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AUGUST 1979

BOSTON AIChE NATIONAL MEETING — FOOD, PHARMACEUTICAL, AND BIOENGINEERING DIVISION, August 19—22, 1979. Session on Synthetic Foods — Research Needs and Status.

A session is being coordinated with the AIChE Research Committee to define and delineate the state of the art of food synthesis. It is desired to assess the time framework of research needs in this area as well as the roles that chemical engineers will serve. A coherent session that will explore these points, as well as present current work, should be most useful in updating the problems and viewpoints in this area.

CALL FOR PAPERS. A letter of intent to present a paper should be sent by December 15, 1978 to Professor Alvin H. Weiss, Department of Chemical Engineering, Worcester Polytechnic Institute, Worcester, Massachusetts 01609, Phone: 617-753-1411, Ext. 380.

AIChE Proposal to Present Forms are due February 1, 1979. Manuscripts are due May 1, 1979. Note that AIChE requires that SI units be used.

CHARACTERIZATION OF ANTIOXIDANTS RESPONSIBLE FOR INHIBITION OF WARMED-OVER FLAVOR IN RETORTED TURKEY

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ABSTRACT

Antioxidant material which has been extracted from retorted turkey was partially characterized as to its chemical nature. Fractionation on Sephadex G-50 indicated the active antioxidant material to have a molecular weight of between 200 and 500. It inhibited oxygen uptake in model systems in concentrations as low as 200 ppm, indicating strong antioxidant properties. It was not volatile, but did exhibit strong reducing properties similar to those found in reductones, which are known intermediates in the browning reaction.

INTRODUCTION

In a previous study (Einerson and Reineccius 1977) evidence was presented which suggested the production of antioxidative substances during the retorting of turkey meat. It was postulated that reductones formed as the result of browning interactions between sugars and amino compounds were responsible for this antioxidative activity. The purpose of this investigation was to further characterize these antioxidative substance(s).

MATERIALS AND METHODS

Sample Preparation

Turkey samples were selected and prepared as described by Einerson and Reineccius (1977).

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Column Chromatography

The freeze-dried diffusate fraction of the aqueous extract of retorted turkey was fractionated on Sephadex G-50 and G-15. Column dimensions were 2.75 cm × 55 cm. The columns were equilibrated with a 0.05 M tris hydrochloride buffer, pH 7.00, containing 0.1 M KCl. Flow rate was maintained at approximately 40 ml/hr. Void volumes were determined using Blue Dextran 2000, and the G-15 column was standardized by passing solutions of glucose, lactose, raffinose, and stachyose through the column and determining the elution volumes. A standard curve of log molecular weight versus elution volume was constructed from these data.

The diffusate and standard sugars were applied to the column by dissolving the material in 2 ml of buffer-salt solution and carefully layering this on top of the column. Fractions were collected with an LKB Ultrorac Fraction Collector. The various fractions were then frozen, freeze-dried, and stored in a desiccator until analysis. Presence of sugars in the effluent fractions was determined by the phenol-sulfuric acid method (Dubois *et al.* 1956).

Evaluation of Antioxidant Effectiveness

The various isolated fractions were tested for antioxidant effectiveness by the oxygen uptake method used by Einerson and Reineccius (1977) with the following modifications. BHA was dissolved in methanol to incorporate it into the model system. In order to adjust the rate of oxidation to optimum levels, the microcrystalline cellulose was impregnated with 50, 200 and 500 ppm of iron. All fractions were tested in duplicate.

Further Dialysis of Extracted Material

As a purification step before further analysis, the diffusate fraction of aqueous extract of retorted turkey was further dialyzed using a Bio-Fiber 50 hollow fiber cellulose membrane. A solution containing the diffusate fraction was placed in the beaker outside the hollow fibers and 100 ml of deionized, distilled water was recycled through the fibers at a rate of 180 ml/hr. Dialysis took place at 4°C and lasted 48 hr. The resulting diffusate and dialysate fractions were concentrated by rotary-evaporation, frozen, freeze-dried, and stored in a desiccator until analysis.

RESULTS AND DISCUSSION

A total of 11 fractions were collected from the fractionation of the

extracted material on Sephadex G-50. Tests of these for antioxidant effectiveness showed fraction 7 to contain the greatest amount of antioxidant activity, followed by fractions 8 and 6. However, examination of K_d values and comparison of elution volumes to those of known compounds indicated that the material present in these fractions was of a molecular weight below the effective fractionating range of G-50, which is from about 500 to 10,000. For this reason, the extract was fractionated again, this time on Sephadex G-15, which has a fractionation range of from 0 to about 1000.

Figure 1 shows oxygen uptake in model systems containing the extract fractionated on Sephadex G-15. A total of 6 fractions were collected, with 0.0208 g of each fraction incorporated into the model systems, representing 1% by weight of the oil in the system.

Fraction 3 showed the greatest amount of antioxidant activity, followed by fractions 4 and 5. The molecular weight of this material was estimated from a standard curve obtained by passing standard sugars through the column and determining their elution volume. This standard curve is illustrated in Fig. 2. As shown in Fig. 2, the molecular weight estimations of fractions 3, 4 and 5 are 460, 270, and 160, respectively.

It should be understood that these molecular weights are only rough approximations, since in gel filtration, compounds are separated on the basis of their molecular configuration, or Stokes radius, as well as size, and there is no guarantee that the unknown substances extracted from retorted turkey possess the same type of configuration as the standard sugars. Nevertheless, these approximations are probably reasonably

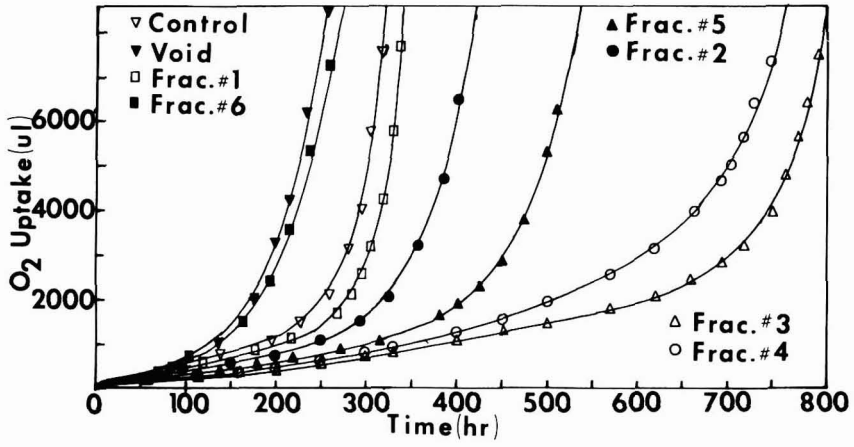

FIG. 1. OXYGEN UPTAKE IN MODEL SYSTEMS (50 PPM FE + 2) CONTAINING MATERIAL FRACTIONATED ON SEPHADEX G-15

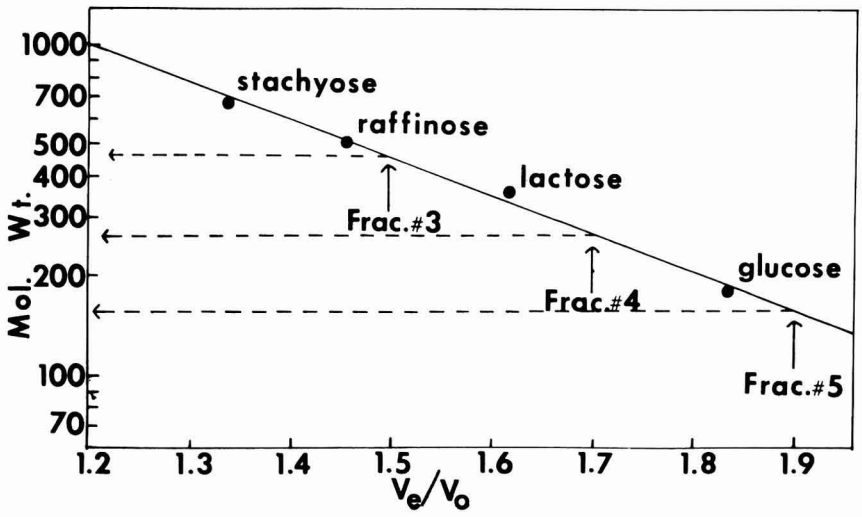

FIG. 2. SEPHADEX G-15 STANDARD CURVE AND THE MOLECULAR WEIGHT ESTIMATIONS OF FRACTIONS 3, 4, AND 5 OBTAINED FROM IT

accurate, and are certainly consistent with the hypothesis that low molecular weight reducing substances are responsible for the antioxidant activity of this material.

It should also be mentioned that Sephadex gels, due to the presence of a small number of carboxyl groups, show some ion exchange properties. Therefore, some types of molecules are retained longer on the gel than would otherwise be expected on the basis of molecular weight. However, this effect is usually eliminated by using solutions of moderate ionic strength, such as the one being used here (Andrews 1964).

In order to further characterize the antioxidant material isolated during gel filtration, it would have been desirable to separate the extracted material from the buffer-salt solution in which it was dissolved. However, ion exchange chromatography, dialysis, extraction with various solvents, and high pressure liquid chromatography all proved unsuccessful in the separation of the components. The extracted material could not be fractionated on the Sephadex column in pure water, due to problems with adsorption of the material onto the packing.

An experiment was done to determine the antioxidant effectiveness of the extracted material as compared to the known antioxidant BHA. Since the fractions obtained from gel filtration could not be separated from the buffer-salt solution and therefore could not be accurately weighed and because the active material was known to be of small molecular weight, the diffusate fraction obtained from dialysis with cellulose dialysis tubing was further purified by dialyzing with a Bio-

Fiber 50 device, which has a molecular weight cutoff of about 5000 as opposed to 10,000 for regenerated cellulose dialysis tubing. Approximately 90% of the material obtained in the diffusate fraction during dialysis with cellulose tubing also passed through the Bio-Fiber membrane.

Figure 3 shows the results of an experiment in which 1000 ppm (lipid basis), 500 ppm, and 200 ppm of the freeze-dried Bio-Fiber diffusate and 200 ppm BHA were incorporated into the model systems. The results demonstrate that this material will inhibit oxygen uptake in the model systems at a concentration as low as 200 ppm.

It is evident that this material exhibits antioxidant effectiveness at low concentrations. Although the activity is not great when compared to a known potent antioxidant such as BHA, it must be remembered that the extracted material was tested in a relatively crude, unpurified form. The active component may be present in even a much smaller concentration. Furthermore, this material is present in the actual retorted meat in a much greater concentration than 200 or even 1000 ppm, so its activity in these meats is likely to be considerable.

In an attempt to further fractionate and characterize the extracted material, the purified material from the Bio-Fiber dialysis was subjected to high pressure liquid chromatography using a μ-Bondapak C_{18} column. The attempt was largely unsuccessful, as the material was separated into only three major peaks. Only one of these peaks could be tested for antioxidant activity, and was found to contain less activity than the material before fractionation, indicating failure to isolate and purify the active antioxidant material.

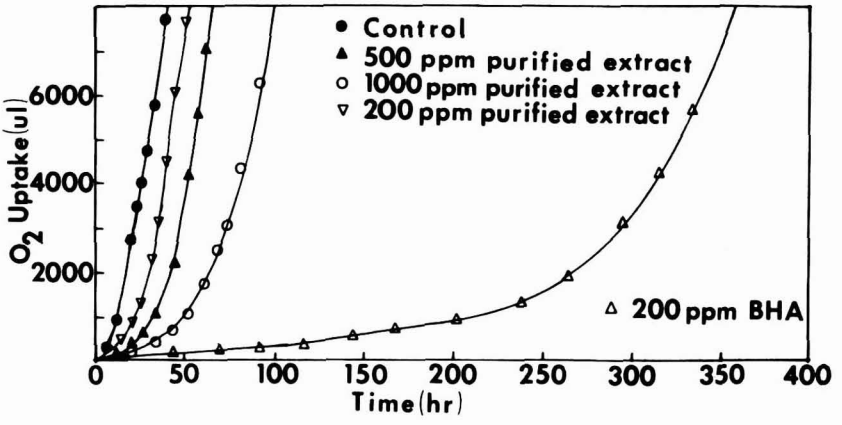

FIG. 3. OXYGEN UPTAKE IN MODEL SYSTEMS (200 PPM FE^{+2}) CONTAINING 200, 500, AND 1000 PPM EXTRACTED MATERIAL, AND 200 PPM BHA

The material which had been extracted from retorted turkey was further analyzed in an attempt to better determine its chemical nature. It was found that the purified dialyzed extract as well as the antioxidative fractions obtained through gel filtration all reduced 2,6-dichloroindophenol and could be titrated to a sharp endpoint with iodine in acid solution, indicating that this material possesses strong reducing properties. Upon heating, the purified extract did not exhibit a distinct melting point, but slowly decomposed between 215 and 230°C. Analysis by gas-liquid chromatography showed no appreciable volatile components. It was not possible to obtain an infrared spectrum of the material, due to water absorption by the sample. Many of these properties are also characteristic of reductones (Hodge and Rist 1953), further indicating that reductones or at least reducing substances are the material responsible for the antioxidative activity found in retorted turkey.

It is evident that the analytical techniques used here were not completely successful in the identification of the antioxidant material produced during the retorting of turkey. The extracted material was extremely hygroscopic and difficult to work with. Complete identification of the active antioxidant material would require the development of analytical techniques not now available, and is likely to be a difficult task.

CONCLUSIONS

The antioxidant material extracted from retorted turkey was further characterized as to its chemical nature. The active material was found to have an approximate molecular weight of between 200 and 500 and to show antioxidant activity at concentrations as low as 200 ppm. It was not volatile, but exhibited strong reducing properties, and was very hygroscopic. All of these characteristics are consistent with the hypothesis that low molecular weight reducing substances formed as a result of browning interactions are responsible for the antioxidant activity found in retorted turkey.

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THE EFFECT OF PROTEIN BLENDING ON CHEMICAL AND FUNCTIONAL PROPERTIES OF SOY GELS[1]

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ABSTRACT

A blending model for improving the protein quality of soy isolate gels has been investigated. The results indicate that the calculated PER (C-PER) of soy gels was improved from 0.9 *to above* 2.0 *after blending with blood plasma protein isolate, wheat protein concentrate, partially delactosed whey and/or casein with the soy isolate. All gels containing protein blends were self-supporting and possessed slightly darker colors than did the control gels which contained only soy isolate. However, the colors the protein blended gels were improved by the incorporation of partially delactosed whey or casein into the blend.*

INTRODUCTION

Heat-induced soy gels have been investigated by many workers (Beckel *et al.* 1949; Glabe *et al.* 1956; Circle *et al.* 1964; Catsimpoolas *et al.* 1970; Catsimpoolas and Meyer 1970, 1971a,b; Yasumatsu *et al.* 1972; Ehninger and Pratt 1974; and Shemer 1973). Circle *et al.* (1964) stated that the viscosity of an unheated soy protein suspension increased exponentially with increased protein concentrations. The firmness of the gel produced from a heated protein suspension was also found to be dependent upon the temperature and time of heating. The authors also found that reducing agents, sodium sulfite and cysteine, lowered the viscosity of the soy protein suspension and prevented its gelation. Catsimpoolas *et al.* (1970) proposed a theory for gel-progel conversion. They believed that a soy gel is converted into the progel

[1] Published as Paper Number 5365, Journal Series, Nebraska Agricultural Experiment Station.

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state by heating and then forms a gel upon cooling. The authors found the formation of the progel and gel were affected by pH, temperature, and ionic strength. Catsimpoolas and Meyer (1971a) also observed that soy globulins, when suspended in water-alcohol and/or water-glycol mixtures, produced gels of higher apparent viscosity than did those suspended in water. Protein-lipid interaction was investigated by Catsimpoolas and Meyer (1971b) and it was suggested that the shorter fatty acid chains of the glyceride or the partially esterified glycerol increased the apparent viscosity of the gel. Phospholipids and cholesterol were also found to enhance the gelation of soy proteins. Ehninger and Pratt (1974) demonstrated that a lower concentration of sucrose than dextrose was needed to form a protein-water-sugar complex, which increased the viscosity of soy isolate gel at pH levels of 6.0 and 6.5.

Soybean protein is a good plant protein both in quality and quantity. However, it is limiting in the sulfur amino acids. This limitation becomes more critical after the soy protein has been isolated, since the methods used for soy isolate production cause a further loss of sulfur amino acids. Shemer (1973) pointed out that methionine was lost during the isolation of soybean protein. He also indicated that 20% of the methionine was lost as a result of heating the protein in boiling water.

