROM CHANNEL CATFISH FRAMES

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### INTRODUCTION

The demand for farm-raised channel catfish (*Ictalurus punctatus*) has grown tremendously in recent years due to year round availability, consistent quality, and healthy attributes of catfish. In 1993, over 459 million tons of catfish were produced in the United States (Anon., 1994). As this industry has grown, so has the amount of offal, trimmings, and other solid waste generated and the need to provide effective means of waste removal and disposal.

According to the USDA (Anon., 1992) 47% of catfish sold is in the form of fillets, 32% is whole fish, and the remaining 21% includes steaks, nuggets, and value added products. Only 68% of the fish is sold for food with the remainder being waste, including skeletons (frames), which account for 18% of the fish (Woodruff, 1984).

Currently, most catfish producers do not have the necessary equipment to use fish frames other than converting them into fish meal. Income processors receive for catfish offal, about \$.05/lb, just covers the cost of transporting the waste materials to rendering plants.

These frames could be utilized more effectively. About 50% of frames is meat, which can be recovered by various means (deboning, cooking, and separating). There are several advantages to finding better uses for even a portion of this waste. Less waste and more useable product means less cost for discharging waste into waste treatment systems, reduces water pollution and strain on the environment. A profit could also be made on the additional muscle meat recovered (McAlpin et al., 1994).

By passing the catfish frames through a mechanical deboner, approximately 25-50% of the weight of the frame is removable meat (Ammerman, 1985). Once removed, methods to process and preserve this mince become important

Antioxidants can be added to increase shelf-life. Several food-grade antioxidants and metal chelators such as sodium citrate, phosphates and sodium erythorbate or in combination may be used to maintain quality of fish mince. Sodium citrate protected ocean trout against lipid oxidation (Shenouda et al., 1979). Citric acid can inactivate enzymes and sequester trace metals (Anon., 1985). Brifisol™, a phosphate mix, can be a useful antioxidant in finely ground meats (Anon., 1990).

Phosphates have been shown to prevent rancidity in fish (Gordon, 1971), and sodium erythorbate has been found to have protective effects on various fish species (Bilinski et al., 1979, Hwang and Regenstein, 1988, Santos and Regenstein, 1990, Licciardello et al., 1977, 1980, 1982). Erythorbates are comparable to ascorbates in antioxidant effectiveness, but cost less (Anon., 1982).

The present research was conducted (1) to explore the feasibility of producing a mince from channel catfish frames and, (2) to study methods of maintaining the overall quality of the frame mince during frozen storage.

## MATERIALS AND METHODS

### Sample preparation

Catfish frames were transported from a commercial processor in Mississippi to the Mississippi State University/National Marine Fisheries Experimental Seafood Processing Laboratory in Pascagoula, MS. Frames were packed on ice (1 to 3 days) prior to transportation and kept on ice until used. Once unloaded, frames were rinsed with filtered water using a rotary fish washer (Model GL300 Ryan Engineering Inc., Seattle, WA) that allowed them to drain as they tumbled. Frames were then passed through a deboner with 5mm holes in the cylinder (Model NDX13 Bibun Machine Construction Co. Ltd., Japan).

Mince used in unwashed treatments (U) was covered with plastic and stored at  $3 \pm 1$  °C until used. Washed mince (W) was prepared by placing mince in wash tanks containing 1 part mince to 4 parts water (5°C). This slurry was stirred for 10 min and allowed to settle for 5 min before water was decanted. This procedure was repeated 3 times to remove lipids, some pigments, and water soluble proteins. Mince slurry was pumped to a rotary screen rinser (Model F32LW, Bibun Machine Construction Co. Ltd., Japan) to remove loose water and then transferred to a screw press (Model YS200, Bibun Machine Construction Co., Ltd., Japan) to remove remaining excess water and reduce the moisture content to 78%.

### Treatments

The mince was held in a refrigerated room ( $3^{\circ}\text{C} \pm 1$  °C) until used. There were 5 treatments for both washed and unwashed mince, consisting of four antioxidant combinations and one control. The following antioxidants and combinations of antioxidants were added to the mince: (1) 0.15% sodium citrate - CI (Haarman and Reimer Corp., Elkhart, IN); (2) 0.15% sodium erythorbate - ER (Pfizer New York, NY); (3) 0.15% sodium citrate and 0.15% sodium erythorbate (CE); (4) 0.15% sodium citrate, 0.15% sodium erythorbate, and 0.4% Brifisol™ 414 (BR) - a mixture of sodium acid pyrophosphate, sodium pyrophosphate, and sodium polyphosphate, glassy (BK Ladenburg, Cresskill, NJ); and, (5) mince alone or control (CO). Sodium citrate and sodium erythorbate were calculated (w/w) based on acid equivalents.

Antioxidants were dissolved in a small amount of water added to unwashed mince and were added to the washed mince in powdered form. All treatments including controls were mixed for 3 min to evenly disperse the antioxidants. Replication 1 used a household mixer (Model K45 Kitchenaid Division, Hobart Manufacturing Co., Troy OH) and replications 2 and 3 used an industrial size mixer (Model A200, Hobart Manufacturing Co.). Mince was then placed in 20 0.45 kg wax coated cardboard boxes (Packaging Production Corp., New Bedford, MA) and frozen to  $-40^{\circ}\text{C}$  in a plate freezer (Dole Freeze-Cell Model 2735-6A, Dole Refrigerating Co., Lewisburg, TN). Frozen mince was stored in a storage freezer overnight ( $-20^{\circ}\text{C}$ ), packed in dry ice, and transported to Mississippi State University for chemical analyses. Samples were stored at  $-14^{\circ}\text{C} \pm 2^{\circ}\text{C}$  for the duration of the study.

