

## ENZYMATIC TREATMENT OF OFFAL FROM FLESHING MACHINES

by M.M. TAYLOR, E.J. DIEFENDORF, T.A. FOGLIA, D.G. BAILEY AND S.H. FEAIRHELLER  
*Eastern Regional Research Center\**  
600 EAST MERMAID LANE  
PHILADELPHIA, PENNSYLVANIA 19118

### Abstract

Pigskin and cattlehide fleshings were treated with four commercially available proteolytic enzymes that had optimal activities at a neutral or alkaline pH. The digestions were carried out at 40°C for 3 hours and then at 60°C for 1 hour. Up to 87% of the available fat was recovered as well as a readily separated protein hydrolyzate fraction. Chemical analysis demonstrated that the fat contained 0.2-6.0% free fatty acid content depending on the treatment. The results suggest that with a small investment in enzymes a good quality fat can be produced from fleshings which will convert a solid waste disposal problem into a value-added by-product.

### Introduction

Treatment of the offal from fleshing machines has traditionally been limited to the chemical process of rendering. This is time consuming and necessitates the use of high amounts of energy, which is costly. Exposure of the fat to high temperatures and strongly acidic or basic conditions will obviously yield products which will not give the most economical return. An alternative method is enzymatic processing, which is becoming increasingly more economical with the advent of biotechnology. These treatments can be carried out at low temperatures, over short periods of time, yielding protein products which could be used for fertilizer or accepted by sanitation departments and yielding fat products which have a low free fatty acid content and thereby have potential value to the chemical industry.

It is the intent of this paper to present data gathered in this laboratory on the evaluation of four commercially available enzymes for digestion of the fleshings. The advantages and disadvantages of each enzyme will be discussed. The conditions described should be applicable to North American tanneries.

### Experimental

#### MATERIALS

Fresh pigskins and fresh cattlehides were obtained from a commercial hide broker. The skins and hides were fleshed on a fleshing machine in our pilot tannery. The fleshings were collected, drained of water, well mixed, and then placed in small plastic bags and frozen until needed.

Pancreatin was obtained from Sigma Chemical Company\*\* (St. Louis, MO). It is described as containing several types of enzymes including amylase, lipase and protease, and has

\*Agricultural Research Service, U.S. Department of Agriculture

\*\*Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

total activity at least equivalent to the U.S. Pharmacopeia (USP) specifications (pancreatin will convert not less than 25 times its weight of potato starch into soluble carbohydrates in 5 min in water at 40°C, will digest not less than 25 times its weight of casein in 60 min at pH 7.5 at 40°C and will release not less than  $2 \times 10^{-6}$  eq of acid per min per mg pancreatin from olive oil at pH 9.0 at 37°C).

Enzeco<sup>®</sup> Alkaline Protease-L (alkaline protease) was obtained from the Enzyme Development Corporation (New York, NY). This product is recommended where a heat stable proteolytic enzyme is required that is functional at an alkaline pH. It has optimal activity at pH 7.0-11.0 at 50-70°C. It is standardized to contain a minimum of 400,000 D.U./g.

Alcalase<sup>®</sup> and Neutrase<sup>®</sup> were obtained from Novo Laboratories, Inc. (Wilton, CT). Alcalase<sup>®</sup> is a proteolytic enzyme and has optimal activity at pH 8.3-9.0 at 55-65°C. It is standardized to contain 1.5 AU/g (Anson Units per g). Neutrase<sup>®</sup> is a neutral protease and has optimal activity at pH 5.5-7.5 at 45-55°C. It is standardized to contain 1.5 AU/g.

#### PROCEDURE

The frozen fleshings were thawed overnight in a refrigerator. They were ground twice in a meat grinder and weighed into one or two quart Mason jars. A mixture of the fleshings (100-500 g samples) and the appropriate amounts of water (50-100%), enzyme (1-5%), and lime (0-0.5%) were added. Control samples were run to which only water and lime were added. The digestions were shaken at 75 RPM for 3 hr. at 40°C and then for 0-18 hr. at 60°C. After the reaction was complete, the warm sample was centrifuged at 2500 RPM for about 15 min. The fat layer (still liquid) was carefully removed by pipet and transferred to a tared bottle. The hydrolyzed protein layer and residue were transferred to an Erlenmeyer flask and 10% by volume of concentrated HCl was added. The sample was heated on a steam bath for 30 min and was then filtered, and protein solution was collected in a tared bottle. The residue was weighed wet so that a mass balance could be calculated, and again in the dry state so that percent residue remaining could be determined.