Heat-induced soy gels have the potential of being developed into a unique gel-type foods with a high protein content and a high nutritional quality, if the sulfur amino acid content in the soy gel can be increased. It was the purpose of this study to enhance the essential amino acid profile of soy protein based gels, using blends of various food proteins. The effect of fortification on the textural and color qualities as well as chemical properties of the gel were investigated.

MATERIALS AND METHODS

Protein Sources

SP-55 (soy protein isolate, Table 1) which was supplied by Miles Laboratories was used as a gel base. This soy isolate (the term soy isolate as used in this manuscript refers to the SP-55 product) was blended with other protein sources, which included partially delactosed whey, blood plasma protein isolate, wheat protein concentrate and casein. Partially delactosed whey and casein are commercial products. Blood plasma protein isolate, prepared according to Tybor *et al.* (1975), was donated by Dr. C. W. Dill, Texas A&M University. Wheat protein concentrate was extracted from wheat bran as described by Woerman and Satterlee (1974).

Table 1. Gel blend compositions

Gel Blends	Ingredients[1]	Composition (% w/w)
Control gel 1	Soy isolate	25.0
	H_2O	75.0
Blend 1	Soy isolate	15.0
	Partially delactosed whey	2.6
	Blood plasma protein isolate	7.4
	H_2O	75.0
Blend 2	Soy isolate	15.0
	WPC	2.5
	Blood plasma protein isolate	7.5
	H_2O	75.0
Control gel 2	Soy isolate	15.0
	H_2O	85.0
Blend 3	Soy isolate	10.0
	Blood plasma protein isolate	5.0
	H_2O	85.0
Blend 4	Soy isolate	9.0
	Partially delactosed whey	1.5
	Blood plasma protein isolate	4.5
	H_2O	85.0
Blend 5	Soy isolate	10.0
	Blood plasma protein isolate	2.5
	Casein	2.5
	H_2O	85.0

[1] A soy isolate specifically developed for soy gel production

Preparing the Soy Gel

During preliminary studies, it was found that a 15% aqueous soy isolate solution gave a self-supporting gel with desirable gel strength. Based upon these results, two differing blend formulations were used:

Formulation 1. 15% soy isolate was used as the gel base and blended with 10% of the other food protein sources (25% solids formulation).

Formulation 2. 10% soy isolate was used as the gel base and blended with 5% of the other food protein sources (15% solids formulation).

To prepare a gel, the protein mixture was homogenized with glass distilled water to obtain a uniform slurry of either 25 or 15% solids. The slurry was degassed under vacuum and the pH and viscosity were measured. The slurry was then heated in a sealed glass jar (48 mm diameter × 62 mm height) for 30 min in a boiling water bath. The jar containing the heated gel was immediately cooled at 5° C.

Measurement of Functional Properties

Viscosity. The apparent viscosity of the unheated protein slurry was measured using a Brookfield (LVT) Viscometer in a 48 mm diameter beaker at 25°C. Since the slurry was non-Newtonian, the apparent viscosity (centipoise) was calculated by dividing shear stress by shear rate and multiplying by 100.

Gel Strength. An Instron Universal testing machine was used to measure the gel strength according to the method described by Kramer and Hawbecker (1966). The extrusion cell consisted of a hollow cylinder, 35 mm ID, with 7 (1.59 mm width) slits and 6 (3.175 mm width) bars on the bottom. The plunger diameter was 34.24 mm. A gel cylinder of 35 mm in diameter and 20 mm high was used for the shear-press test. (See Fig. 1 for photographs showing the extrusion cell.)

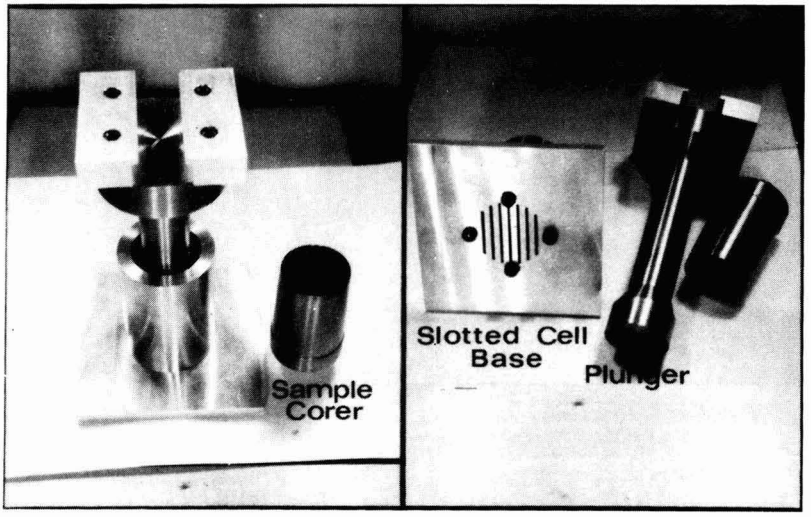

FIG. 1. THE EXTRUSION CELL FOR SHEAR-PRESS TESTING OF GELS

The cell was used on an Instron Universal Testing Machine.

Gel Color. A color difference meter (Gardner Model C-4) was used for all color measurements. A standard white plate, L — 94.5, a = —1, b = +3, was used to adjust the instrument. Three color values; L (lightness0, a ("+" is redness, "—" is greenesss), and b ("+" is yellowness, "—" is blueness) were measured. A gel, 5 mm thick and 50 mm in diameter was placed on a petri dish. The color of the gel was measured using a white paper background which was placed on top of the gel.

Protein Content and Amino Acid Composition

Protein content was determined by Kjeldahl method (AOAC 1975), using a Kjeldahl factor of 6.25. For amino acid analyses, the proteins were hydrolyzed for 24 hr using 6N HCl, at 110°C under vacuum. The sulfur amino acids were obtained by pretreating the proteins with performic acid followed by a standard 6N HCl acid hydrolysis (Moore 1963). Tryptophan, which was destroyed in the acid hydrolysis, was released using $Ba(OH)_2$ (Pataki 1968). All amino acids were analyzed using a Beckman Model 120°C amino acid analyzer.

In Vitro Digestibility and Computer-Predicted-PER (C-PER)

The *in vitro* digestibilities of control and blend gels were determined by the multienzyme digestion technique of Hsu *et al.* (1977). The C-PER of all samples was calculated using the method described by Satterlee *et al.* (1977) and Hsu *et al.* (1978).

RESULTS AND DISCUSSION

Composition of Gel Blends

The fortification of the soy isolate with other protein sources was performed using linear programming to select protein sources that would optimize the PER of the soy gel. Table 1 lists the ingredients of each gel blend and their final protein content. It was observed that with the exception of the control gels 1 and 2, blood plasma protein isolate was selected for all the five gel blends. The selection of blood protein isolate was probably due to its high protein and high sulfur amino acid content. To counterbalance the high level of soy isolate in the gel, a protein source high in both protein and sulfur amino acid content had to be used.

Gel Viscosity, Strength and Color

Table 2 shows the viscosities of various gel slurries prior to heating. The data indicate that the control gel slurries had the lowest viscosities at both the 15 and 25% solids level. The viscosities of all gel blends were higher than those of the respective controls. Since the protein contents of control gel 2 and blend gels 3 and 5 were very close, the increase in the viscosities of blend gels was probably due to the presence of blood plasma protein. The possible effect of blood plasma protein on viscosity was further evidenced by the fact that the gel slurry with the highest viscosity was blend gel 3, the gel fortified solely

Table 2. Viscosity of the unheated gel slurries

Gel Blends	pH	% Solids	% Protein	Viscosity[1] (cP)
Control gel 1	7.0	25	21.71	173.36
Blend 1	7.3	25	20.21	1555.97
Blend 2	7.2	25	22.18	1589.76
Control gel 2	7.0	15	13.03	23.1
Blend 3	7.3	15	13.22	74.08
Blend 4	7.2	15	12.13	48.46
Blend 5	7.2	15	13.17	59.01

[1] Measured with a Brookfield (LVT) Viscometer at 25°C. Shear rates for the slurries of blends 1 and 2 was 6.30 sec^{-1} and for the remaining gel slurries it was 7.49 sec^{-1}

with blood protein isolate.

Table 3 lists the strengths (gel breaking force) of the control and blend gels. The gel breaking forces indicate that the control gel 1 was the strongest of those gels having a 25% solids base. Blend gel 3 was the strongest gel of those having a 15% solids base, followed by blend gels 5 and 4. Control gel 2 was the weakest of the gels having the 15% solids base. The gel strength results of 15% solids base gels related well to the respective individual viscosities of unheated protein slurries. The gel strengths of the 25% solids base gels showed a reversed relationship to their individual slurry viscosities. Control gel 1 had the lowest viscosity prior to heating, but it became the strongest gel upon heating. This was possibly due to the fact that the binding forces between the soy isolate protein molecules are stronger than that between soy isolate and other protein molecules. The 25% solids soy isolate gel had a larger number of possible binding sites between the soy protein molecules resulting in a stronger gel. Possibly, incorporation of other protein molecules blocked the binding sites between the soy protein molecules and therefore reduced the strength of the blended gels. The lower solids base (15%) soy

Table 3. The breaking force of the various gels as measured using the Instron Universal Testing Machine

Gel Blends	Solid (%)	Gel Breaking Force (Newtons)
Control gel 1	25	544.23
Blend 1	25	509.91
Blend 2	25	446.11
Control gel 2	15	24.52
Blend 3	15	54.91
Blend 4	15	34.32
Blend 5	15	36.28

isolate gel did not have a sufficient number of binding sites between soy protein molecules. Therefore, the gel strength was increased by the binding forces between soy protein and other protein molecules. All control and blend gels were self-supporting (Fig. 2).

Color is one of the primary functional characteristics that affect food acceptability. Therefore, it was deemed necessary to understand the effect of protein blending on gel color. Table 4 illustrates the color parameters of the gels, as measured by the Gardner Color Difference Meter. Three values, L, a, and b were taken. However, only L values, which determined the lightness of the gel were discussed since these values related well to the visual evaluations. The L values indicate that the control gel 2 had the lightest color, followed by control gel 1, blend gels 4, 5, 1, and 3. Blend gel 2 had the darkest color. The dark color in the blend gels was due to the presence of the blood plasma protein isolate and wheat protein concentrate. When a portion of the blood protein was substituted with partially delactosed whey or casein, the final gel color became lighter.

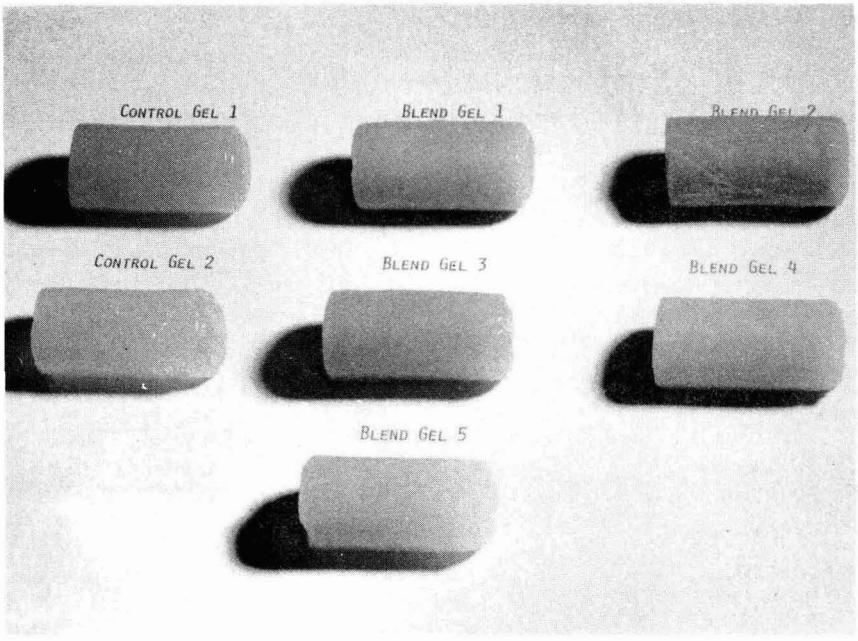

FIG. 2. PHOTOGRAPHS OF THE CONTROL AND BLEND GELS

In Vitro Protein Apparent Digestibility and C-PER

Table 5 shows the digestibilities and C-PERs of control and blended

Table 4. Gardner Color Difference Meter values of various gels

Gels	Gardner Color Difference Meter Values[1]		
	L	a	b
Control 1	49.4	+2.9	+20.6
Blend 1	45.3	+6.6	+18.6
Blend 2	35.0	+2.7	+15.4
Control 2	63.5	+4.0	+ 6.3
Blend 3	41.0	+4.9	+ 7.6
Blend 4	46.5	+3.7	+ 7.9
Blend 5	46.3	+1.2	+ 7.2

[1] A white standard plate (L = 94.5, a = —1, b = +3) was used for the calibration. The gel, 5 mm thick, was measured against a white background

Table 5. The effect of thermal processing on the *in vitro* protein apparent digestibility and C-PER of the various gels

	Ingredients		Product		Changes Due to Thermal Processing	
Gel Blends (1)	Digestibility (%)	C-PER	Digestibility (%)	C-PER	Digestibility	C-PER
Control gel 2	81.22	0.12	88.81	0.92	+7.6	+0.80
Blend 1	83.56	2.38	87.90	2.41	+4.3	+0.03
Blend 2	85.55	2.35	89.53	2.39	+4.0	+0.04
Blend 3	84.83	2.28	90.08	2.41	+5.3	+0.13
Blend 5	84.79	1.97	89.71	2.15	+4.9	+0.18

gels both before and after heating. The results indicate that heat enhanced the protein digestibility of all gels, especially the control gel, which was increased by approximately 9%. The increase in protein digestibility was probably due to the denaturation of soy protein and the destruction of inherent proteolytic enzyme inhibitors. Because the soy isolate was obtained using mild processing conditions, it may still have active enzyme inhibitors (Shemer 1976).

The C-PER of the control gel 2 was 0.92. After blending with blood protein isolate the PER of blended gels was increased above that of the control gel (Table 5). When comparing the C-PER of heated and unheated gels, it was found that heat processing improved the C-PER of all gels. The increased C-PER of heated gels was possibly due to the enhanced protein digestibilities.

Amino Acid Losses During Thermal Processing

Table 6 lists the essential amino acid compositions of four gel

Table 6. The effect of thermal processing on essential amino acid composition

Amino Acid	Ingredients Profile (g/100g Protein)	Product Profile (g/100g Protein)	Change (%)
	Control Gel 2		
Lysine	6.32	6.14	— 2.8
M + C	1.45	1.38	— 4.8
Threon	2.00	2.08	+ 4.0
Isoleu	4.46	4.34	— 2.7
Leu	7.44	7.51	+ 0.9
Valine	3.74	3.43	— 8.3
P + T	8.14	8.59	+ 5.5
Tryptophan	0.38	0.40	+ 5.3
	Blend 1		
Lysine	6.46	6.21	— 3.9
M + C	3.82	3.04	—20.4
Threon	3.32	3.50	+ 5.4
Isoleu	3.96	3.97	+ 0.3
Leu	7.74	7.98	+ 3.1
Valine	4.62	4.55	+ 0.2
P + T	8.40	8.64	+ 2.9
Tryptophan	0.87	0.74	—10.3
	Blend 2		
Lysine	6.25	6.31	+ 0.9
M + C	3.86	3.46	—10.3
Threon	3.19	3.04	— 4.7
Isoleu	4.05	4.01	— 0.9
Leu	7.78	7.86	+ 1.0
Valine	4.68	4.66	— 0.4
P + T	8.42	8.48	+ 0.7
Tryptophan	0.77	0.75	— 2.5
	Blend 3		
Lysine	6.81	6.71	— 1.5
M + C	3.48	3.51	+ 0.9
Threon	3.25	3.29	+ 1.2
Isoleu	3.94	3.95	+ 0.3
Leu	7.82	7.71	— 1.4
Valine	4.63	4.55	— 1.7
P + T	8.57	8.29	— 3.3
Tryptophan	0.72	0.70	— 2.8
	Blend 5		
Lysine	6.62	6.70	+ 1.2
M + C	2.97	2.75	— 7.4
Threon	2.74	2.88	+ 5.1
Isoleu	4.34	4.36	+ 0.4
Leu	7.82	7.87	+ 0.6
Valine	4.54	4.70	+ 3.5
P + T	8.66	8.58	— 0.9
Tryptophan	0.60	0.64	+ 6.6

samples, both heated and unheated. The essential amino acids of the control gel 2 are also listed for comparative purposes. The only amino acids that showed significant losses during heating were the sulfur amino acids present in blend gels 1, 2, and 5. Since blend gels 1 and 2 contained partially delactosed whey and wheat protein concentrate, respectively, the carbohydrates in these proteins may have caused the loss of the sulfur amino acids. Blend gel 3 contained only the soy isolate and blood plasma protein isolate. Both of these protein sources had a low carbohydrate content and these gels showed essentially no loss of essential amino acids upon heating. Control gel 1 and blend gel 4 were not analyzed because the composition of the ingredients in control gel 1 was identical to that of the control gel 2, and the composition of the ingredients in blend gel 4 was identical to that of the blend gel 1.