### Analyses

Oxidative rancidity was determined by the 2-thiobarbituric acid reactive substances (TBARS) test (Tarladgis et al, 1960). Optical density was multiplied by a factor of 7.8 to express the results as mg malonaldehyde/kg mince according to Sinnhuber and Yu (1958). Enzymatic rancidity (free fatty acids, FFA) was determined by the method described by Woyewoda et al (1986). Color was determined by a Hunterlab Model D25 Color Meter (Hunter Associates Laboratory, Inc., Reston, VA), standardized with a white plate standard no. LS-13601. All preceding tests were performed in duplicate after 0, 1, 2, 3, and 4 mo. of frozen storage.

Moisture, fat, protein, ash, copper, iron, phosphorus, and erythorbate contents were determined at time 0 for each experimental observations by the Mississippi State Chemical Laboratory. All analyses, with the exception of erythorbate, used AOAC (1990) methods. A standard curve was set up to evaluate erythorbate results using Vitamin C (ascorbate) procedures (Strohecker and Henning, 1966). In addition to AOAC methods, the phosphate analysis used procedures from Clesceri et al. (1989).

### Statistical Analysis

The data were analyzed using analyses of variance for a split-plot in a randomized complete block design. Wash treatment (w and u), whole plot and antioxidant treatment (5), and storage time (0 to 4 mo.) were the subplots. Data was analyzed using PROC GLM of the Statistical Analysis System (SAS, 1985). If significant differences were found, means were separated using Fisher's Protected least significant difference (LSD) (Steel and Torrie, 1980) at the 5% level of significance.

## RESULTS AND DISCUSSION

washed mince had lower ( $P < 0.05$ ) fat, phosphorus, and ash, but higher ( $P < 0.05$ ) moisture and Protein (Table 1). Washing removes lipids, blood, and other prooxidants along with water soluble proteins (Miyachi and Steinberg, 1970). Proximate composition of the unwashed mince was similar to that reported by Silva and Ammerman (1993) for catfish muscle and by Freeman and Hearnberger (1993) in the skin side and lateral portion of catfish fillets; washed mince was in composition to the internal portion and visceral side of fillets.

Table 1. Effect of wash treatment on proximate composition and selected nutrients (wet basis) in catfish mince averaged over antioxidant treatment.

Nutrient	Washed	Unwashed
Phosphorous (%)	0.22 b	0.30 a
Copper (mg/kg)	0.65 a	0.58 a
Iron (mg/kg)	5.29 a	5.21 a
Erythorbate (mg/g)	0.57 a	0.58 a
Moisture (%)	82.20 a	74.25 b
Fat (%)	3.24 b	14.09 a
Ash (%)	0.51 b	0.66 a
Protein (%)	14.20 a	11.22 b

ab - Means within row not followed by same letter differ ( $P < 0.05$ ).

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There were differences ( $P<0.05$ ) between washed and unwashed minces in phosphorus, iron, erythorbate, and ash due to antioxidant treatment (Table 2). Mince containing Brifisol™ (BR) was **higher in** phosphorus and ash, due to Brifisol™ phosphates. Erythorbate levels were higher ( $P<0.05$ ) in minces containing added erythorbate (ER, CE, BR), as would be expected. Iron levels were higher ( $P<0.05$ ) in minces containing citrate (CI, CE, BR) and was lower ( $P<0.05$ ) in the control (CO) and erythorbate (ER) treated minces.

### Color

Washed minces had higher ( $P<0.05$ ) “L” (lighter product) and lower ( $P<0.05$ ) “a” and “b” values than the unwashed mince (Table 3). Miyauchi and Steinberg (1970) noted that washing leads to improved color and flavor stability of mince by removing blood and heme pigments.

Though “b” values differed ( $P<0.05$ ) between wash treatments, both washed and unwashed mince were more Yellow in hue than blue. There was also a difference ( $P<0.05$ ) in “b” values among antioxidant treatments, regardless of wash treatment or storage time (data not shown). Mince containing Brifisol™ had higher ( $P<0.05$ ) “b” value than the other treated minces.

There was a gradual ( $P<0.05$ ) decrease but significant shift in “b” values over storage time, regardless of wash or antioxidant treatment (Fig. 1). Nakayama and Yamamoto (1977) noted a shift toward yellowness in unwashed minces made from unskinned short spine thornyhead, turbot and dogfish. Oxidation of dark muscle lipids can result in yellow-to-brownish discoloration in fish known as rusting (Licciardello, et al., 1982). Gordon (1971) reported that phosphate treatments in fish retards rancidity and prevents development of yellow appearance.

There were no changes in Hunter “L” values over time. Moledina et al (1977) found “L” values to decrease over time in mechanically deboned flounder meat, and found that preservatives could greatly minimize this. Minced turbot darkens while minced pollock lightens during storage (Nakayama and Yamamoto, 1977). Fish should have an off white to cream color, a whiter, less red color is desired (Jahncke et al. 1992).

### TBARs

Washed mince had lower ( $P<0.05$ ) TBARs than unwashed mince regardless of antioxidant treatment or storage time (Fig. 2). This was probably due to lower lipid and heme pigment contents (Table 3). Fischer and Deng (1977) indicated heme iron as the major catalyst of lipid oxidation in mullet dark muscle. Hiltz et al. (1976) attributed variations in TBARs of silver hake to varying amounts of the lipid-rich red muscle. Silberstein and Lillard (1978) found hemoprotein content to influence prooxidant activity in extracts of minced mullet.