#### ANALYSES

The fat isolated from the enzyme treatments was characterized using the following analyses: saponification number<sup>(1)</sup>, iodine number<sup>(2)</sup>, peroxide value<sup>(3)</sup>, and percent free fatty acids<sup>(4)</sup>. The protein in the fleshings and in the isolated hydrolyzate was determined by total Kjeldahl nitrogen (TKN). Moisture in the fleshings was determined by heating the sample in a gravity oven at 80°C for 16 hr. Ash in the fleshings was determined by heating the moisture sample at 600°C for 2 hr. Total fat in the fleshings was determined by hydrolysis of the sample and extraction of the hydrolyzate with chloroform<sup>(5)\*</sup>.

### Results and Discussion

The literature suggests that crude preparations of pancreatin were successful in the digestion of pigskin fleshings<sup>(6)</sup>. These preparations, as described, contained a high amount of lipase, which gave a fat product whose free fatty acid content was reported to be as high as 50%. Our preliminary experiments were run to confirm these treatments. However, it was felt that we could achieve the same separation with a pancreatin preparation low in lipase resulting in a product with a more desirable low free fatty acid content. These

\*Care must be taken in using chloroform because of its toxic nature.

experiments were also designed to achieve maximum separation of the fat and the protein by experimentally optimizing the enzyme concentration, pH, temperature and time. Low amounts of unreacted residue were desirable.

A protocol was established to determine how effective the enzyme treatments were in recovering the maximum amount of fat and hydrolyzed protein with a minimum amount of residue. A variety of experiments were run to optimize isolation of the two fractions. The method was designed to recover the fat quickly with little alteration, to stabilize the protein fraction by the addition of acid and to facilitate mass balances so that all materials could be accounted for: water, enzyme, lime and acid added, as well as fat, protein and residue recovered.

Control samples, which were treated in water or with lime and water, were run routinely. The recovered fat from these samples was 50% less than that from the samples to which the enzyme had been added. Moreover, chunks of tissue remained in the protein fraction, giving high residue values and making recovery of the two fractions rather difficult.

Preliminary experiments were also run to confirm a literature reference on the use of an alkaline protease for fleshing treatment<sup>(7,8)</sup>. The procedure described used urea and ammonium sulfate for pH adjustment and acid had to be added to effect separation of the fat from the protein after enzyme treatment. Enzeco<sup>®</sup> Alkaline Protease was available to us and proved to be effective in the separation of the fat and the protein without the use of acid. Various concentrations of this enzyme were employed, and it was found that 2% enzyme and 0.5% lime (for pH adjustment) for 3 hr at 40°C, and then for 2 hr at 60°C gave the optimal recovery of fat with low residue.

The fat isolated from treatment with pancreatin vs. alkaline protease was characterized, and the results are compared in Table I. As seen in this table, the most significant difference found was in the free fatty acid content for fat from the pancreatin reaction. Accordingly, the free fatty acid content was determined for all the treatments. The peroxide values show differences from the two treatments. The peroxide values show differences from the two treatments. This value, however, is also reflecting the history of the hides and skins from which the fleshings were removed, and was not routinely examined.

**TABLE I**

**Characterization of Fat Isolated from Pigskin Fleshings**

Analysis <sup>b</sup>	Treatment <sup>a</sup>	
	2% Alk. Protease	5% Pancreatin
Saponification Number	196.71	196.66
Peroxide Value	23.88	28.41
Free Fatty Acids	0.36	3.10
Iodine Number	70.90	67.20

<sup>a</sup>Reactions were run in 50% water, at 40°C for 3 hr and then at 60°C for 1 hr.

<sup>b</sup>N = 3

The saponification number and the iodine number are typical of lard samples<sup>(9)</sup>. The free fatty acid content of the fat from both the pancreatin and alkaline protease treated samples meet the standard grades of Choice White (maximum 4% free fatty acid) and White A (8%) for greases<sup>(9)</sup>.

In these initial experiments, the samples were agitated by hand. Since agitation would appear to be an important factor, a shaker bath was used in the remaining experiments. In the first of these experiments, the effect of reaction time at 60°C on the recovery of fat was studied. Five percent pancreatin was added to four fleshings samples and the latter were heated at an initial temperature of 40°C for 3 hr. The temperature was raised to 60°C and the first sample was removed immediately and packed in ice. Reactions in the second, third and fourth samples were stopped at 30, 60, and 120 min respectively. The fat was isolated, the free fatty acid content was determined, and a materials balance was calculated; these results are shown in Table II. As expected, the percent recovery of fat in sample #1 was low as was the percent overall recovery of materials. When the residues were hydrolyzed with hydrochloric acid, sample #1 produced 15.8% additional fat. The recovery of fat in the other samples did not increase significantly beyond the 30 min reaction, thus indicating this as the optimal reaction time. The free fatty acid content does not indicate a trend. The 120 min sample gave the highest value.