CONCLUSION

Soy isolate is a potential base ingredient for gel-type protein food. However, the deficiency in sulfur amino acids in a soy isolate is the prime factor responsible for its protein quality. This study on soy gel blending demonstrated that the nutritional quality of soy gels could be improved by blending the soy isolate base with other protein sources such as blood plasma protein isolate, wheat protein concentrate, partially delactosed whey, and casein. The C-PER of soy gel increased from 0.9 (control gel) to above 2.00 after blending. All blended gels were self-supporting gels. Gels with a 15% solids base had gel breaking forces stronger than that of the control gel. Those with a 25% solid base were weaker than the control gel. The color of the blended gels was darker than that of the control gels as a result of incorporating blood plasma protein isolate in the blend gels. However, the color was improved by blending the gels with some partially delactosed whey and/or casein.

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INFLUENCE OF WATER ACTIVITY ON PELLICLE CHAFFING, COLOR AND BREAKAGE OF WALNUT (*JUGLANS REGIA*) KERNELS

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ABSTRACT

The moisture sorption characteristics of walnut kernel at 27 and 37°C and that of the pellicle at 27°C have been determined. The equilibrium moisture content of the pellicle at water activities studied was much higher than that of the kernel, however, its water activity at the BET monolayer moisture level is close to that of the kernel. Storage studies of the kernels equilibrated to different relative humidities (RH) indicate that the kernel color and flavor, as indicated by TBA value, are more stable at a moisture content of 3.2 to 3.6% rather than at the monolayer level of 2.2%. Vibration and drop tests on kernels equilibrated to different RH's indicate that pellicle chaffing and kernel breakage decrease with water activity of the kernel particularly above 58% RH.

Pellicle chaffing which occurs mostly during handling and transportation of the kernels can be minimized by maintaining the kernels around 58% RH during handling and transportation. Subsequently the moisture content can be brought down to 3.2 to 3.6%, which is the optimum range for storage of kernels.

INTRODUCTION

Marketing of walnut kernels is governed by statutory regulations based on their color, extent of chaffing and breakage in the USA (USDA 1962) and India (Agmark, India 1972). Each of these parameters has considerable influence on the market value of the kernels.

Among these parameters color of the kernel is perhaps the one most susceptible to deterioration. As such, there have been a number of studies aimed at minimizing the post-harvest darkening of the kernel color (Sorber and Kimball 1950; Sibbett *et al.* 1974; Martin *et al.*

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1975). Rockland (1957) observed that the moisture content is one of the factors which influences the kernel color during storage. He found a moisture level of 3.5 to 3.8% to be optimum for storage of kernels, and any deviation on either side of this moisture range made the kernels increasingly prone to discoloration and rancidity.

Pellicle chaffing and kernel breakage mostly take place during handling and transportation of the kernels. It is known that these factors, besides imparting unsightly appearance to the kernels, also renders them more susceptible to autoxidation by exposing fresh surfaces of the kernel meat. Little is known about the factors or conditions which influence pellicle chaffing and kernel breakage. In view of the vital role of these factors in commercial marketing and storage of kernels, a study of the conditions which influence pellicle chaffing and kernel breakage was considered important. In this paper the moisture sorption characteristics of the kernel, BET monomolecular moisture level and the optimum water activity of the kernel with respect to kernel color, Pellicle chaffing and kernel breakage are presented.

MATERIALS AND METHODS

Walnut Kernels

Medium shell walnuts from seedling trees were procured from Kashmir valley. The nuts were hand cracked and the kernel halves of the "Extra light" and "Light" grades (USDA 1967) were used in the studies.

Equilibration of the Kernels to Different Humidities

Walnut kernel halves were divided into 8 lots and each lot containing about 1000 g was placed in a vacuum desiccator over a saturated salt solution of known relative humidity (RH). To facilitate equilibration, the desiccator was evacuated and left at 26 ± 1°C. Equilibration of the kernels to the desired RH was checked periodically by weighing a test sample kept separately in a petri dish inside the desiccator. The desiccator was re-evacuated each time the sample was removed for test weighing. Equilibration was considered complete when the weight gain or loss by the test sample was not more than 0.1%, over a 24-hr period. The equilibration of samples was complete in 5 days at 32 to 75% RH and in 10 days at 11% RH.

Vibration Test

The kernels equilibrated at each RH were filled (250 g each) into 3

one-liter cylindrical lever-lid tin containers and the lids were closed tightly. The tins were fastened vertically on the table of the vibration tester and subjected to a circular simple harmonic vibration in the vertical plane at 7 Hzs and 5 mm amplitude giving a peak acceleration of nearly 1 × g for 15 min. This extent of vibration was equivalent to about 600 km rail transport under Indian conditions (Veerraju, unpublished data). After the test the contents of each container were emptied into a tray and the chaff on each kernel was carefully brushed, collected and weighed. Percent chaff was defined as total chaff × 100 ÷ 250.

Drop Test

After the vibration test, unbroken kernels from each lot were reconditioned to their respective relative humidities as described earlier. The cylindrical tin container was filled with 250 g of the equilibrated kernels (two containers for each RH) and the lid was closed tightly. Each of these containers was dropped from a height of 915 mm onto a concrete floor once each on the bottom and on the side (along the axis of the cylindrical can). After the drop, test kernels from each tin were emptied into a tray and those broken were separated and weighed. Percent breakage was defined as weight of brokens × 100 ÷ 250.

Determination of the Pellicle Content in the Chaff

The chaff obtained during the vibration test consisted of the pellicle as well as the abbraded kernel meat. Hence, to determine the quantity of pellicle lost by the kernel during the vibration test, the following procedure was used. The chaff (C) obtained during the vibration test was extracted with petroleum ether (40—60) for 6 hr in a soxhlet extractor. The ether was driven off using a water bath, the extract dried for 6 hr at 60°C at 100 mm Hg pressure and weighed and reported as total lipids. The pellicle chaffed off from the kernel was computed as follows.

$$\text{Meat chaffed off from the kernel along with the pellicle (M)} = \frac{\text{Total lipids extracted from chaff (C)}}{\text{64 (the average percent lipids in the kernel meat)}} \times 100$$

$$\text{Pellicle chaffed off from the kernels} = P = (C - M)$$

The percentage of pellicle chaffed out of the total pellicle covering the kernel was calculated by taking the total pellicle content of the kernel as 5% (Prabhakar 1977).

$$\text{Percent pellicle chaffed off} = \frac{P}{\text{weight of kernels (250 g)}} \times 100 \times \frac{100}{5}$$

Attempts to compute the pellicle content of the chaff by estimating polyphenols, the main constituents of the pellicle, gave erratic results probably due to complexing of the polyphenols with constituents like proteins in the chaff.

Equilibrium Moisture Content (EMC) at Different Humidities

The moisture content at different humidities was determined for the kernel, kernel meat and pellicle at 27°C and for the kernel and the pellicle at 37°C using the Wink's weight gain/loss method, over saturated salt solutions (Rockland 1960). The moisture content in the samples was estimated by drying at 100°C at 100 mm Hg pressure according to the methods of the AOAC (1970). All the moisture values reported in the paper are on dry weight basis.

Kernel Color

The USDA walnut color chart (1967) was used to grade the kernels into 4 classes consisting of (1) Extra light, (2) Light, (3) Light amber, and (4) Amber. These color classes were not adequate however, for expressing the observed color differences in different experimental lots. Hence the USDA color scale was expanded to include an additional class viz super extra light which is lighter than extra light. For color estimation 100 kernels were used in each sample and each kernel was compared against the USDA walnut color chart for recording the kernel color.

Thiobarbituric Acid (TBA) Value

For determining the TBA value the steam distillation procedure of Sidwell *et al.* (1955) was adopted using a 5 g sample. The color development with the TBA reagent was carried out according to the procedure described by Holland (1975). The TBA value as μg malonaldehyde was read off from a plot of the absorbance versus μg malonaldehyde obtained with a working standard of 1-1-3-3-tetra ethoxy propane.

RESULTS AND DISCUSSION

Sorption Characteristics and the BET Monomolecular Moisture Content

The moisture sorption isotherms of walnut kernel at 27 and 37°C and of kernel meat (without pellicle) at 27°C are shown in Fig. 1. The moisture sorption characteristics of the pellicle at 27°C is presented in Fig. 2. It is seen from Fig. 1 that the EMC of the kernel with pellicle (Curve A) is higher than that of the kernel meat (without pellicle, curve C). The EMC of the pellicle (Fig. 2) was much higher than that of the kernel (Fig. 1) at any RH. This might be expected since the pellicle contains a large fraction of hydrophilic constituents such as polyphenols, unlike the kernel meat which contains an average of 64% of hydrophobic lipids.

The moisture sorption isotherms reveal that ERH of the kernel changes significantly for small changes in kernel moisture between water activities of approximately 0.2 to 0.6. The moisture content of the kernel at 37°C as expected was lower than at 27°C (Fig. 1 curves A and B).

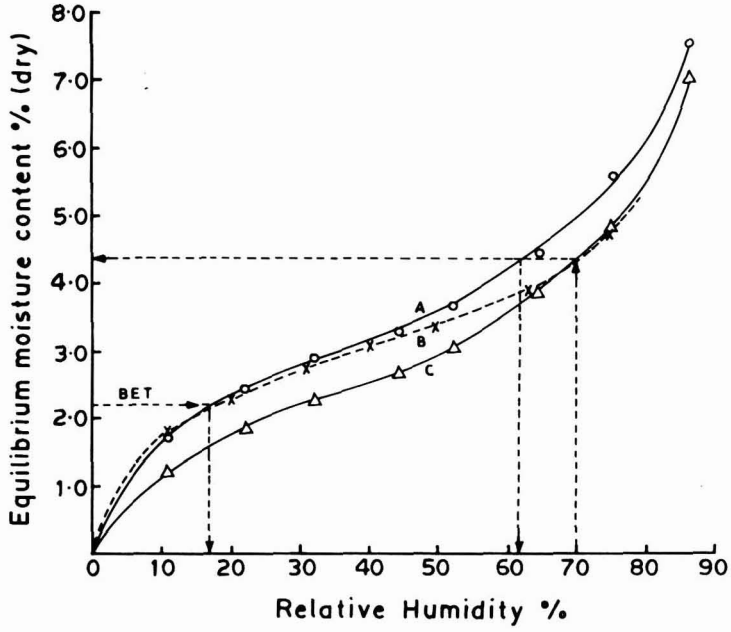

FIG. 1. MOISTURE-SORPTION ISOTHERM FOR WALNUT KERNEL

A. Kernel (with pellicle) 27°C
B. Kernel (with pellicle) 37°C
C. Kernel meat (without pellicle) 27°C

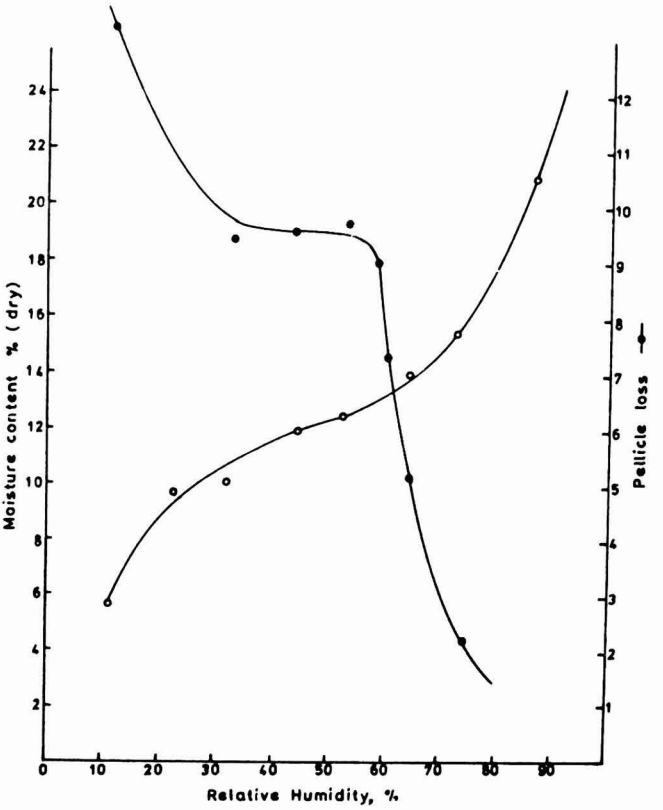

FIG. 2. MOISTURE SORPTION ISOTHERM FOR PELLICLE AT 27°C

When the kernels are stored in a closed container under fluctuating ambient temperatures this factor of variation of ERH of the kernel with temperature assumes importance. This is so since kernels with a moisture content for example of 4.3% equilibrate to a RH of 62% at 27°C but at 37°C they equilibrate to an RH of 70%, noted as critical moisture content in Fig. 1. This is the ERH at which they are susceptible to microbiological spoilage.

BET Monomolecular Moisture Content of Walnut Kernel

The monomolecular moisture content for walnut kernels computed from the BET moisture sorption isotherm is 2.2% at 27°C. The moisture sorption characteristics of the pellicle differs from that of the kernel meat (Fig. 2). The BET monolayer moisture content for the pellicle was calculated at 9.5% which however equilibrates to an RH of

22%, that is, close to the monolayer water activity of the kernel. Therefore, walnut kernels with a moisture content of 2.2% or with a water activity close to 20% RH might be expected to have the longest shelf life (Salwin 1962).

Effect of Water Activity on the Commercial Color Grade and TBA Values

Water activity had noticeable influence on the color of kernels (Table 1). During a storage period of 200 days the kernels stored between 40% and 49% RH retained the maximum percentage of SEL + EL grades. At lower relative humidities of 11% and 20% the kernels acquired a pinkish tinge, although the later RH is nearer to the BET monolayer moisture level. The pinkish tinge acquired by the kernels at the lower humidities confirms the observation of Rockland (1957) that the discoloration in walnut kernels during storage is of two types, one occurring at low moisture levels being different from the discoloration that takes place at higher moisture levels of the kernel. After a long period of storage (400 days) difference in color grades of the samples with different water activities was much less.

The TBA values which is an index of flavor in walnut kernels, (Prabhakar 1977) of kernels at 52% RH were slightly lower than those at 11 and 32% RH's (Table 2). At 63% RH the TBA values tended to be low. However, this RH cannot be considered as safe for storage of kernels since it is higher than the critical RH of 62%. Also, at 63% RH, retention of superior color grades was not as good at a lower RH of 52%.

This indicated that for preservation of the color of the kernel at its

Table 1. Influence of water activity on commercial color grade of walnut kernels at 27°C

	Color Grade[a]					
	SEL	EL		L	LA	
	Days					
% ERH	200	200	400	200	400	400
11[b]	Nil	32	26	68	45	29
20[b]	Nil	50	25	50	43	32
32	16	32	35	52	26	39
40	42	24	28	34	42	30
49	42	32	28	26	48	24
63	18	25	37	57	35	28

[a]Each sample consisted of 100 kernels. The kernels were individually graded using the USDA walnut color chart (USDA 1967). The values are expressed as a percentage of the total. SEL: Super Extra Light. EL: Extra Light. L: Light. LA: Light Amber. Initial color grade of kernels: 100% SEL.