Freeman and Hearnberger (1993) found the flesh located along the lateral line of catfish fillets to have higher TBARs than flesh from other parts of the fillets. Absence of the lateral line in catfish frames would produce mince with less heme pigments and could partially explain the relatively low TBARs reported here for both washed and unwashed mince.

There was an increase ( $P<0.05$ ) in TBARs during storage (Fig. 2). Woodruff (1987) reported slightly higher TBARs values in catfish fillets during frozen storage as did Silva and Ammerman

Table 2. Effect of antioxidant treatment on proximate composition and selected nutrients in catfish mince averaged over wash treatment.

Nutrient	TREATMENT <sup>1</sup>				
	CO	CI	ER	CE	BR
Phosphorous (%)	0.20a	0.20a	0.22a	0.18a	0.50b
Copper (mg/kg) <sup>NS</sup>	0.47	0.62	0.54	0.49	0.97
Iron (mg/kg)	3.67a	7.05b	3.12a	5.23ab	7.18b
Erythorbate (mg/g)	0.03a	0.03a	0.94b	0.95b	0.94b
Moisture (%) <sup>NS</sup>	78.32	78.53	78.22	78.16	77.89
Fat (%) <sup>NS</sup>	8.68	8.39	8.49	9.03	8.73
Ash (%)	0.48a	0.53a	0.51a	0.49a	0.93b
Protein (%) <sup>NS</sup>	12.70	12.46	12.80	12.96	12.65

ab-Values within row not followed by the same letter differ (P<05).

NS - No significant differences.

<sup>1</sup>CO = Control, CI = sodium citrate, ER = erythorbate, CE = citrate + erythorbate, BR = **brifisol™**.

Table 3. Effect of wash treatment on TBARs and Hunter Color of catfish mince averaged over antioxidant treatment and storage time.

<b>Variable</b>	<b>Washed</b>	<b>Unwashed</b>
TBARs (mg malonaldehyde/kg fish)	0.20b	0.30a
Hunter L value	66.42a	61.35b
Hunter a value	-0.25a	1.87b
Hunter b value	8.36b	9.48a

ab - Means within row not followed by the same letter differ (P<0.05).

(1993) for whole dressed catfish. Ciarlo et al. (1985) found initial TBARs values to be lower in minced hake than hake fillets. There were no differences ( $P < 0.05$ ) in TBARs due to antioxidant treatment (Table 4). TBARs reactive material is generally produced in substantial amounts only from fatty acids containing three or more double bonds (Nawar, 1985) found in relatively low concentrations in channel catfish (Silva et al., 1993; Worthington et al., 1972).

### FFA

Among antioxidant treatments, BR offered more protection ( $P < 0.05$ ) against the formation of free fatty acids than CO regardless of storage time or wash treatment (Table 4).

There was an interaction ( $P < 0.05$ ) between wash treatment and storage time (Fig. 3) for free fatty acids. In both washed and unwashed mince, free fatty acids increased ( $P < 0.05$ ) over storage time. After the first month there was also a difference ( $P < 0.05$ ) between washed and unwashed mince each month. These increases in free fatty acids are very similar to those found in catfish fillets over the same storage time (Nguessan, 1992). Hiltz et al. (1976) found the rate and extent of free fatty acid increase to be the same for fillets and minced silver hake.

Silva et al. (1993) found oxidation to occur primarily in phospholipids but not neutral lipids of catfish fillets. Eun et al. (1993) reported an active system of peroxidases in catfish microsomes, and concluded that phenolic antioxidants, followed by phosphates and finally natural antioxidants were more effective in preventing oxidation of catfish muscle during storage.

Both the unwashed and washed mince made from catfish frames had acceptable color and low TBARs and FFA values during frozen storage. In future experiments, other quality parameters such as sensory and microbiological evaluations would be necessary to determine overall acceptability.

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Table 4. Effect of antioxidant treatment on TBARs and free fatty acid (FFA) values in catfish mince averaged over wash treatment and storage time.

Antioxidant	TBARs Value (mg malonaldehyde/kg fish)	FFA Value ( $\mu$ mol/log fish)
Control (CO)	0.33 <sup>NS</sup>	3.39 a
Citrate (CI)	0.28	3.33 a
Erythorbate (ER)	0.25	3.53 a
Citrate + Erythorbate (CE)	0.19	3.40 a
Brifisol + Citrate + Erythorbate (BR)	0.20	3.05 b

ab - Means within column followed by different letter differ ( $P < 0.05$ ).

NS - No significant differences.

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## GAS CHROMATOGRAPHIC/OLFACTOMETRIC EVALUATION OF FLAVOR EXTRACTS PRODUCED FROM CRAWFISH PROCESSING BY-PRODUCTS

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Crawfish processing plants produce over 85 million pounds of picking table scraps annually, with recovery of only 15% (by weight) of edible tail meat (Meyers et al., 1990). Potential exists for the utilization of these by-products for the production of marketable flavor extracts. This approach may generate additional revenue for crawfish processors, while at the same time significantly reduce their waste volume.

Enzymatic hydrolysis of protein followed by heat treatment can be applied to produce Maillard reaction flavor. The production of certain meat and savory flavors employs **this** technology (Dziezak, 1986). Many recoverable volatile flavor compounds and precursors are present in crawfish processing by-products (PBs). Baek (1994) selected a suitable protease for hydrolysis of PBs and optimized the processing conditions.