**TABLE II**

**Effect of Time at 60°C on Separation of Fat from Protein**

Parameter	Time in min at 60°C <sup>a</sup>			
	0	30	60	120
	(%)			
Fat Isolated Initially <sup>a</sup>	25.0	52.0	52.0	50.0
Free Fatty Acids	5.4	4.5	4.2	6.0
Fat after Hydrolysis	15.8	0.5	0.6	0.1
Material Balance	87.0	93.0	91.0	92.0

<sup>a</sup>Pigskin fleshings treated with 5% pancreatin, 50% water, and at 40°C for three hr before the temperature was raised.

<sup>b</sup>Weight of recovered fat as percentage of pigskin fleshings.

**TABLE III**

**Characterization of Fat Isolated from Cattlehide Fleshings**

Analysis <sup>a</sup>	Treatment <sup>a</sup>	
	2% Alk. Protease	5% Pancreatin
% Fat Isolated <sup>c</sup>	39.70	40.00
Saponification Number	198.30	197.10
Peroxide Value	7.44	11.95
Free Fatty Acids	0.46	4.56
Iodine Number	57.20	54.46

<sup>a</sup>Reactions were run in 50% water at 40°C for 3 hr and at 60°C for 1 hr.

<sup>b</sup>N = 3

<sup>c</sup>Weight of recovered fat as percentage of cattlehide fleshings.

**TABLE V**
**Effect of Time and Treatment on Recovery of Fat and Protein from Pigskin Fleshings**

Parameter	Treatment <sup>a</sup>		
	Control	2% Alk. Protease	5% Pancreatin
		(%)	
<b>1-Hour Reaction</b>			
Fat Recovered <sup>b</sup>	28.5	74.2	87.4
Protein Recovered	59.4	94.8	103.6
Residue	47.6	12.3	8.1
Free Fatty Acids	0.6	0.2	3.8
Material Balance	90.5	92.9	94.4
<b>18-Hour Reaction</b>			
Fat Recovered	29.8	80.9	86.2
Protein Recovered	58.0	94.3	106.6
Residue	44.7	5.8	7.5
Free Fatty Acids	0.6	0.2	6.0
Material Balance	94.5	93.6	95.1

<sup>a</sup>Reactions were run in 50% water at 40°C for 3 hr before the temperature was raised to 60°C for either 1 or 18 hr.

<sup>b</sup>Data obtained from the analysis of the fleshings (See Table IV, Batch A) were used to calculate the percent recoveries.

The Novo enzymes, having activities at pH's similar to pancreatin and alkaline protease, were tested for their ability to separate the fat and the protein in pig fleshings. A batch of pig fleshings was thawed and ground, and prior to treatment a portion was analyzed for moisture, ash, TKN and total fat. These results are shown as batch B in Table IV. Comparison of these data show the variations one can expect in different batches of fleshings. These data will be used to determine the percent recovery of fat and protein.

For comparison, pancreatin and alkaline protease, whose effectiveness at the 5 and 2% percent levels had been demonstrated, were also run on this new batch of fleshings. Alcalase<sup>®</sup> and Neutrase<sup>®</sup> were used at 1, 2 and 3% level, even though other concentrations may be more effective. From the isolated products, percent fat, percent protein, percent residue, and free fatty acid content were determined. Material balance, percent fat recovered and percent protein recovered were also determined.

Table VI shows the effectiveness of three concentrations of pancreatin on the treatment of pig fleshings. Ideally, a high fat recovery, high protein recovery, low residue, and a low free fatty acid content are being sought. Because of more tightly controlled experimental conditions, not only in the reaction itself, but also in the isolation of the products, the 3% concentration of pancreatin yields the highest recovery of fat with the lowest percent residue. To date, the pancreatin has been the most effective.

Table VI also gives the results obtained with alkaline protease. A 2% concentration had been used in the preliminary studies, along with a 2 hr reaction time at 60°C. These data indicate, however, that a 1% level for 1 hr at 60°C would be equally effective. Again, tightly controlled experimental conditions are probably contributing to the higher effectiveness. Moreover, the free fatty acid content was quite low, which is highly desirable. The percent fat recovered, however, is not as good as that obtained with pancreatin.