[b]All grades of kernels at these water activities had a pinkish tinge

Table 2. Influence of water activity on the TBA value of walnut kernels at 27°C

Water Activity as % RH	Storage Period (days)				
	30[a]	60	120	270	365
	TBA values as μg malonaldehyde/100 g kernel				
11	700	1050	1100	1350[c]	1600
32	690	800	850	1000[d]	1600[c]
52	650	650	750	1000[d]	1300[c]
63	420	800	800	850[e]	650[e]
75	350[b]	900			

[a]Initial TBA value, 190 μg malonaldehyde/100 g kernel; the values are averages of duplicates
[b]Visible fungus growth
[c]Highly rancid
[d]Slightly stale
[e]Musty odor, no rancid smell

best as well as retarding development of rancidity, a water activity higher than that corresponding to the BET monolayer moisture content was necessary. Therefore from considerations of retarding deterioration of color and flavor of the kernels during storage an RH of 40 to 50% corresponding to a moisture content of 3.2 to 3.6% appears to be most desirable. This is in general agreement with the results of Swarthout *et al.* (1958), who suggested an optimum range of 3.0 to 3.5% moisture for maximum stability of kernels.

Pellicle Chaffing and Kernel Breakage in Relation to Moisture Content of the Kernels

In kernels subjected to drop and vibration tests, chaffing of the pellicle and breakage of the kernel were maximum at low humidities as is evident from Fig. 3. At the relative humidity corresponding to the BET monomolecular moisture content chaffing, pellicle loss and kernel breakage were 2.3%, 12.0% and 41% respectively as against 0.8%, 6.0% and 26% at 62% RH. It was observed during these trials that in the walnut kernels equilibrated to 32% or lower RH's the pellicle got loosened from the kernel meat easily. In such instances re-equilibration of the kernels to higher RH's did not restore the native state of the adherence of the pellicle to the kernel meat. Pellicle chaffing and kernel breakage decreased steeply above 58% and 62% RH respectively. This indicated that maintaining the ERH of the kernel as high as possible is beneficial for minimizing pellicle chaffing and breakage.

From the foregoing discussions, the desirable water activity for preserving each of the quality attributes of the walnut kernels may be summed up as follows:

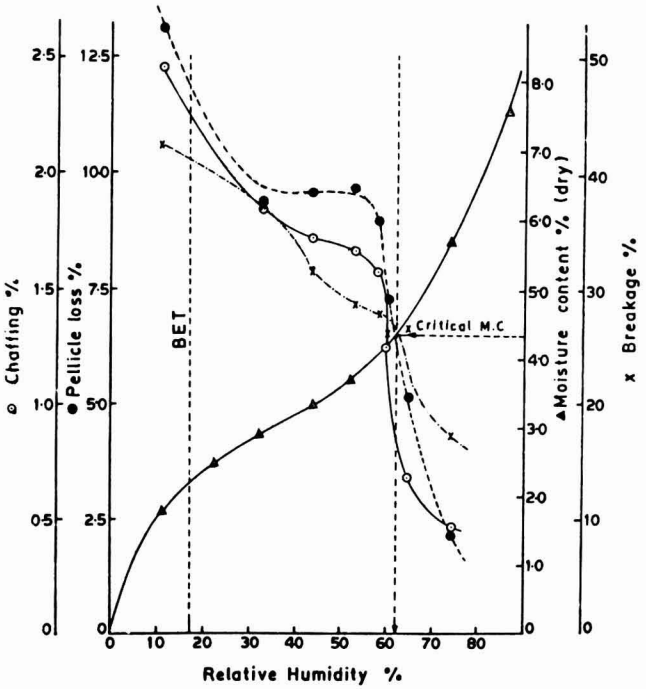

FIG. 3. EFFECT OF MOISTURE CONTENT OF THE KERNELS ON PELLICLE CHAFFING AND KERNEL BREAKAGE

Chaff includes pellicle and kernel meat fines separated from the kernel.

Quality Attribute	Optimum Limit of Water Activity (% RH)	Kernel Moisture %
Guarding against humidity fluctuations during storage due to changes in ambient temperature	62	4.3
Color retention	40—50	3.2—3.6
Retarding onset of rancidity	40—50	3.2—3.6
Significant lowering of chaffing and pellicle loss	58	4.1
Significant lowering of kernel breakage	62	4.3

This data shows that the low breakage of kernels at high relative humidities cannot be taken advantage of fully since significant lowering

in breakage of kernels occurs only beyond the critical RH of 62%. Pellicle chaffing declines steeply beyond a RH of 58%. It is important to take advantage of this factor, since pellicle chaffing, besides contributing to the color and appearance of the kernel functions as a protective coating against autoxidation. This can be accomplished by maintaining the kernel at high water activities (above 58% RH) only during the short periods of bulk handling and transportation of the kernels. Later, prior to unit packing the moisture content of the kernels can be brought down to the desirable 3.5% level. In this way increased protection to the kernels against pellicle chaffing during transportation as well as against color darkening and rancidity during storage can be accomplished.

These studies bring to the fore the inadequacy of maintaining the moisture content nearer to the monolayer level for providing adequate protection to walnut kernel, since at this moisture level the desirable characteristics of kernel color, absence of rancidity and minimum of pellicle chaffing are not retained at their best.

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ENGINEERING A PLANT FOR ENZYMATIC PRODUCTION OF SUPPLEMENTAL FISH PROTEINS[1]

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INTRODUCTION

Over the past decade, there has been much discussion regarding the need for developing new sources of protein from the sea. Actually, raw materials of considerable magnitude that can be utilized much sooner than the new resources exist in the present world fisheries. Effective utilization of (1) discarded by-catches from specific fisheries, (2) industrial fish that can be upgraded to products of higher value than fish meal, and (3) waste currently available from processing of edible fish will have an immediate impact on world animal protein supplies. Hence, more emphasis should be placed on "total utilization" of present resources (Pigott 1974, 1976).

The presently discarded by-catch is probably a significant percentage of the world catch. In fact, it is estimated that the shrimp fisheries alone will account for 10—20 million tons of this category by 1985. Of the 70 million metric ton annual world catch, between 30 and 40% is used directly for production of animal feed (FAO 1974). Much of this is from species too small, too boney, or too oily for direct human use, and is reduced to fish meal (Peterson *et al.* 1974). Fish meal is a good animal food supplement and will continue to be an important use of

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fish protein; however, it is not suitable for direct human use due to fishy odor and taste, unhygienic production, high oil content, and reduced nutritional value due to processing techniques (Dreosti 1974; Myklestad 1974). Therefore, industrial fish must be processed by methods different from conventional fish meal production if it is to be upgraded for human food.

Of the remainder of the world catch, which is processed in one way or another for human food, up to 65% becomes a by-product or waste, resulting in serious local pollution or, at best, is reduced to low grade animal feed (Pigott 1974). A salmon or cod fillet line, for example, uses no more than 35% of the whole fish (Perkins 1975; Finch 1970). Canning lines are more efficient, utilizing up to 80% of the whole fish (Peterson *et al.* 1974). Ironically, the proximate analysis of this waste portion (viscera, head, filleted frame, etc.) is very similar to the edible portion. If handled in a sanitary manner, it is equally nutritious to the portion being marketed.

Another area of waste not normally considered is the large amount of fish solubles lost due to inefficient handling of raw materials or plant operations. For example, yield of fish meal from the industrial fisheries as compared to raw material input indicates that several percent of the fish is lost as waste solubles. Although no adequate survey has ever been made of the protein lost through unnecessary solubles loss, it is known that large amounts of the important nutrients are lost in this manner.

Major sources of both edible fish flesh and supplemental protein for formulated food products are available in the categories discussed above, and new research efforts should be vigorously pursued to recover these ingredients so badly needed by much of the world.

There are three basic categories that require concentrated effort to meet the goals of increasing the yield from presently available fish resources: (1) Using presently available machinery and procedures for removing fresh flesh from processed carcasses or from acceptable industrial or by-catch fish. Accelerated work is necessary to ensure that formulated products are prepared that will be acceptable to the concerned markets. (2) Improving or developing systems of shipboard preprocessing, pick-up and transfer of fish on the high seas, stabilization of fish, both on shipboard and on shore, and improving raw material transportation. These development programs will result in large amounts of presently unavailable fish for direct consumption and for processing into fish meal, extracted supplemental protein products, and deboned fish flesh.

(3) Re-emphasizing development of techniques for extracting high-quality supplemental fish proteins for both animal and human food. There are good techniques for producing these products, but refine-

ments must be made, along with development of the engineering technology necessary to assure success on commercial scales. In fact, the scientific community must realize that the final practical solution to implementation of new ideas resulting in economic commercial processes is completely dependent on the application of basic engineering principles. The downfall of many research projects has been due to a failure to develop practical applied technology along with the laboratory program.

For several years, researchers at the Institute for Food Science and Technology at the University of Washington have been investigating methods of utilizing both marine food processing wastes and fish stocks normally not used for human consumption. The primary goal of this research has been to produce products of high nutritional value while, at the same time, minimizing pollution through "total utilization" of raw materials. The purpose of this paper is to report the final phase of a project that culminated in a pilot plant for investigating the commercial feasibility of a pepsin digestion process that yielded a high quality supplemental fish protein material under laboratory conditions. Final testing of the product included formulation and sensory testing, as well as chemical and biological evaluations.

PILOT PLANT DEVELOPMENT

The background work for design and construction of the pilot plant included not only evaluation of basic unit processes and operations necessary for product manufacture, but many of the support areas associated with raw material and final product handling, storage, and distribution. The basic pepsin digestion technique was reported by Tarky (1971, 1973) and Heggelund (1975). The procedure for preparing a soluble, dried hydrolysate consists of deboning, enzyme hydrolysis, centrifuging to remove sludge and oil, ion exchange to neutralize and remove certain by-products of the digestion, and spray drying. Laboratory-prepared samples of the supplemental fish protein prepared from English sole fillet waste were soluble in water, had no objectionable taste or odor, and had Protein Efficiency Ratios (PER) as high as 3.35, compared to 2.63 for the casein reference protein.

Raw Materials

A wide variety of raw materials was processed in the pilot plant. These included shrimp, bottom fish, herring, and Pacific hake (*Merluccius productos*) ranging from low to high lipid content and from fresh

caught to several months in cold storage.

Commercial 1:10,000 N.F. pepsin was obtained from American Laboratories, Inc. and Sigma Chemical Company. Crude pepsin preparations were made from whole hog stomachs obtained from the Cudahy Food Products slaughterhouse, Seattle, Washington, and from whole cow fourth stomach (abomasum) obtained from the Hygrade Corp. slaughterhouse, Tacoma, Washington.

Analyses

Standard methods of analysis for proximate analyses, heavy metals, amino acids, microbial count, Protein Efficiency Ratio, and pepsin activity were utilized in the work. Detailed discussion on materials and procedures have been discussed by Bucove (1976).

Pilot Plant

General Description. Construction of the pilot plant, and especially the enzyme reactor, was based on information in the literature review and on experience gained during the laboratory work mentioned above. A homogenizer was chosen that allowed variable degrees of mixing. By replacing a cutting bar ring with several optional sizes,and by recycling, the degree of homogenization could be varied almost infinitely. A batch type, mixed tank reactor was built, with ability to convert to a continuous stirred tank reactor (CSTR) provided. The stirred tank configuration was chosen over the tube or plug flow type reactor (PFR) for a number of reasons — in spite of the literature review findings that a PFR would likely be more efficient. A PFR could only be properly designed with a good reaction kinetic rate model, which was lacking. The reactants in this case have to be well mixed prior to the reaction, which favors a STR type reactor. The mixture becomes very viscous, almost jelly-like, upon acidification, and handling of the mixture would be difficult in a PFR. Since mass transfer is important in this reaction, a stirred reactor is required. The further the reaction proceeds, the more liquified the mixture becomes, so that in spite of the product concentration being higher, the mass transfer rates would also be higher, also favoring a STR. Finally, stirred tank reactors are much more common, require less sophisticated engineering and less technical upkeep, and therefore are more adaptable to the fish processing industry. The choice of a batch operation was further influenced by the simplicity of this mode compared to a continuous operation, as well as the fact that most of the fish processing industry is already operating on a batch basis. Much of the raw material for this process would only be available in batches. The most probable immediate possibility for a continuous

operation of this process would be in conjunction with a fish meal plant. In this case, a portion of the incoming raw product could be separated prior to the continuous cooker and diverted to the enzyme plant. The sludge and deboner waste could be added back into the meal operation.

To properly provide mixing in the reactor throughout the reaction was a problem. The fish flesh-water mixture falls by gravity into the reactor. Acid is added in the reactor, and its distribution requires mixing to establish a uniform pH of 2. However, at this point the mixture becomes quite viscous, and a greater amount of mixing effort is required. Later in the reaction, when the mixture is much more liquid, less mixing effort is required, in fact demanded, to not damage the pepsin and to minimize formation of emulsions. To accommodate the variable mixing effort required, the reactor was designed with a conical bottom so that the slurry could be mixed in two fashions: by pumping out at the bottom and recirculating and by mixing with a paddle. The pump and recirculation plumbing provide the additional amount of mixing and shear needed at the beginning of a batch. After the reaction proceeds for ½ to 1 hr, a paddle alone is sufficient. The paddle mixer was a commercial milk tank stirrer and was adapted to the pilot plant.

The reactor itself was constructed from a stainless steel kettle. The bottoms and legs were removed and a 60° cone welded on the bottom, using a tungsten inert-gas welder. The legs and plumbing outlet were replaced and 50′ of ½″ copper tubing was wound around the circumference of the cone to provide for steam heating. The copper coil was insulated and lagged in with asbestos material, and an additional heating coil was installed on the upper surface. The heating coils were attached to a low pressure steam line in series, with a pressure regulating valve, filters, and traps. Pressure control was provided for 0 lb to 30 lb gauge. A photograph of the reactor appears in Fig. 1.

A positive displacement rotary gear pump was chosen to meet the requirements of digest recycles or transfer. Several centrifuges were tried, including a solid bowl, a disc bowl, and a desludging basket. The entire pilot plant was assembled on a welded framework of 2″ pipe and 5″ steel I beams. A diagram of the pilot plant is shown in Fig. 2. The drying of the final product was accomplished by an Anhydro Laboratory-size spray drying, which can be seen to the left of the pilot plant in the photograph (Fig. 3).

Equipment List

(1) **Grinder.** Garbage grinder type G406, 5 H.P. motor; Gruender Gusher and Pulverizer Co., St. Louis, Missouri. This grinder was in-

FIG. 1. ENZYME REACTOR

stalled in the pilot plant to allow use of frozen raw material, or large pieces of flesh.

(2) **Homogenizer.** Hobart Model MCV-12 Emulsifier, capacity 100 lb/min on coarse adjustment; 12 H.P. motor; 3,500 RPM. Hobart Manufacturing Co., Troy, Ohio.

(3) **Reactor.** Capacity 2.6 ft^3 (19.5 gal.), 16″ diameter × 30″ height, type 304 stainless steel. Fitted with 5.5 ft^2 of steam heat transfer surface, steam trap and fittings, thermometer attachment, and 1-½″ Acme thread stainless sanitary pipe fittings; custom made by author.

(4) **Holding Tank.** Thirty gallon type 304 stainless, fitted with 1-½″ Acme sanitary fittings; United Utensils Co., Inc., Port Washington, N.Y.

(5) **Mixer.** Milk tank mixer, ¼ H.P., 2 speed; General Electric Corp., Penn.

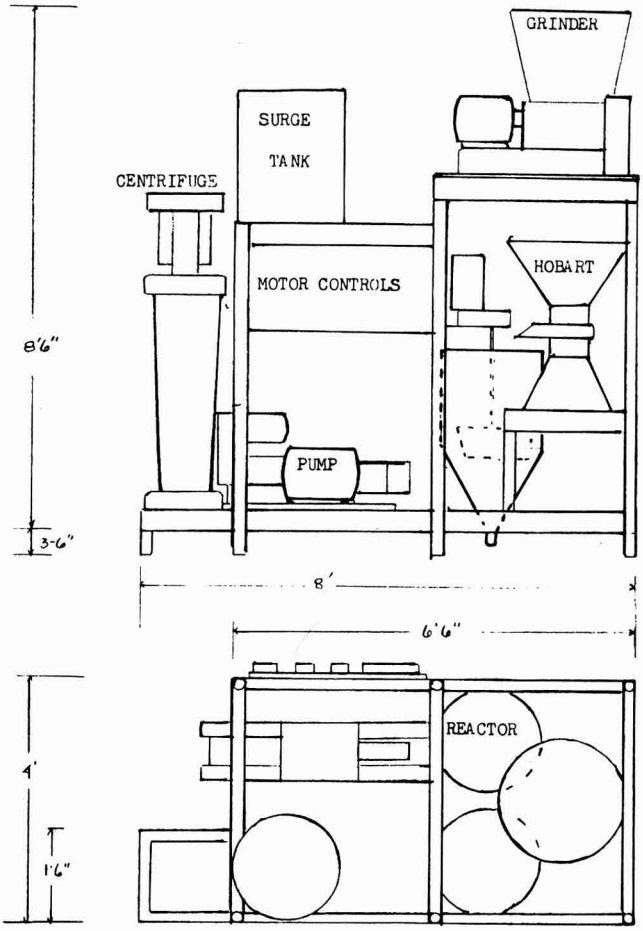

FIG. 2. DIAGRAM OF THE PILOT PLANT

(6) **Pump.** Tri-Clover Model 10 positive displacement rotary pump, with Reeves ½ H.P. geared variable speed drive. Capacity 0 to 12 gal/min. Stainless steel case, rubber impellors, sanitary fittings, and sanitary enclosed motor housing. Ladish Co., Chicago, Illinois.