Gas chromatography/olfactometry (GC/O) involves the use of human subjects as GC detectors. The most commonly used GC/O techniques are dilution analysis methods such as CharmAnalysis™ (Acree et al., 1984) and aroma extract dilution analysis (AEDA) (Schieberle and Grosch, 1987). However, it takes a long time to complete the analysis in dilution analysis. Osme developed by Miranda-Lopez et al. (1992) needs only one run to obtain osmeogram, reducing analysis time.

The objective of this research was to evaluate and compare aroma properties of flavor concentrates prepared with and without protease treatment using gas chromatography/olfactometry (GC/O).

## MATERIALS AND METHODS

### 1. Materials

Live crayfish (*Procambarus clarkii*), hereafter referred to as crawfish, were purchased from a seafood processor in Baton Rouge, LA, and temporarily stored in a 4°C walk-in cooler. After being washed in tap water, live crawfish were boiled for 7 min at 100°C (Marshall et al., 1987). Tailmeat was removed from the boiled crawfish by hand. Composite crawfish processing by-products (PBs) and claws (CPBs) were collected separately. PBs was composed of claw, viscera, and shell. Both PBs and CPBs were ground with addition of distilled water to make final concentrations of 75 % (PBs) and 60 % (CPBs). These were the highest concentrations that could be effectively mixed. The ground PBs and CPBs were vacuum-packaged in polyethylene bags (Koch Supplies, Inc., Kansas City, MO) and then stored at -20°C.

### 2. Enzymatic Hydrolysis of PBs and CPBs

After thawing at 4°C overnight, one kg of PBs (or CPBs) was placed into a 1-L jacketed reaction vessel (Cat. No. 991780, Wheaton, Millville, NJ). Stirrer was attached to thoroughly mix PBs(or CPBs) during reaction. Reaction was performed under optimal reaction conditions (pH 8-9, 65 °C, and 2.5 hr reaction time) with 0.3% of alkaline protease APL-440 (Solvay Enzymes, Inc.). The pH was not adjusted because intrinsic pHs of both PBs and CPBs were in the optimum range. A control was run under the same conditions except for absence of APL-440 Each hydrolysis was performed in duplicate.

### 3. Aqueous Extraction of Flavor Compounds

After hydrolysis, aqueous extraction using boiling water was carried out. This is the most common method for obtaining seafood extracts (Ochi, 1980). Flavor compounds were extracted in a closed system consisting of a water-cooled condenser attached to a S-L round bottom flask. A heating mantle was used to heat the flask. As soon as the hydrolysis was completed, PBs (or CPBs) hydrolysate was put into the flask containing boiling water (1 L) to inactivate protease, and an additional 1 L of distilled water was added to the flask. Extraction was carried out for 2 hr after returning to boil. For control, APL-440 was added to boiling water 20 mm before PBs (or CPBs) hydrolysate was placed into the flask, and the extraction procedure was similar to that described previously. Filtration was performed using two consecutive steps after cooling the aqueous extract in the walk-in cooler (4°C): 1) cheese cloth (2-layers) and 2) filter paper (Whatman #41).

#### 4. Atmospheric Evaporation and Vacuum Evaporation

Filtrates were concentrated to  $\approx 25\text{-}30^\circ\text{Brix}$  using two evaporation methods, atmospheric evaporation and vacuum evaporation. For atmospheric evaporation, an externally heated stainless steel container was used to concentrate the filtrate. Vacuum evaporation was performed at  $60^\circ\text{C}$  using a Rotavapor (Büchi, Switzerland). It took approximately 1 hr for atmospheric evaporation and 6 hr for vacuum evaporation to concentrate to  $25\text{-}30^\circ\text{Brix}$ , which was measured using a hand refractometer (Cambridge Instruments Inc, Buffalo, NY).

#### 5. Vacuum Simultaneous Steam Distillation/Solvent Extraction (SDE)

The SDE apparatus was modified to perform under vacuum ( $\sim 30$  in. Hg; b.p.  $60\text{-}65^\circ\text{C}$ ) in order to minimize artifact formation during extraction. The standard SDE apparatus (Cat. No. K-5230101-0000, Kontes, Vineland, NJ) was modified as described by Cadwallader et al (1994). Each concentrate was placed into a 5-L round bottom flask and brought up to a total volume of 2 L with distilled water. Glass beads and  $45.4\ \mu\text{g}$  of 2,4,6-trimethylpyridine (TMP) as the internal standard were added to the flask. Extraction was carried out for 4 hr using 100 mL of redistilled dichloromethane as solvent. Air was evacuated for 30 min prior to heating of the sample flask. SDE extracts were kept at  $-20^\circ\text{C}$  overnight to facilitate water removal. The volume of SDE extract was reduced to 10 mL under a gentle stream of nitrogen, dried over 3 g of anhydrous sodium sulfate, and then further reduced to 100  $\mu\text{L}$  prior to analysis.

#### 6. Gas Chromatography/Mass Spectrometry (GC/MS)

A Hewlett-Packard (Palo Alto, CA) GC/mass selective detector (HP5790 GC/5970B MSD) was used for analysis of SDE extracts. A 5  $\mu\text{L}$  aliquot of each SDE extract was injected in the splitless mode. Volatile components were separated using a fused silica capillary column (Supelcowax 10, 60 m x 0.25 mm i.d. x 0.25  $\mu\text{m}$  film thickness; Supelco, Inc., Bellefonte, PA). The other conditions are the same as mentioned by Cadwallader et al. (1994).