The data obtained from the pancreatin and alkaline protease treatments, were compared

**TABLE VI**
**Treatment of Pigskin Fleshings with Commercial Enzymes<sup>a</sup>**

Pancreatin	Concentration of Enzyme <sup>b</sup>		
	3%	4%	5%
		(%)	
Fat Recovered <sup>c</sup>	78.8	78.0	76.2
Protein Recovered	103.5	94.5	101.7
Residue	12.8	14.0	12.6
Free Fatty Acids	3.4	4.2	4.3
Material Balance	94.4	94.8	94.8
<b>Alkaline Protease</b>	<b>1%</b>	<b>1.5%</b>	<b>2%</b>
Fat Recovered	73.5	70.5	69.8
Protein Recovered	81.2	74.4	78.2
Residue	11.6	13.4	15.0
Free Fatty Acids	0.2	0.2	0.2
Material Balance	92.5	91.5	91.8
<b>Alcalase<sup>®</sup></b>	<b>1%</b>	<b>2%</b>	<b>3%</b>
Fat Recovered	73.7	78.7	75.2
Protein Recovered	79.9	98.3	101.7
Residue	16.0	11.2	12.6
Free Fatty Acids	0.2	0.2	0.2
Material Balance	93.9	93.6	93.2
<b>Neutrase<sup>®</sup></b>	<b>1%</b>	<b>2%</b>	<b>3%</b>
Fat Recovered	68.6	67.4	77.3
Protein Recovered	81.7	92.7	107.3
Residue	17.3	17.3	17.6
Free Fatty Acids	0.7	0.8	0.7
Material Balance	94.3	94.3	99.0

<sup>a</sup>Fleshings treated with enzyme, in 50% water at 40°C for 3 hr and then at 60°C for 1 hr.

<sup>b</sup>Concentration based on wet weight of fleshings.

<sup>c</sup>Data obtained from the analysis of the pigskin fleshings (See Table IV, Batch B) were used to calculate percent recoveries.

with the two enzymes that were obtained from Novo, Alcalase<sup>®</sup> and Neutrase<sup>®</sup>. Alcalase<sup>®</sup> is also used in the food industry, for it acts on all kinds of protein commonly found in food materials, whether from animal or plant sources. Table VI shows the results obtained with this enzyme on the treatment of pigskin fleshings. Best results were obtained with the 2% concentration and again the free fatty acid content is quite low. This enzyme, like the Enzeco<sup>®</sup> Alkaline Protease, requires a pH adjustment, and this was carried out with 0.5% lime.

Neutrase<sup>®</sup>, familiar to the tanning industry for other uses, was used at the 1, 2 and 3% concentrations. Table VI shows the results of these treatments. The 3% concentration is the most effective, and again the free fatty acid content is still quite acceptable.

Of the four different enzymes examined, pancreatin stands out as having the highest percent recovery of fat with the lowest percent residue, under the conditions which were

used in this study. The free fatty acid content is higher than that found in the fat recovered from the other enzyme treatments. Depending on the type of product that will be derived from this fat, this value may be satisfactory. If a lower value is desirable, the other enzymatic treatments give quite good recoveries of fat with low free fatty acid contents. The effectiveness of Alcalase<sup>®</sup> and Neutrase<sup>®</sup> could possibly be improved, but this will have to be the subject of future research.

### Conclusion

It has been demonstrated that offal generated from fleshing machines can be treated enzymatically at low temperatures and in a relatively short time to give by-products which have potential value to the chemical industry. The use of commercially available enzymes, from sources familiar to the tanning industry, proved to be successful in the isolation of the fat and protein hydrolyzate.

### Acknowledgment

The authors would like to acknowledge Dr. George Na for his critical review of the manuscript and for his encouragement during its preparation.

### References

1. ALCA Official Methods of Analyses H31 (1954).
  2. ALCA Official Methods of Analyses H32 (1954).
  3. American Oil Chemists' Society, Official and Tentative Methods Cd 8-53 (1960).
  4. ALCA Official Methods of Analyses H30 (1954).
  5. Taylor, M.M., Diefendorf, E.J., Phillips, J.G., Fairheller, S.H. and Bailey, D.G., *JALCA*, **81**, 4 (1986).
  6. Bronowski, K., Sagata, J. and Studniarski, K., *Leder*, **30**, 8 (1979).
  7. Braeumer, K., Eckmayer, Z., Monsheimer, R. and Pfeleiderer, E. Ger. Offen. 2,705,671 (1978).
  8. Braeumer, K., Eckmayer, Z., Monsheimer, R. and Pfeleiderer, E., *Leder- und Hautemarkt. Gerbereiwissenschaft und Praxis*, **30**, 100 (1978).
  9. Swern, D. "Bailey's Industrial Oil and Fat Products," Volume I, Fourth Edition. John Wiley and Sons, New York, New York, (1979).
- Received December 15, 1988.
-