(7) **Sharples Centrifuge.** Type M312H-33B, solid bowl centrifuge 4″ ID × 36″ bowl, 2 H.P., 15,000 rpm; Pennwalt Corp., Warminster, PA.

(8) **DeLaval Centrifuge.** Gyro-Tester bench top laboratory centrifuge, bowl volume 410 cc, sludge capacity 93 cc, maximum capacity 90 gal/hr, 1/3 H.P., 12,000 rpm.

(9) **Alfa Laval Centrifuge.** Type BRPX 207-105 Industrial separator 8″ bowl, maximum capacity ca. 2,000 gal/hr, 10 H.P., 6,000 rpm; DeLaval Separator Co., Poughkeepsie, N.Y.

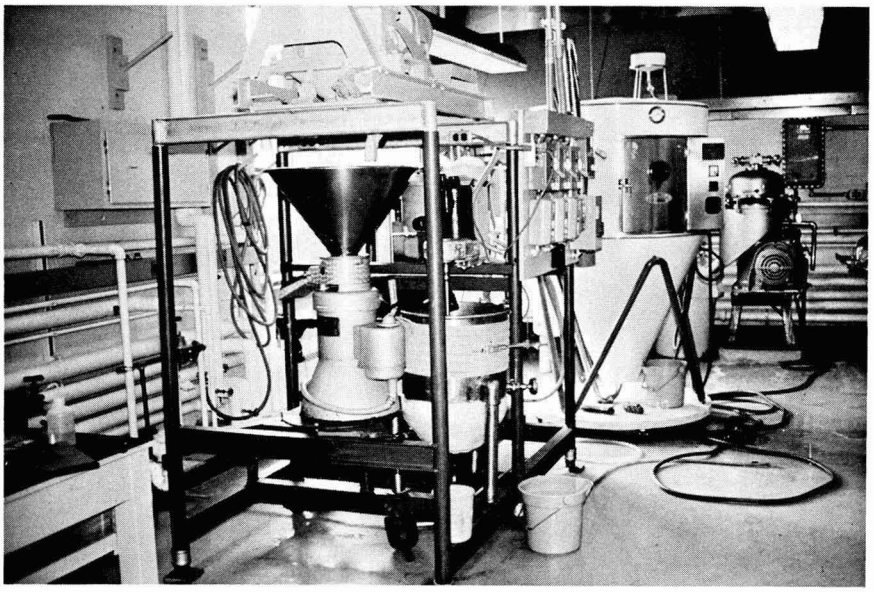

FIG. 3. PILOT PLANT

(10) **Motor Controls.** Each motor was protected by a G.E. General Duty fuse box of the appropriate amperage, and a Westinghouse De-Ion Motor Watchman circuit breaker.

(11) **Plumbing.** Steam lines are ½′ black iron schedule 40 pipe, with a Cash Acme 0—30 lb regulator. Fluid lines are 1-½″ ASB plastic with GSR (Sun Valley, Cal.) ball valves, and type 304 S.S. Acme thread sanitary fittings for the pump, tank, and reactor.

Operational Procedures. Prior to use, all equipment must be cleaned and plumbing fittings in place. The raw material must be deboned and thawed.

(1) Weigh the deboned fish waste (DFW) in containers and add ½ wt/wt water; mix lightly.

(2) Pass the DFW-H_2O through the Hobart on coarse (no cutting ring).

(3) Open the recycle valve (make sure the centrifuge feed valve is closed).

(4) Turn on the pump at maximum speed. Switch the mixer on high.

(5) Turn on the steam (25 psig).

(6) Slowly add concentrated HCl to the slurry (about 10 ml per lb of DFW — not DFW + H_2O). Adjust pH to 2 with continued mixing.

(7) When the pH is uniformly 2, mix the enzyme powder with sufficient water to form a slurry. Add the slurried enzyme in at the return.

(8) When the reacting mixture liquifies sufficiently, cut back the pump speed and put the mixer on slow.

(9) Adjust the steam pressure to maintain 45—50°C (maximum 55°C).

(10) At 2—4 hr of digestion time, the separator can proceed. Bring the Sharples to speed and prime with water. When the centrifuge is primed, immediately connect the slurry flow. Adjust flow to ca. 3/4 gal/min. Run 5—6 gal. through the Sharples, then cut the flow and flush with water. Dismantle and clean the bowl. Repeat the centrifugation until all the slurry is separated.

(11) Neutralize the liquid fraction in stainless steel or plastic containers by addition of the ion exchange resin. Filter to assure no carryover of resin.

(12) Concentrate and spray dry.

Experimental Procedure. Data on the pilot plant operation was obtained by operating the plant in the manner described above and collecting samples at various points throughout the procedure. Zero hour samples were taken and additional samples were removed from the reactor at time intervals for analysis. Samples of the liquid separated by the pilot plant centrifuge were placed in a Sorvall laboratory centrifuge at 10,000 G for 10 min to evaluate the plant separation. Temperature come-up versus time was recorded, as were flow rates and general observations.

RESULTS OF PILOT PLANT OPERATION

Processing

The previously described pilot plant was designed to optimize the procedure developed in the laboratory in order to obtain a complete material balance, information on reaction kinetics, and economic evaluation of the process. In conjunction with obtaining the above information, runs were made to prove the equipment and to provide sufficient final product for proximate analyses, amino acid analyses, nutritional assays, and product formulation. The process flow diagram and material balance for hake are shown in Fig. 4. The proximate analyses of final and intermediate products are presented in Table 1.

Reaction Kinetics

During the operation of the pilot plant, it was discovered that the amount of enzyme could be substantially reduced without significant loss in yield if the operating temperature was increased from 37 to

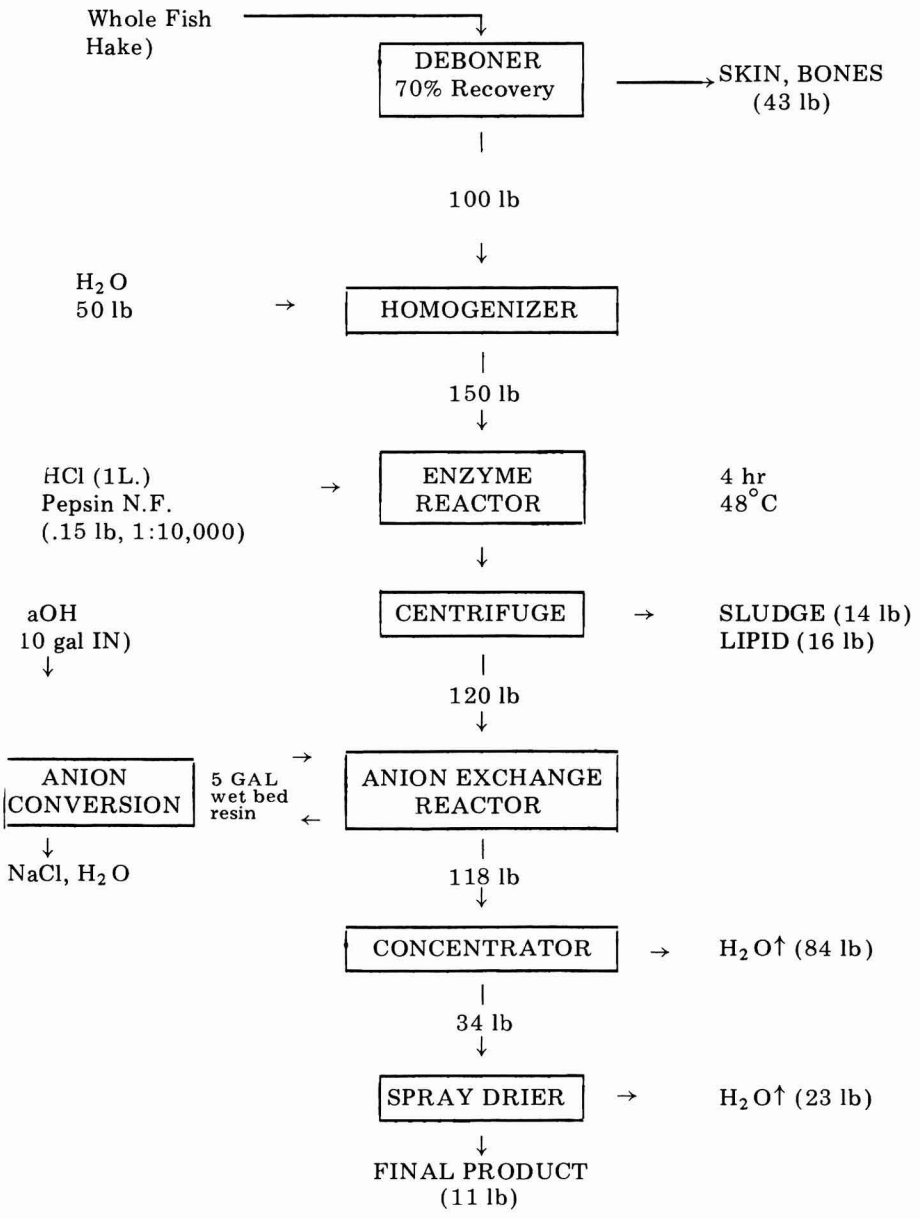

FIG. 4. PILOT PLANT MATERIAL BALANCE AND FLOW CHART
[Basis: 100 lb deboned fish]

Table 1. Material balance and proximate analysis chart[1]

Deboned Fish (Hake)	%	lb
H_2O	78.60	78.60
Protein	13.19	13.19
Fat	7.56	7.56
Ash	0.48	0.48
	99.83	99.83

Deboned Fish and H_2O Slurry	%	lb
H_2O	85.74	128.61
Protein	8.79	13.19
Fat	5.04	7.56
Ash	0.32	0.48
	99.89	149.84

Centrifuge Fractions	Liquid		Lipid		Sludge	
	%	lb	%	lb	%	lb
H_2O	90.95	110.20	56.70	9.09	77.34	10.78
Protein	7.62	9.23	9.37	1.50	14.48	2.02
Fat	0.19	0.23	32.40	5.19	3.62	0.50
Ash	0.21	0.25	1.52	0.24	4.56	0.64
	98.97	119.91	99.99	16.02	98.90	13.94

Concentrated Liquid (at 30% Solids)	%	lb
H_2O	70.00	24.01
Protein	26.91	9.23
Fat	0.26	0.09
Ash	2.83	0.97
	100.00	34.30

Dried H_2O Product	%	lb
H_2O	5.30	0.58
Protein	85.00	9.23
Fat	0.80	0.09
Ash	8.90	0.97
	100.00	10.87

[1] Basis: 100 lb deboned fish

48° C. Previous literature has reported that the optimum reaction temperature of pepsin is 37° C. The higher temperature is being used by commercial chemical companies that are producing enzyme from bovine stomach (Phalen 1976).

The yields of solubilized protein up to four hours of digestion are shown in Fig. 5. The average 68% yield after 4 hr is a conservative figure for that which can be obtained commercially. The centrifuges available for this work were certainly not optimum, and an industrial size disc-bowl centrifuge equipped with intermittent sludge discharge capabilities will increase the yield to perhaps 75% with better solid-liquid separation.

Attempts to develop a rate equation over the entire digestion period

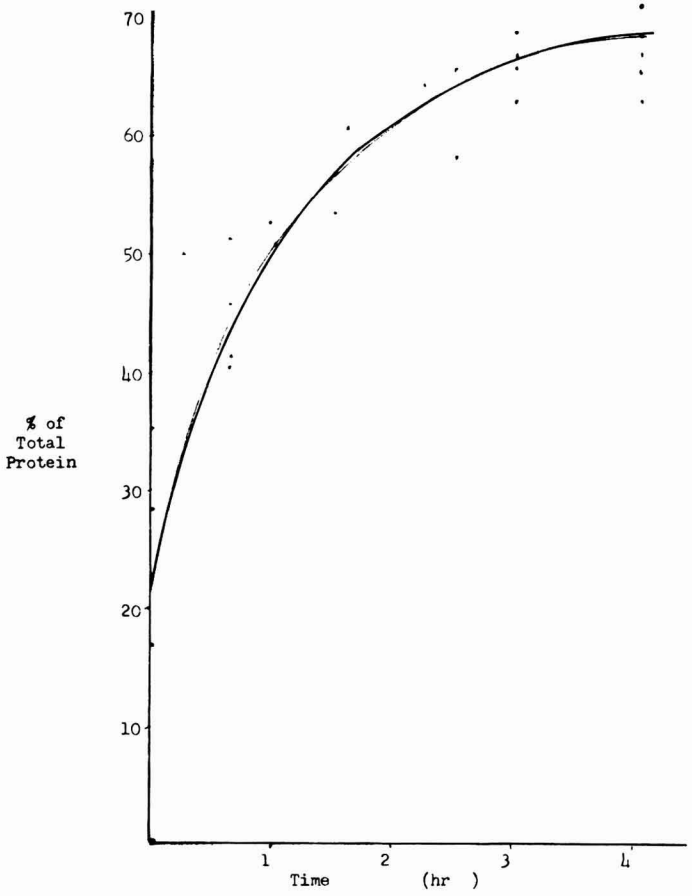

FIG. 5. YIELD-SOLUBILIZED PROTEIN VERSUS TIME (PILOT PLANT DATA)

were not successful. The temperature come-up and the breaking-down of the jelly-like texture caused by initial addition of acid require that the enzyme be added ½ hr after charging the reactor. Therefore, for purposes of developing a rate equation, the curve (Fig. 5) was extrapolated to the protein axis with the same curvature exhibited after the original ½ hr (Fig. 6). This modified curve, while not fitting the initial data as well, still remains within the experimental scatter. Furthermore, this certainly will be the better representation of a commercially designed process where acid would be more rapidly dispersed and the enzyme can be added soon after the acid.

The change of protein solubilization with time (dP/dt) versus time was calculated from Fig. 6. From this modified solubilization rate plot

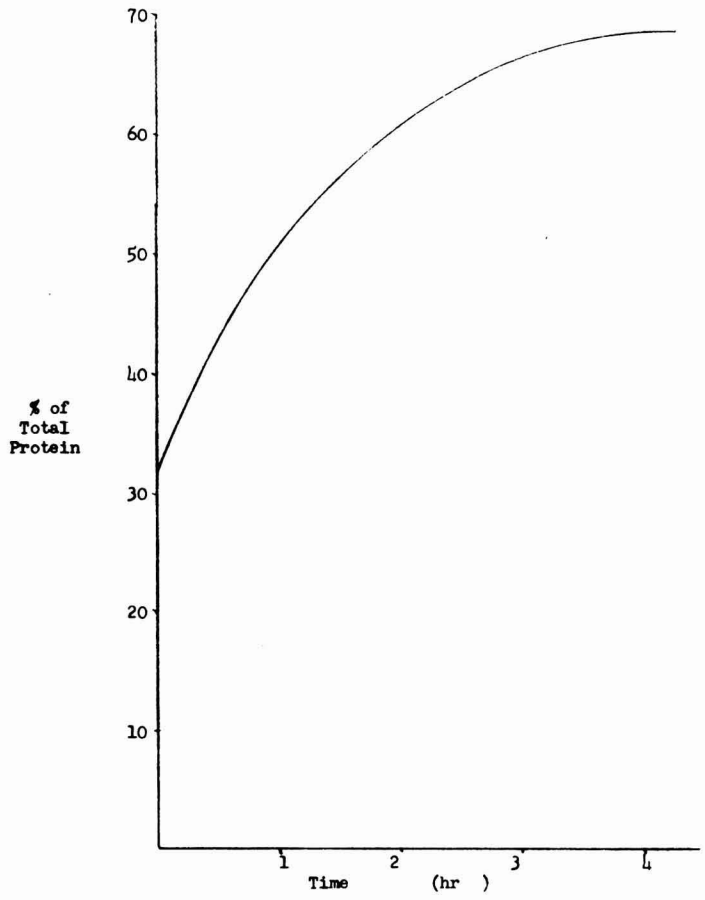

FIG. 6. YIELD-SOLUBILIZED PROTEIN VERSUS TIME (EXTRAPOLATED CURVE)

(Fig. 7), a rate expression was developed as follows:

$$\frac{dP}{dt} = (1 - t/t_i)_a \tag{1}$$

$$\int_{P_o}^{P_i} dp = \int_{t_o}^{t_i} (1 - t/t_i)\, a\, dt \tag{2}$$

$$P = \int a\, dt - \int \frac{at}{t_i}\, dt \tag{3}$$

$$P = at - \frac{1}{2}\frac{at^2}{t_i}\Bigg]_{t_o}^{t_i} \tag{4}$$

$$P = a\left(t - \frac{t^2}{2t_i}\right)\Bigg]_{t_o}^{t_i} \tag{5}$$

From Fig. 6:

$$a = 22$$

$$t_i = 3.4$$

From Fig. 7:

$$P_{total} = P + P_o$$

and $P_o = 31.5$

Therefore:

$$P_T = 22\left(t - \frac{t^2}{(2)(3.4)}\right) + 31.5 \tag{6}$$

$$P_T - 22\left(t - \frac{t^2}{6.8}\right) + 31.5 \leftarrow \tag{7}$$

This rate expression is plotted in Fig. 8 and, with the exception of the first ½ hr, fits the pilot plant curve to within 2%. The accuracy is certainly sufficient for most engineering purposes.