#### 7. Gas Chromatography/Olfactometry (GC/O)

Sensory properties of individual volatile compounds in SDE extracts of flavor concentrates were evaluated by sniffing the GC effluent. Prior to sniffing, each extract was diluted to possess the same intensity of the internal standard peak. Each sample was evaluated by an expert panelist who was asked to record the description and intensity (strong, medium, and weak) of each odorant detected. This may be classified as a type of osme analysis (Miranda-Lopez et al., 1992). GC/O conditions are the same as described by Cadwallader et al. (1994).

## RESULTS AND DISCUSSION

Aroma profiles of flavor concentrates of PBs and CPBs were evaluated and compared using GC/O. Table 1 lists the odor active compounds and their description in flavor concentrates of PBs and CPBs. Considerable sulfury, nutty and baked potato, crabby and grainy, and raw marine-like notes were detected. A larger number of odor active compounds were present in hydrolysate of PBs than in control (Figure 1). This may be due to the improved flavor extractability or increased precursors by enzymatic hydrolysis. Kim et al. (1994) reported that the flavor extractability was improved by proteolysis in crab processing by-products. Of these odor active compounds, dimethyl trisulfide (peak no. 14, cooked cabbage), unknowns having sulfury and crabby odors, and an unknown (peak no. 54, raw marine-like) had strong odors. Naphthalenes may contribute to raw marine-like odors, which play an important role in crawfish meat (Chen, unpublished data). The unknown peak no. 54 was thought to be a naphthalene derivative based on high RI value and odor description. 2-Acetyl-1-pyrroline (2-AP, peak no. 12, popcorn) and 2-ethyl-3,5-dimethylpyrazine (peak no. 18, baked potato) also are thought to contribute to overall aroma quality.

After evaporation, a large number of odor active compounds disappeared (Figure 2). The most intense compounds after atmospheric evaporation were 2-AP dimethyl trisulfide and an unknown (peak no. 54). 2,3-Butanedione (peak no. 1, buttery), (Z)-4-heptenal (peak no. 8, fishy and rancid), and an unknown (peak no. 20, nutty and baked potato) were detected after atmospheric evaporation of PBs hydrolysate. Flavor concentrates after vacuum evaporation exhibited lower odor intensity.

Figure 3 indicates that there was no difference in the aroma profiles between hydrolysate and control in CPBs. However, 2-ethyl-3,5-dimethylpyrazine (peak no. 18) was detected only in hydrolysate. 2-AP was perceived as the only strong aroma in hydrolysate. After evaporation, another nutty and baked potato aroma (peak no. 20) was detected (Figure 4). These nutty and baked potato aroma notes may play an important role in flavor concentrates of CPBs.

When compared with CPBs, number and intensity of odor-ants in the flavor concentrate of PBs were higher. This may be due to the small amount of lipid in CPBs. Lipid plays an important role in production of volatiles. Lipid decomposition products can react with Maillard reaction intermediates to form heterocyclic compounds (Ho et al., 1989).

## CONCLUSION

Aroma properties of flavor concentrates prepared with and without protease treatment from composite crawfish processing by-products (PBs) and from separated claws (CPBs) were evaluated and compared. More odorants were present in PBs

Table 1. Odor active compounds in flavor concentrates of PBs and CPBs

Peak no.	Compounds name	RI <sup>a</sup>	Odor description
1	2,3-Butanedione	985	Buttery
2	Unknown	1015	Sour, Onion
3	Dimethyl disulfide	1070	Sour, Sulfury
4	Unknown	1092	Sour, Sulfury
5	Unknown	1148	Chocolate
6	Unknown	1156	Chocolate
7	Unknown	1167	Rancid, Pungent
8	(Z)-4-Heptenal	1239	Baked potato, Rancid
9	Unknown	1258	Sulfury, Onion
10	Unknown	1293	Mushroom
11	Unknown	1302	Nutty, Peanut
12	2-Acetyl-1-pyrroline	1337	Popcorn
13	Unknown	1349	Nutty, Peanut
14	Dimethyl trisulfide	1380	Cooked cabbage, Sour
15	(E)-2-Octenal (?)	1421	Raw peanut skin
16	Unknown	1429	Mushroom
17	Unknown	1445	Nutty, Peanut skin
18	2-Ethyl-3,5-dimethylpyrazine	1455	Nutty, Baked potato
19	Unknown	1469	Nutty, Stale
20	Unknown	1483	Nutty, Baked potato
21	Unknown	1520	Sulfury, Sour
22	Unknown	1570	Sweet, Grainy
23	(E,Z)-2,6-Nonadienal	1585	Cucumber
24	Unknown	1601	Nutty, Peanut
25	Unknown	1605	Sour
26	Unknown	1633	Burnt
27	Unknown	1639	Sulfury, Sour
28	2-Acetylthiazole	1646	Popcorn, Chocolate
29	Unknown	1662	Sweet, Grainy
30	Unknown	1668	Grainy, Nutty, Crabby
31	Unknown	1673	Nutty, Meaty
32	Unknown	1691	Nutty, Crabby
33	Unknown	1717	Fishy, Fresh fish
34	Unknown	1731	Crabby, Grainy
35	Unknown	1747	Cucumber
36	Unknown	1751	Fishy, Fresh fish
37	Unknown	1768	Sweet, Grainy
38	A Schiff base (?)	1800	Plastic, Sweet
39	Unknown	1814	Burnt, Sulfury
40	Unknown	1857	Catty
41	Unknown	1881	Mushroom
42	Unknown	1897	Sulfury, Vegetable-like
43	Unknown	1935	Sulfury, Sour
44	Unknown	1994	Sweet, Crabby, Grainy
45	Unknown	2004	Sulfury, Sour
46	Unknown	2014	Skunky
47	Unknown	2025	Sweet
48	Unknown	2057	Sweet, Grainy
49	Unknown	2079	Sewage
50	A naphthalene (?)	2135	Mothball
51	Unknown	2170	Sweet, Melon
52	Unknown	2201	Sweet, Floral
53	Unknown	2214	Cooked mushroom
54	A naphthalene (?)	2220	Naphthalene, Raw marine

<sup>a</sup> Retention Index

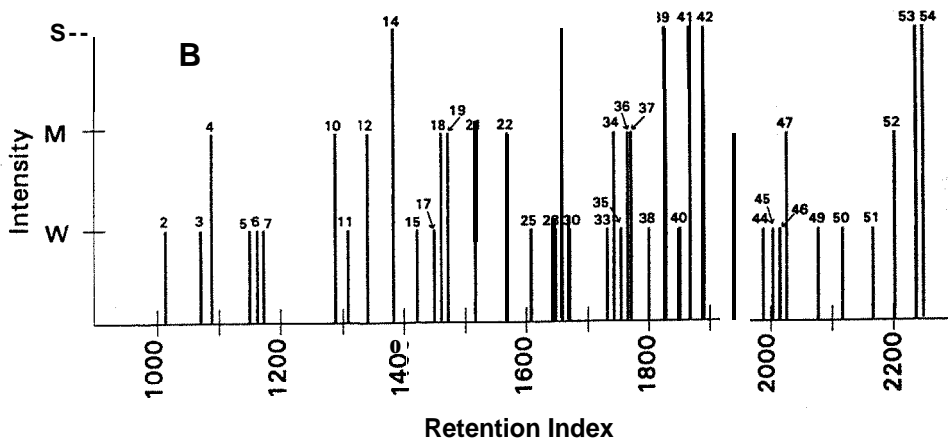
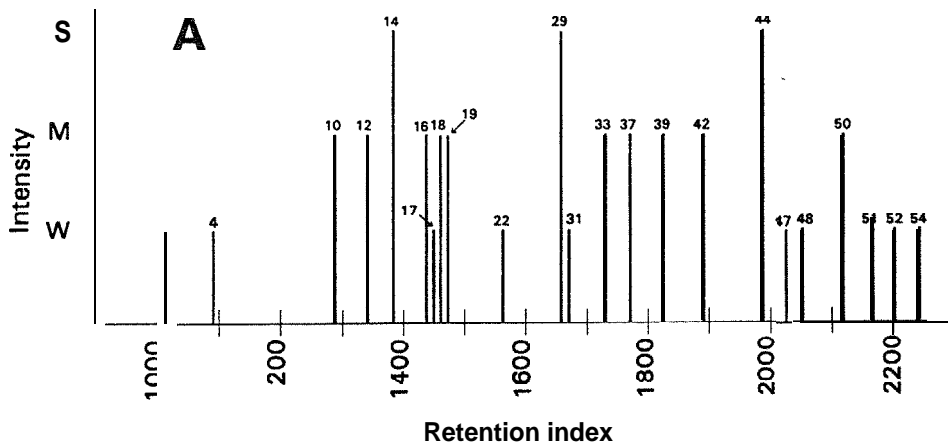


Figure 1. Aromagrams of volatile flavor components in PBs control (A) and PBs hydrolysate (B) before concentration (Peak numbers correspond to those in Table 1, S: Strong, M: Medium, W: Weak).