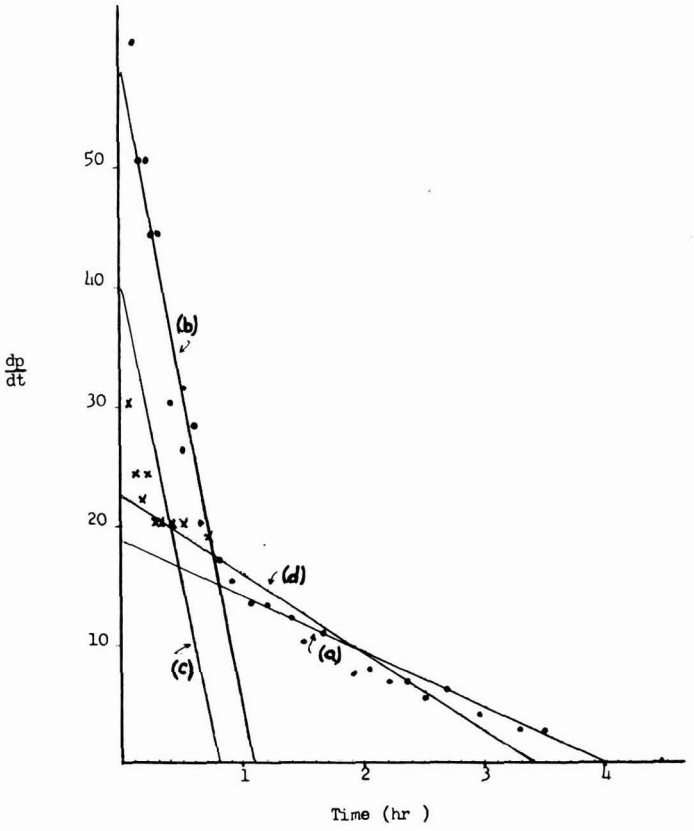

FIG. 7. SOLUBILIZATION RATE VERSUS TIME

SUPPLEMENTAL FISH PROTEIN (SFP) PRODUCT

The product resulting from the pepsin enzyme process described is a white fluffy powder. It has been christened with the name Supplemental Fish Protein (SFP) in order to divorce this product from the FPC (Fish Protein Concentrate) stigma that was attached to the ill-fated product resulting from development of organic solvent extraction techniques.

In contrast to the non-functional granular FPC, SFP is a fluffy, whit water-soluble material. The product has the characteristic bitter taste c hydrolysates containing polypeptides and amino acids; however, th ion-exchanging technique and the short hydrolysis time greatly min mizes this property.

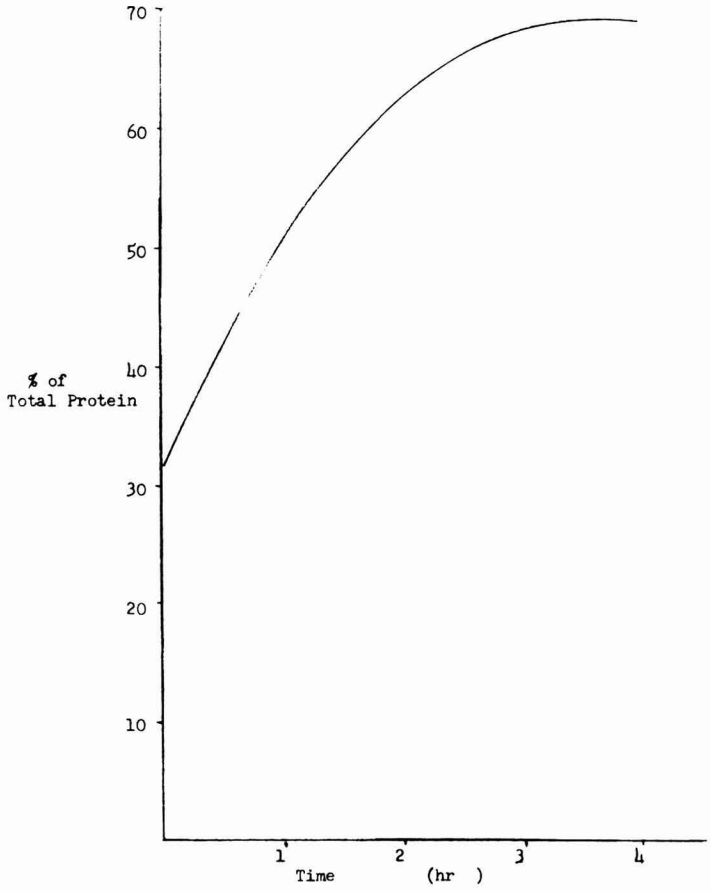

FIG. 8. YIELD-SOLUBILIZED PROTEIN VERSUS TIME MODEL RATE EXPRESSION

A proximate analysis of SFP is presented in Table 2. The material is slightly hydroscopic and varies from 5—8% water. It should be noted that the protein content, based on the total amino acids of 84.6% (Table 3), is almost identical to the 85.0% calculated from the Kjeldahl nitrogen determination.

The high biological value of SFP is shown in Table 4, where the Protein Efficiency Ratio is compared to that of standard reference casein. The identical growth of rats consuming SFP in which there was an addition of 1% (of total amino acids) tryptophan indicated that the common drawback of low tryptophan in enzyme hydrolysates is not a problem with this product.

Table 2. Proximate analysis of enzyme digested hake

Total Amino Acids	84.6%
Kjeldahl Protein	85.0%
Moisture	8.2%
Ash	8.9%
Lipid	0.8%
Lead	<0.01 ppm
Mercury	<0.5 ppm
Fluorides	Negative (AOAC)

Table 3. Amino acid analysis (Compared to reference casein)

	SFP	ANRC Reference Casein
Isoleucine	3.9	5.0
Leucine	7.3	7.5
Lysine	8.5	6.7
Phenylalanine	3.3	4.0
Tyrosine	2.7	5.2
Cystine	1.0	0.3
Methionine	2.8	2.3
Threonine	3.8	4.0
Tryptophane	1.1	1.0
Valine	4.5	5.9
Total Amino Acids	84.7	89.8

Table 4. Protein Efficiency Ratio

PER	FPC-Enzyme Digest	FPC-Enzyme Digest plus Tryptophane	Casein Control
Weight gain/Protein consumed	3.44	3.45	3.00
Percent of Casein Control	1.15	1.15	1.00

Evaluation of SFP for Food Supplementation

There are two potential uses of SFP in food products. It can be incorporated into various staples such as bread or pasta, thereby increasing the amino acid quality, or it can be used to replace in foods the functional roles of more costly proteins. The nutritional value of SFP has been well established. However, it was necessary to further test the

degree of functionality, as well as the consumer acceptance. For this work, 3 different SFP products were prepared, designated as Grades 1, 2 and 3. Grade 1 is the fluffy white material representing the highest quality of product that the pilot plant can produce. Grade 2 SFP, representing a cheaper production cost product, was centrifuged a minimum time period and was slightly off-white and more dense. The Grade 3 material was slightly brown in color, resulting from minimum dwell time during the various processing stages, including concentration of final liquid (before spray drying) at high temperatures. These three products represent the spectrum of qualities that can be found in SFP prepared under the most expensive to the least expensive operating conditions.

The details of procedures and methods for evaluating the various grades of SFP are being reported elsewhere (Ostrander 1976). However, a summary of some test results indicates the potential of this product for food supplementation.

Functional Properties

The functional properties investigated were aeration, or foam formation, and foam stability (both in unheated and heated products), gelation, solubility, emulsifying ability, and stability of emulsions, as shown in Table 5.

Table 5. Functional roles and supplementation of SFP

SFP Grade	Function	Level of Use	Product
1	Foam		
	refrigerated	100%	Mousse
	heated	15, 25%	Angelfood cake
2	Foam		
	heated	5, 15, 30%	Sponge cake
	Supplement	5, 10, 15%	Cornbread
	Replacement for milk	3, 7, 12%	Pudding
3	Foam		
	refrigerated	100%	Mousse
	heated	5, 10, 15%	Spoon Bread
	Emulsion	100%	Mayonnaise

Foam Formation and Stability in Non-Heated Products. Foams were prepared and tested at 4 and −18°C. A trained sensory panel determined that the level of refinement of SFP is of much greater importance than the conditions under which the product was used for foaming properties. The Grade 1 SFP had a much larger cell size upon whipping with significantly more air incorporation, better stability, and

increased volume as compared to the lower grade products. At the higher temperature, the foam had little stability.

Foam Formation and Stability in Heated Product. (A) Angel food cake, using Grade 1 SFP. The highly refined SFP was used to replace 15 and 25% of the egg albumin in angel food cakes. Preliminary work indicated that foam stability and flavor were not satisfactory at the higher level of substitution. The cake formulas are given in Table 5.

Sensory Tests and evaluation indicated that there were no significant differences caused by the SFP on the color or tenderness of the cakes or the specific gravity of the batter. The cakes with 15% SFP were scored as being moister and with a pleasing, delicate odor and taste. As reflected by sand retention and texture scores, the crumb characteristics were finer and with a more uniform cell size in the cakes with the 25% level. The volume of the cakes decreased slightly at the higher level of SFP. This change in volume is also reflected in the increasing specific gravity values. In future studies, a stabilizer could be used to obtain a more stable foam. However, the SFP can definitely be used to partially replace egg albumen in a heated foam product.

(B) Sponge cake, using Grade 2 SFP. Preliminary tests indicated that this SFP can be used to replace 5 and 15% of the egg albumen in sponge cake. The taste and odor of the cooled cake is acceptable. However, the low degree of heat coagulability limits the amount of replacement, since a third test, using 30% SFP as egg albumen replacement, allowed the cake to fall during the final baking stage.

(C) Spoon Bread, using Grade 3 SFP. Another traditional food product based on an egg white foam is spoon bread, a moist, eggy cornmeal bread commonly used in the South. In preliminary tests, the foams produced lacked stability after whipping, indicating that the protein is not mechanically denatured. Nor do the proteins appear to be denatured by heat, and therefore the baked products collapse upon cooling.

It was determined that at 5, 10 and 15%, replacement of egg albumen with the lowest grade SFP results in an acceptable product. Again, as in the case of the higher grade products, the percentage of egg albumen is restricted to these lower levels, due to foam instability.

Emulsification and Emulsification Stability in Mayonnaise. The yolk in a standard mayonnaise recipe was replaced with solutions of SFP. It was determined that Grade 1 SFP could easily be used to completely replace the egg yolk in mayonnaise, thus yielding a product low in cholesterol. It required considerably less SFP than egg yolk to give the desired viscosity and stability.

Supplementation. A standard recipe for cornbread was prepared with SFP replacing 5, 10, and 15% of the flour. Lemon juice and brown sugar were added to mask any bittering or aftertaste. The conclusions

were that supplementation up to at least the 10% level is satisfactory and most probably the Grade 1 product would allow larger addition.

Other tests indicated that SFP can be used in hot cereal products or in wintertime (i.e. whole-wheat pudding) desserts.

ECONOMICS

Figure 9 presents the material costs to produce one ton of the final product (Grade 1 SFP). Listed in the figure are values for both an under-utilized species (hake) and fish waste (cod fillet trimmings). Hake is higher in protein and gives a better deboning recovery, but costs more. Hake is currently being landed north of Seattle for between 1 and 2 cents a lb. More fillet waste is required for the same amount of protein product, but is generally cheaper, varying from a few cents a lb to a negative cent or two to haul it away. One cent a lb is a good average cost figure.

Pepsin is the largest process expense at the current price of $28 per lb for N.F. 1:10,000 grade. The current ongoing research involving pepsin preparations is expected to provide a reduction in this cost. The raw material being used for pepsin production is pig stomach mucosa at

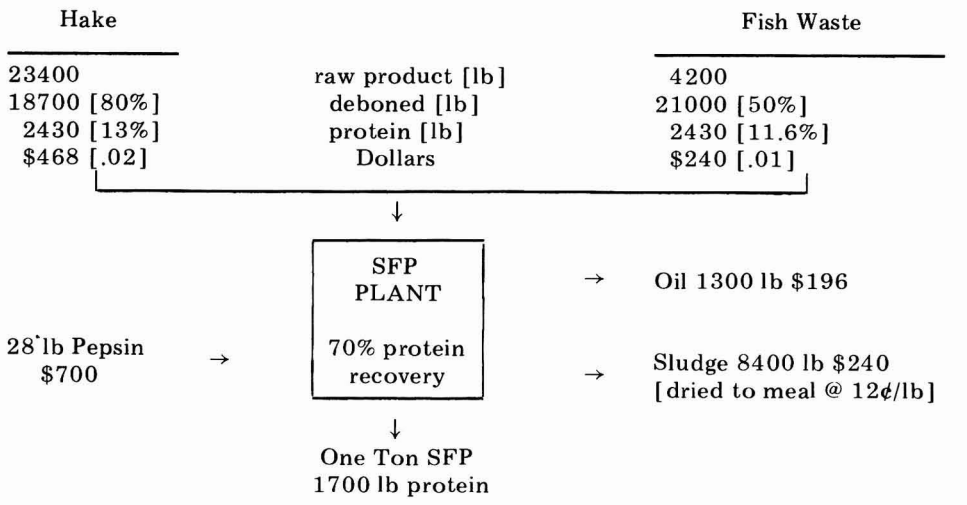

FIG. 9. MATERIAL COSTS

[Basis: 1 ton final product]

about 50 cents per lb. With commercial yields of 4% pepsin, the raw product cost is $12.50 per lb of pepsin. The use of less expensive bovine and ovine material may lower this cost, and development of better processing methods may prevent loss of native enzyme activity and lower processing expenses.

The sludge has between 10 and 15% protein, has a good amino acid array (Heggelund 1975), and is proposed as an animal feed, especially in formulations such as aquaculture fish feed, where insolubility is a positive factor. The lipid fraction can be polished and purified by further centrifugation and the oil sold as a valuable by-product.

On the basis presented here, the final product costs per lb for pepsin are $0.35; for raw material, $0.23; and estimating $0.20—0.25 for processing, it is expected that this product could initially be produced at a cost on the order of $0.80 per lb, with subsequent reduction by decreasing enzyme costs. This compares well with non-fat milk solids selling at over $0.55 per lb at 35% protein, and with casein selling at $1.65 per lb.

CONCLUSIONS

This investigation has shown that high quality supplemental fish protein (SFP) can be commercially produced from whole fish or fish waste, economically and with relatively simple engineering.