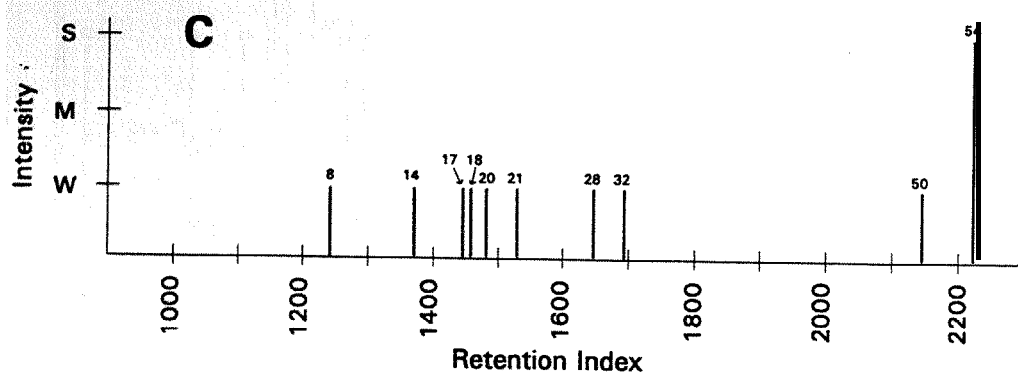
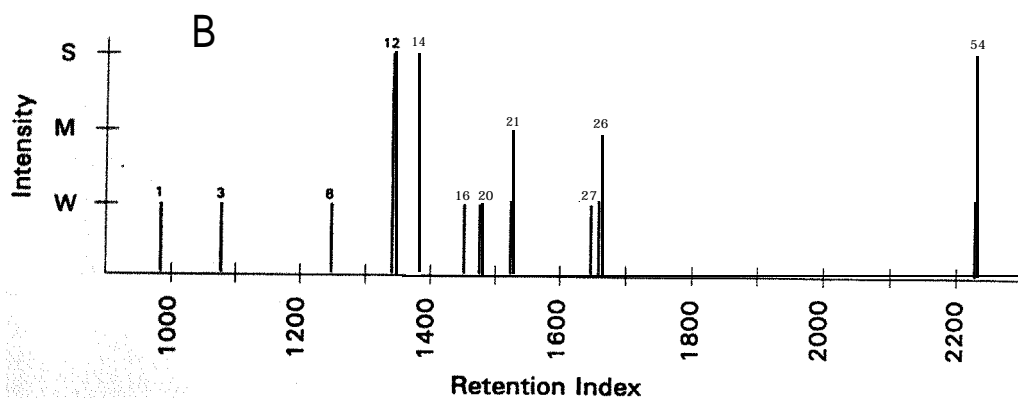
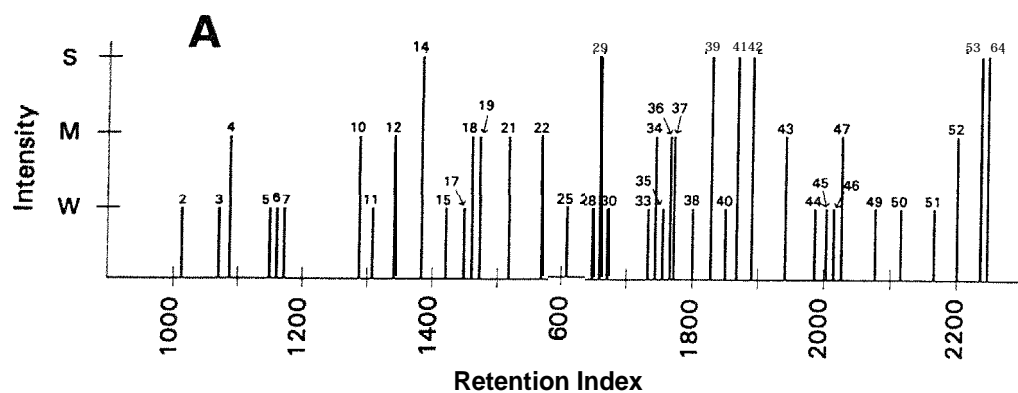


Figure 2. Aromagrams of volatile flavor components in flavor concentrates of PBs hydrolysate. A: Before concentration; B: Prepared by atmospheric evaporation; C: Prepared by vacuum evaporation (Peak numbers correspond to those in Table 1, S: Strong, M: Medium, W: Weak).

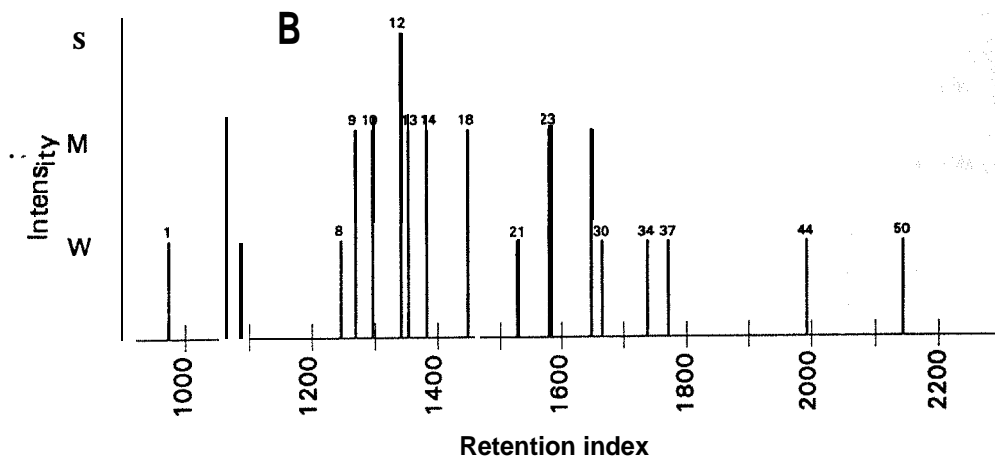
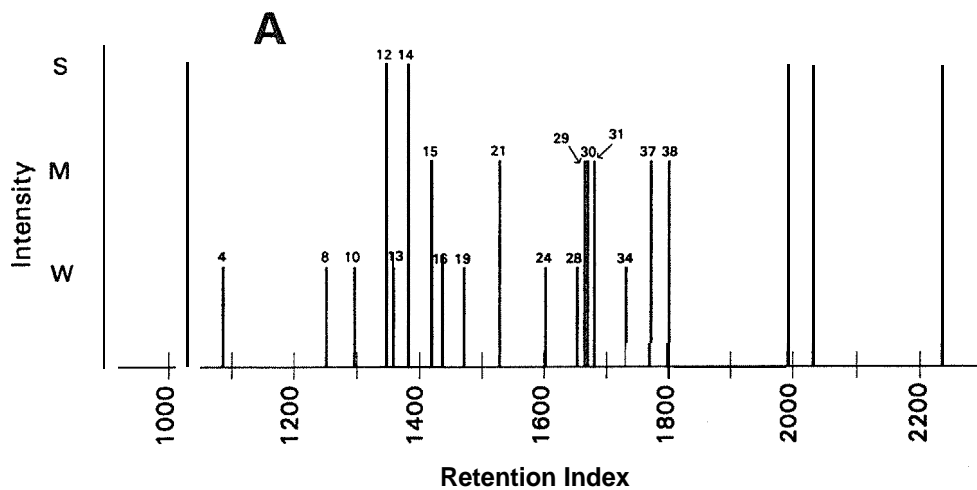