Products prepared in the pilot plant under simulated commercial conditions were comparable to those prepared during the small-scale laboratory development. Testing of the products indicated that SFP has excellent emulsification and foam formation qualities. Emulsion stability is also very good. However, foam stability is lacking. Neither mechanical nor heat treatment will induce denaturization, thus requiring a stabilizer to be added to the product. At higher levels of refinement, any bittering or aftertaste of the SFP can be easily masked with substances containing acids, e.g. brown sugar and lemon juice. In heated products, caution should be taken to avoid an undesirable amount of carbonylamine reaction which can result in a product that is too dark in color and with a burned taste. SFP shows highly desirable characteristics for the replacement of other protein in functional roles in food products and for use as a nutritional supplement of low cost.

ACKNOWLEDGMENT

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ington Sea Grant program under contract No. 04-5-158-48. National Oceanic and Atmospheric Administration.

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BULGUR — SHELF LIFE STUDIES[1]

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ABSTRACT

Cereal grains are the major source of both protein and calories in the world. In this regard bulgur is an ancient wheat food of Near Eastern origin. USDA engineers in the Agricultural Research Service have developed it as part of a national effort to increase the use of wheat domestically and in foreign lands under the Foods for Peace Program.

Reportedly, bulgur cereal keeps well, but there are few systemized storage studies available which relate sensory testing with storage life to this product. The aim of this study, sponsored by the Defense Civil Preparedness Agency, was to perform this task using as products a 100% bulgur wheat cereal and an 85% bulgur/15% soy grits, defatted, toasted blend. These were air and nitrogen packaged in 401 × 411 cans, stored for 0, 1, 3, 6, 9, 12, 18 and 24 months at 38°C, withdrawn, and evaluated at these designated times. Studies indicate that these products are very stable, even when stored in air packs. In a presoaked, uncooked bulgur cereal served at 21°C sweetening with a minimum of 6% sugar raised its acceptance significantly to a more desirable level, nearly comparable to its cooked counterpart. Other product data are discussed including a low cost presweetened bulgur wheat product formulation.

INTRODUCTION

At the request of the Defense Civil Preparedness Agency, the U.S. Army Natick Research & Development Command began conducting developmental work on substitute foods for shelters to replace or augment existing items. Hopefully this action would result in cost savings to the government.

Bulgur was selected for this investigation because it is processed from

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a basic agricultural commodity whole-grain wheat which is plentiful in the U.S., low in cost, highly palatable, and reportedly very stable (Neufield *et al.* 1957; Vail 1962; Sheperd *et al.* 1963). Indeed a long shelf life may well be the single most important criterion for choosing bulgur in a stockpiling program. Sheperd *et al.* 1964 suggested that factors such as deterioration, preparation under shelter conditions, and acceptability of a presweetened product merited further study.

Bulgur has been traditionally processed by a series of steps of soaking, cooking, drying, milling to remove the outer bran coating, and cracking (Brown 1962; Smith *et al.* 1964; Anon. 1963). It is eaten like rice and is shipped to foreign lands in the Foods for Peace Program and it is considered a unique, economical survival food because the cost of a daily ration recently developed containing bulgur and sugar does not exceed $0.40 using one pouch per day as a base (Harris 1974).

From a nutritional standpoint this bulgur food when consumed will not induce keto-acidosis since the total daily weight derived from carbohydrate exceeds 100 g (Gamble 1946). Correspondingly, if the water supply is limited, a high protein food is contraindicated in order to minimize renal activity (Olson *et al.* 1960). The proximate protein content of bulgur is relatively low — 11.2% (Anon. 1972; Watt and Merril 1963); presweetened bulgur with sugar is even lower — 8.1%.

METHOD

Both bulgur and an 85% bulgur/15% soy grits product were purchased from a large milling company in 22.7 kg bags in 1972. Duplicate samples, each containing 500 g of product were immediately containerized in duplicate in either air or nitrogen packs in hermetically sealed 401 × 411 cans. For the air pack no gas was added to the headspace. To achieve low oxygen (nitrogen pack), the contents of the can were purged 3 times in a closed chamber by pulling a 0 Pa vacuum, holding 30 sec to remove any atmospheric gases, and then backflushing with nitrogen gas.

Headspace Gas Analysis

Residual oxygen was run in duplicate on the gases in the can headspace prior to sensory testing. Residual oxygen content was determined by a specially developed chromatographic method (Bishov and Henick 1966). Sampling was achieved with a can-lid piercing needle on a gas-tight syringe. The sampling area on the can lid was first covered with a

self-adhesive tape of closed-cell polyurethane foam. A sealant was used to cover the external patch to prevent leakage before sensory evaluation.

PANEL TESTING

A consumer panel of 32 judges randomly selected at each session reported preferences on a 9-point scale known as a hedonic rating (Peryam and Pilgrim 1957).

PREPARATION

Unsweetened Cereals

Bulgur cereals, either 100% bulgur or the soy-fortified bulgur, were prepared by adding 300 g of cereal and 10 g of salt to 1430 ml of water, and simmering 20 min in a covered saucepan. Cereals were served at 71°C.

Sweetened Cereals

The same preparation procedure as above was followed except that 107 g of sucrose was added to the product before simmering.

Water Reconstituted Cereals (Recipe for % sugar mixtures)

	Grams		
Bulgur	270	270	270
Sugar	50 (3%)	105 (6%)	220 (12%)
Water	1420	1420	1420

This was allowed to stand in water for at least 4 hr to reconstitute.

Withdrawals

Cereals were withdrawn from storage and evaluated after being stored for 0, 1, 3, 6, 9, 12, 18, and 24 months at 38°C.

RESULTS AND DISCUSSION

As shown in Table 1, headspace analysis of cereal products was

Table 1. Headspace analysis[1]

Sample	Time (months) 0	1	3	38°C 6	9	12	18	24
	% Oxygen							
Bulgur, Air Pack	20.9	16.7	7.7	1.2	1.2	1.2	1.2	1.2
Bulgur, Nitrogen	0.5	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Bulgur/Soy, Air	20.8	15.3	6.2	1.2	1.2	1.2	1.2	1.2
Bulgur/Soy, Nitrogen	0.5	0.1	0.1	0.1	0.1	0.1	0.1	0.1

[1] Mean of 2 samples

performed eight times during 24 months of storage at 38°C. Data indicate that if cereal is air packed in cans, it eventually reaches a point where static equilibrium exists between the product and oxygen. In other words, oxygen diffuses into the product until no more absorption takes place. Assays indicated losses of oxygen in the headspace of both bulgur and bulgur/soy products packed initially in air. Oxygen decreases occurred after 1 and 3 month's storage time, but at 6 months at 38°C, the products packed in air were in equilibrium with oxygen in the headspace gas. The equilibrium level was found to be 1.2% headspace oxygen. On the other hand, when the cereal products were initially packed in nitrogen, they were virtually inert to gaseous exchange during the entire storage period, indicating that the degree of autoxidation was thought to be considerably less than in their air-packed counterparts. The question this posed was will increased product respiration result in a more rapid quality loss in the products under test?

This proved to be a complicated question to answer for as seen in Table 2, there was some significant quality loss discernible between samples. This occurred after 3 and 24 months at 38°C. However, these differences were not reproducible at the 6, 9, 12 and 18 month withdrawal periods. In addition the products which showed increased respiration (air packs — Table 1) did not necessarily rate lower. Nevertheless, at 24 months at 38°C the panel rated the bulgur/soy product significantly poorer than the other treatments but not bulgur packed in air. Notwithstanding the air packed product containing soy grits still rated 5 — "neither like nor dislike" compared to the other products' rating of 6 — "like slightly." Some of the ratings were higher than the initial values and can be partially accounted for to test-retest reliability error.

Table 2. Acceptance testing;[1] Mean hedonic rating of unsweetened bulgur

	Time (months)			38°C				
Sample	0	1	3	6	9	12	18	24
Bulgur, Air	5.6	5.4	5.8	5.4	6.4	6.2	5.2	6.1[2]
Bulgur, Nitrogen	5.6	5.4	6.5[2]	5.8	6.7	6.2	5.4	6.2[2]
Bulgur/Soy Air	5.9	5.3	5.7	5.5	6.2	6.1	5.4	5.3
Bulgur/Soy, Nitrogen	5.9	5.8	6.1	5.6	6.2	5.9	4.8	6.0[2]
L.S.D.			0.5					.5

[1] N = 32
[2] Significant difference at 5% level
5 = Neither like nor dislike
6 = Like slightly

As Peryam and Pilgrim (1957) point out many factors can affect individual ratings such as variation in human judgments, time, feelings, etc. Overall the bulgur products were rated as being in the hedonic range of "neither like nor dislike" to "like slightly" categories. There was no particular advantage to nitrogen packing these products except possibly in the case of the product containing defatted soy grits at a 15% level. Hedonic ratings in the range of 5.3 to 6.5 appear to be at target level because if foods are too acceptable some people could overeat at any given meal defeating the purpose for which these foods are intended.

In order to help overcome monotony in survival situations, it was decided to sweeten one-half of the bulgur products and test them under the same consumer acceptance panel conditions. In this case bulgur was sweetened with 6% sugar based on total formula weight and cooked in boiling water. Overall acceptance is shown in Table 3 with the grand mean hedonic ratings increasing 0.3. Statistical differences on sweetened bulgur (Table 3) closely parallelled the results obtained on unsweetened bulgur except at the 6 and 24 month withdrawal periods. At 6 months the sweetened bulgur product packed in air was judged to be significantly poorer than any of the other 3 treatments. Despite this, the sweetened bulgur at this withdrawal rated higher than its unsweetened counterpart shown in Table 2. It was rated as "like slightly" as compared to the unsweetened treatment of "neither like nor dislike" (Table 2).

The unsweetened bulgur/soy product at 38°C which rated significantly poorer from the other samples was not judged to be different

Table 3. Acceptance testing;[1] Mean hedonic rating of sweetened bulgur

	Time (months)			38°C				
Sample	0	1	3	6	9	12	18	24
Bulgur, Air	6.0	6.2	5.5	6.3	5.7	5.8	6.1	6.0
Bulgur, Nitrogen	6.0	6.7	6.2[2]	6.9[2]	6.1	5.8	6.6	6.4
Bulgur/Soy, Air	6.1	5.9	5.3	6.4	6.0	5.7	6.1	6.1
Bulgur/Soy, Nitrogen	6.1	6.2	5.9[2]	6.8	5.8	5.7	6.5	6.3
L.S.D.			0.6	0.5				

[1] N = 32
[2] Significant difference at 5% level
5 = Neither like nor dislike
6 = Like slightly

when it was sweetened. This indicated that sugar somehow masked off flavor and can be used advantageously to cover up off flavors in bulgur.

In an emergency situation there is a possibility that no heating facilities will be available. Therefore, the feasibility of preparing bulgur without cooking was explored; that is, soaking it in water for a predetermined time period. It was found that bulgur cereal must be soaked in water for at least 4 hr to soften it adequately for consumption. Soaking bulgur in water without cooking results in a cereal which has an uncharacteristic "raw starchy taste" and lacks the typical gelatinized texture. In a preliminary test (Table 4) the acceptance panel found that bulgur

Table 4. Acceptance testing;[1] Mean hedonic ratings of cooked versus uncooked bulgur

Treatment	Rating	% Dislike
Control, cooked bulgur, served 77°C	6.1	12
Control, cooked bulgur, served 21°C	5.0[2]	33
Bulgur soaked overnight, served 21°C	3.8[2]	69
L.S.D.	0.6	

[1] N = 32
[2] Significant difference at 5% level
6 = Like slightly
5 = Neither like nor dislike
4 = Dislike slightly

soaked in water overnight and served at 21°C was unacceptable. In fact 69% of the ratings were below 5, the borderline acceptability level on the hedonic scale. Even if cereal were cooked and then allowed to cool to room temperature, it rated borderline in acceptance ("neither like nor dislike").

Since presweetened cooked cereal rated higher in previous tests (Table 3) a factorial design was setup whereby the sugar content was varied from no sugar to 12% sucrose based on the weight of total formula. In Table 5 the acceptance panel results are shown. The data indicate that presweetening to a minimum of 6% sucrose is necessary to obtain a product nearly comparable to its cooked but unsweetened counterpart. Those water soaked cereal products containing no sucrose or 3% level were rated as being significantly poorer than those containing 6 or 12% sucrose. Of the total panel of 32, 8 members expressed the judgment that they would dislike even the 6% sucrose sweetened, unsoaked bulgur cereals. However, in a survival situation sweetened cereals seem desirable. This was considered a major finding.

Based on these results a survival packet was developed for the Defense Civil Preparedness Agency containing 150 g of bulgur and 58 g of sucrose. This blend was packaged in a 4-sided sealed 19 cm × 21.5 cm laminate paper/foil/polyethylene pouch with a tear notch. The pouch is large enough so that 690g of water can be added. This food after soaking in water for 4 hr can be consumed and provides approximately 760C (3180kJ). Two or perhaps even one pouch of food would provide enough energy to sustain life under survival conditions.

Table 5. Acceptance testing;[1] Mean hedonic rating of different sucrose levels in soaked bulgur

% Sucrose	Rating	% Dislike
12	5.8	22
6	5.4	25
3	4.6[2]	32
None	3.2[2]	81
L.S.D.	0.7	

[1] N = 32
[2] Significant difference at 5% level
6 = Like slightly
5 = Neither like nor dislike
4 = Dislike slightly
3 = Dislike moderately

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SYNTHETIC CARBOHYDRATES FROM FORMALDEHYDE

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ABSTRACT

Chemical food synthesis research is currently in progress as part of the US-USSR Research Collaboration in Catalysis for closed loop production of glycerol as a source of carbohydrates in sustained space flight and for direct synthesis of carbohydrates. Experimental results are presented which show the feasibility of producing not only glycerol but also potentially edible sugars from formaldehyde by means of the autocatalytic formose condensation reaction. The speculative possibility of large scale food refineries producing edible carbohydrates and purifying them by chromatographic methods is considered, as well as economically growing single cell protein, using formose sugars as substrate. If protein derived from synthetically produced carbohydrate can eventually be used for animal feed, farmland can be released for human foods.

INTRODUCTION

Current population growth is such that it is predicted that manufacture of foods will be required within the next generation. The availability of H_2 and CO from natural gas, petroleum and proposed coal refineries make CO a preferred raw material for producing both

[1] Deceased

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carbohydrates and single cell protein. Conversion of CO to CH_3OH and HCHO is presently commercialized. HCHO can then be converted to "formose" sugars by the formose reaction. These sugars can then serve as substrate for single cell protein growth.

Beiber (1969) recognized that the most serious problem in single cell protein growth directly from methane or crude oil is oxygen transfer. He pointed out the need for developing equipment with high oxygen transfer capabilities if economic single cell protein from these sources is to be a reality. *Pseudomonas* on methane requires 1.67 g of carbon to produce 1 g of cells, but the oxygen requirement is 5.26 g. On the other hand, *Candida utilis* on glucose, while it does need 1.97 g of carbon, only requires 0.77 g of oxygen (XI Int. Botanical Congress, Seattle, 1969). The ability to chemically oxidize carbonaceous material to formaldehyde, and then produce sugars, solves one major problem in single cell protein manufacture, that of supplying oxygen. However, it does introduce another problem, that of toxicity to living organisms. Mizuno and coworkers (1971, 1972) tested the nutritive value of formose sugars by feeding young and adult rats. They found that replacement of all, or half, of natural sugars by formose caused profuse diarrhea, and bleeding, ending in death for all the rats. They found that the animals grew with slight intestinal disorders when 25, 10, or 5% formose sugars were used. They, as well as Chermside *et al.* (1970) theorized that formose sugars, except for D-hexoses, were only slowly adsorbed from the intestines. Partridge *et al.* (1972) found that branched sugars, which do not normally occur in nature, are present in formose. They postulated that the toxicity of formose is due to these branched sugars blocking glucose oxidase sites. Mizuno and Weiss, (1974) in a review of formose chemistry, provide a background on the efficacy of formose for feeding microorganisms, such as yeast. Early studies, as well as more recent studies described by Mizuno (1975) and Bok and Demain (1974) show that cell growth is significantly lower with formose than with D-glucose.

FORMOSE CHEMISTRY

The homogeneous $Ca(OH)_2$ catalyzed condensation of formaldehyde to glycolaldehyde initiates the formose reaction. Once glycolaldehyde is formed, an α-hydrogen is present. The reaction then behaves as the well known base-catalyzed aldol condensation; and subsequent formaldehyde addition at the α-hydrogen proceeds readily, to form glyceraldehyde. Glyceraldehyde can then undergo the Lobry de Bruyn-van Eckenstein (L-V) rearrangement to dihydroxyacetone. Formaldehyde

addition to the α-hydrogen of glyceraldehyde forms a branched chain tetrose, to those of dihydroxyacetone a straight chain keto-tetrose which then isomerizes to the aldose form (Fig. 1). Build-up to higher molecular weight sugars proceeds analogously. As mentioned, the branched chain sugar species may be responsible for the toxicity found in feeding studies of formose products (Shapira 1968).