Figure 3. Aromagrams of volatile flavor components in CPBs control (A) and CPBs hydrolysate (B) before concentration (Peak numbers correspond to those in Table 1, S: Strong, M: Medium, W: Weak)

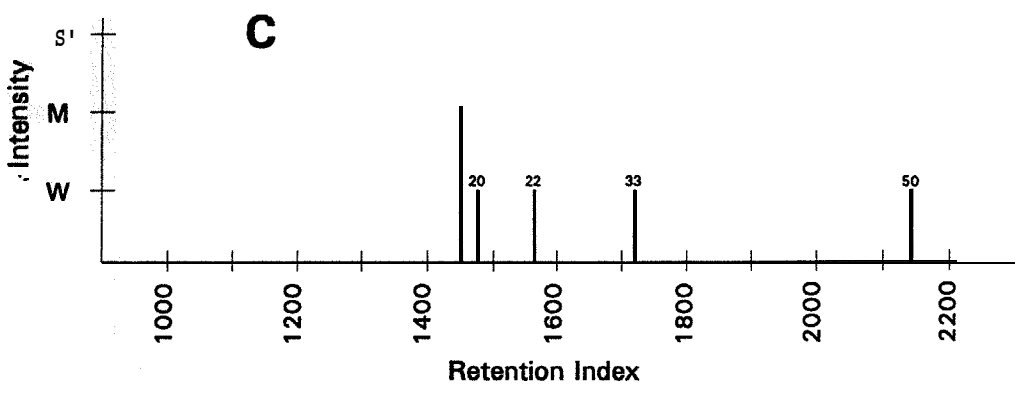
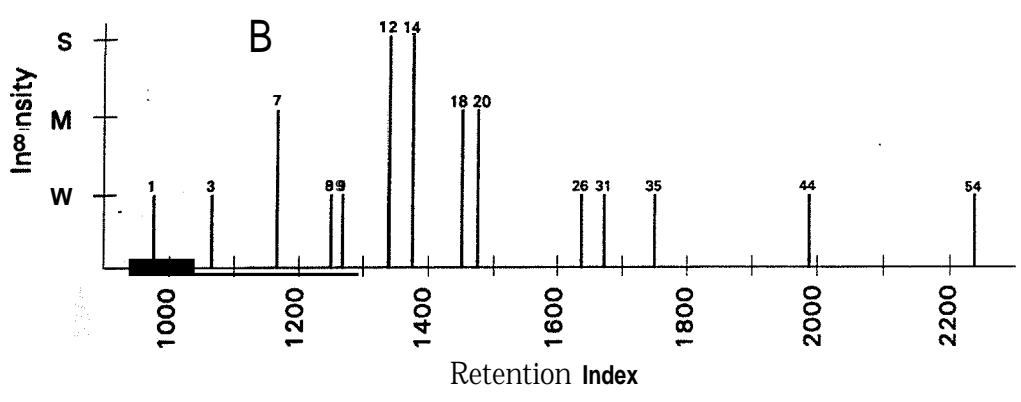
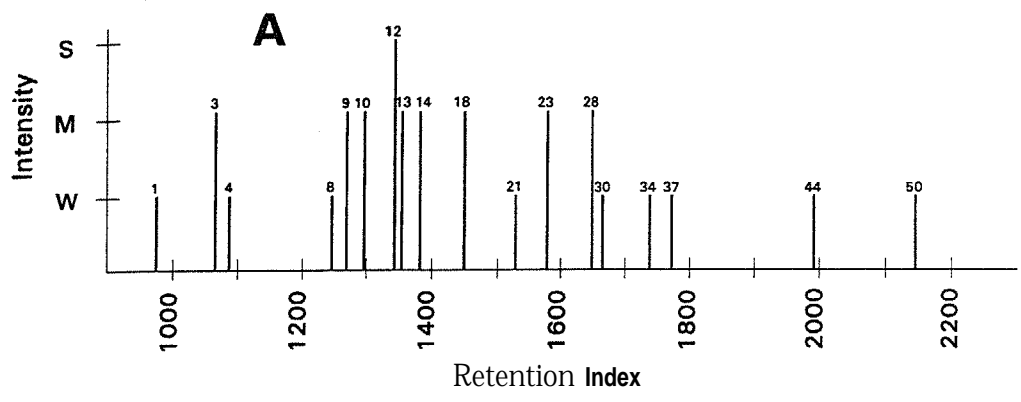


Figure 4. Aromagrams of volatile flavor components in flavor concentrates of CPBs hydrolysate. A: Before concentration; B: Prepared by atmospheric evaporation; C: Prepared by vacuum evaporation (Peak numbers correspond to those in Table 1, S: Strong, M: Medium, W: Weak).

hydrolysates than non-enzyme treated PBs flavor concentrates. Number and intensity of odorants in PBs flavor concentrates were higher than for CPBs. Evaporation (either atmospheric or vacuum) of flavor concentrates decreased the number and intensity of the odorants. Predominant odorants were identified as 2-acetyl-1-pyrroline (popcorn), 2-ethyl-3,5-dimethylpyrazine (baked potato), 2,3-butanedione (buttery), (Z)-4-heptenal (fishy and rancid), and dimethyl trisulfide (cooked cabbage).

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