EXPERIMENTATION

Typical reaction conditions for practical formose condensation are 40—60°C, 1-12 M HCHO solution and an HCHO/$Ca(OH)_2$ mole ratio of 10—20. A slurry of $Ca(OH)_2$ and the HCHO solution are pumped separately into a CSTR operated near complete HCHO conversion. By this technique, side reaction to form CH_3OH and $Ca(OOCH)_2$ by Cannizzaro reaction is minimized to less than 2%. Since HCHO is completely converted, it is not necessary to have a separation scheme for unconverted HCHO. HCHO, H_2O, and CH_3OH are analyzed by TC-GC over Carbosieve B. Sugars are first converted to volatile trimethyl silyl ethers for subsequent FID-GC analysis using an OV-17 SCOT column.

Studies using a continuous stirred tank reactor (CSTR) (Weiss and Shapira 1970; Weiss *et al.* 1970; Weiss *et al.* 1971; Tambawala and Weiss 1972; Weiss *et al.* 1974) gave quantitative results on the kinetics of the $Ca(OH)_2$ catalyzed formose reaction and its associated Cannizzaro effects. The observed autocatalytic and zero order nature of the kinetics of the homogeneously catalyzed formaldehyde condensation reaction were explained by using rate expressions which are analogous to Langmuir-Hinshelwood relationships. Product decomplexing is the

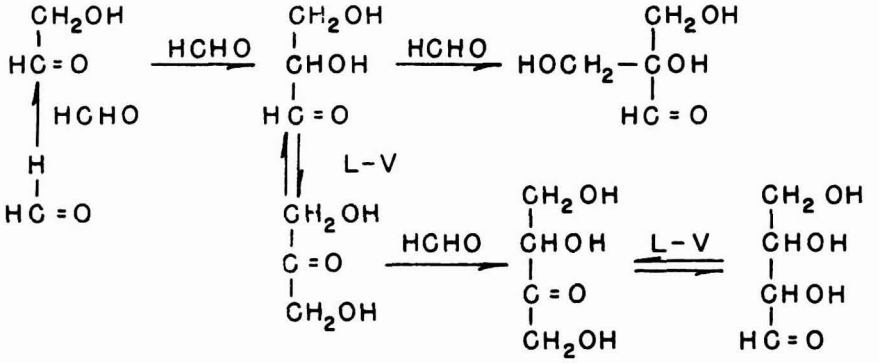

FIG. 1. FORMATION OF BRANCHED AND STRAIGHT CHAIN ALDOSES AND KETOSES THROUGH C_4

rate limiting step, under the conditions studied. The rate of the formose condensation reaction at intermediate conversion levels at 60°C, expressed as moles of HCHO converted/min/liter of reaction volume, is 3.15 × $Ca(OH)_2$ molarity. The Cannizzaro reaction rate (Fig. 2) passes through a maximum near 50% conversion. Near zero conversion, almost no formose reaction and only Cannizzaro reaction occurs in the formose system. Near complete conversion, Cannizzaro rate is minimized and a locus is drawn on Fig. 2 showing minimum Cannizzaro selectivity.

Product distributions at various conversion levels are provided on Fig. 3 which illustrates that manipulation of operating temperature will permit control of either branched chain or straight chain isomeric structure or of molecular weight distribution.

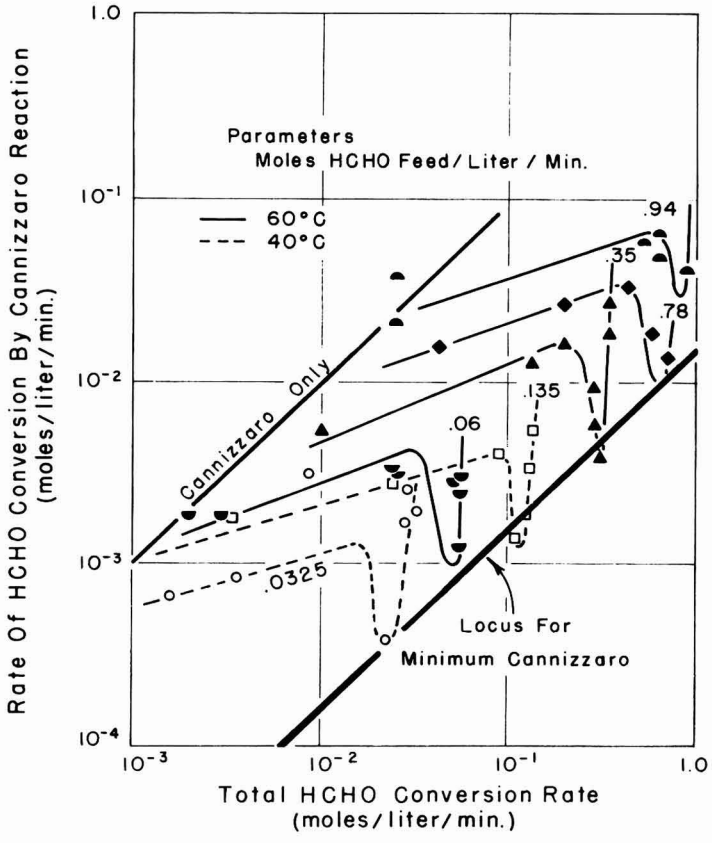

FIG. 2. ONLY CANNIZZARO REACTION PROCEEDS AT LOW CONVERSION IN A CSTR. NEAR COMPLETE CONVERSION A LOCUS OF MINIMUM CANNIZZARO CONVERSION CAN BE DRAWN THAT IS ONLY 2% OF THE TOTAL HCHO CONVERSION

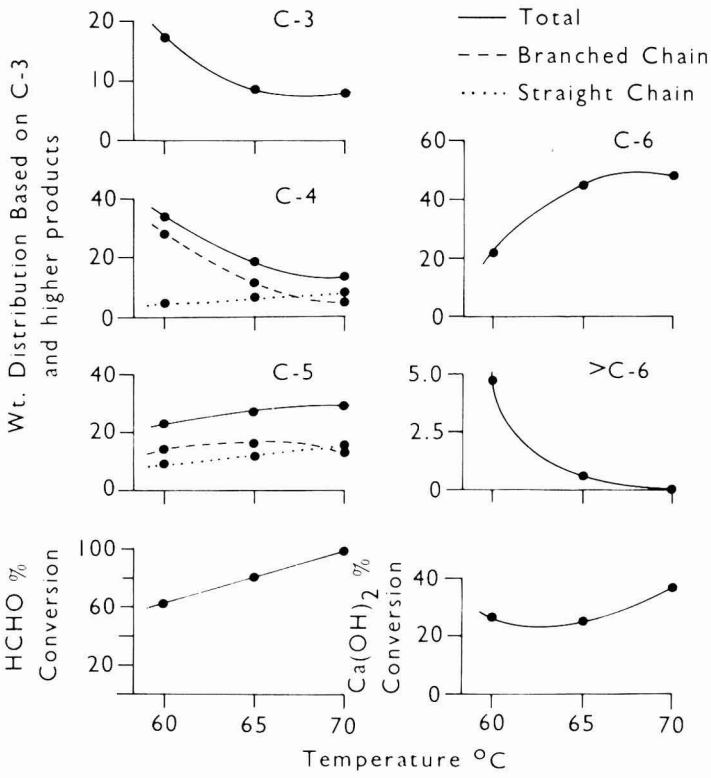

FIG. 3. PRODUCT DISTRIBUTIONS IN THE FORMOSE REACTION AND BRANCHED AND STRAIGHT CHAIN DISTRIBUTION AT VARIOUS HCHO CONVERSIONS

$Ca(OH)_2$ Conversion is due to Cannizzaro reaction

Reduction of the formose sugars to the corresponding polyols suggests the feasibility of a glycerol process at 40° C (Weiss *et al.* 1971). Glycerol is metabolized by the body to D-glucose. Shapira, in a 90-day study of glycerol as a human diet supplement, observed no nausea or ill-effects, although there was a rise in the subjects' urine-free glycerol content (Shapira 1970). Figure 4 shows a flow scheme in which the synthetic formose sugars could be formed, hydrogenolyzed with nickel catalyst in a battery of autoclaves, and then separated into glycerol and by-products, such as ethylene glycol and propylene glycol. Such a process could also proceed with natural sugars. Products with C_4 and higher molecular weight are a mixture of polyols, useful, e.g., as plasticizers. These could either be sold or recycled to extinction in the system. This completely chemical process system has the potential of producing edible glycerol (e.g. for use in a spacecraft or for single cell protein) by non-biological routes.

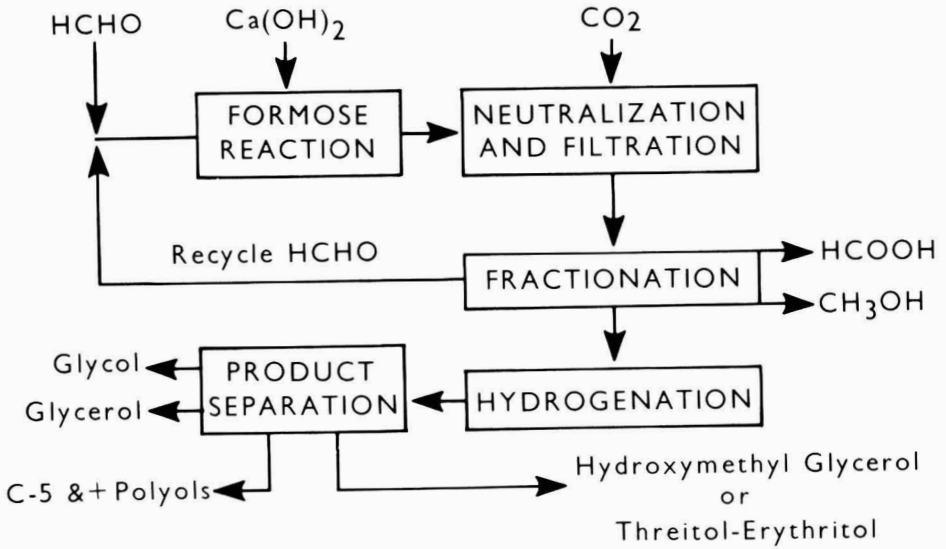

FIG. 4. HYDROGENOLYSIS SCHEME TO PRODUCE GLYCEROL FROM FORMOSE

Higher molecular weight polyols could be sold or recycled to extinction

Process Concept for Edible Sugars

A scheme for producing metabolizable sugars from formaldehyde is shown in Fig. 5 where A_n implies aldoses and ketoses of carbon number n. Cannizzaro products and unconverted HCHO are separated from the neutralized formose product by fractionation, acids and polyols by ion exchange and adsorption chromatography. Gel permeation chromatography is suggested as the basis for separation of inedible from edible

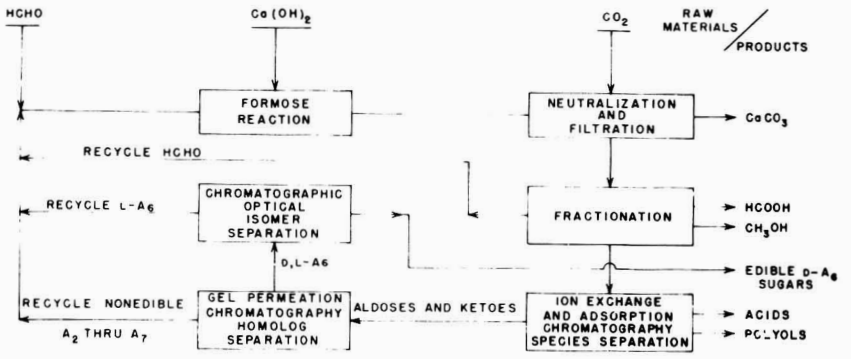

FIG. 5. FLOW SHEET FOR SUGAR MANUFACTURE

aldoses and ketoses. By not placing a demand of individual isomer separation, but rather, homolog separation, on a column, capacities far in excess of the analytical capacity can be anticipated. Timmins, *et al.* (1969), describe operations on production scale chromatography columns as large as 4 feet in diameter. It is still difficult to project that chromatographic separation of carbohydrates will eventually be an economic process.

If the hexose product has an equal D and L distribution, an overall reaction selectivity of formaldehyde to metabolizable sugars of 50% by weight is the maximum possible. This in principle could be improved by recycling L-isomers. A review of optical isomer separation has been published by Buss and Vermeulen (1968). Bauman, A. J., (1975) notes other separation techniques. Exotic techniques mitigate against economic direct production of edible sugars. A difficult process breakthrough in the formose catalysis is needed to produce edible D-sugars only, or at least straight chain, rather than branched sugars.

Process Concept for Single Cell Protein

Figure 6 shows a flow sheet based on a coal refinery for a single cell protein process. In this process, formaldehyde is condensed to formose at conditions corresponding to Cannizzaro minima. The formose then is prepared for single cell protein growth (which is mainly the removal of Cannizzaro products and pH adjustment). Undigested substrate, presumably L-species, would be recycled to extinction in the formose reaction. This represents the overall concept of coal to carbon monoxide to methanol to formaldehyde to sugar to single cell protein, hope-

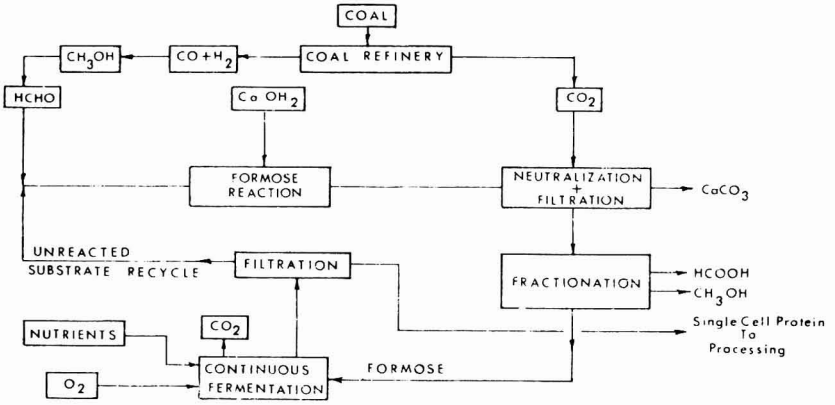

FIG. 6. FLOW SHEET FOR SINGLE CELL PROTEIN MANUFACTURE FROM FORMOSE SUGARS

fully projected on the order of cents per pound. Feeding studies will ultimately be needed on the single cell protein produced. The process has the advantage of not requiring the homolog or stereoisomer separation by chromatographic techniques, since the enzymes perform this task.

CONCLUSION

The utility of the formose sugars will depend on two factors: (1) Reducing or eliminating toxic branched species so that formose is not toxic to human or lower organisms. In addition, the fact that the sugars are racemic raises the experimentally unsolved question of whether non-metabolized sugars can be recycled to extinction as indicated in Fig. 6. (2) HCHO must become cheap enough so that it is economically advantageous to use formose instead of natural sugar. Chemical uses for formose, such as hydrogenolysis to glycerol, do not have the toxicity limitation.

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BOOK REVIEW

Chemische Lebensmittelkonservierung, (Chemical Preservation of Foods). Erick Lück, Springer-Verlag, Berlin. 280 pp., 1977. Price is not available.

The book is divided into a general part in which the author treats principles, and a second part devoted to specific preservatives. The general part is very brief. The overall strategy of chemical preservation is introduced briefly, and problems of additive analysis are mentioned in a 1-½ page long section. The introduction to health aspects and to legal aspects of preservatives is brief but well done, and up to date. The review of principles of antimicrobial action concentrates on phenomenological aspects. Synergism, effects of substrate, solubility effects, pH effects and effects of water activity are mentioned. There is, however, no discussion of modern research into the biochemical, biophysical and genetic basis of the effects of chemicals on microorganisms (or on other organisms, including humans).

The section devoted to specific additives occupies 190 of the 242 pages of text (there are some 40 pages of references). In each case pertinent data including trade names, history of use, properties, legal aspects, antimicrobial effectiveness spectrum, and suggested applications are presented in a clear manner. The information seems to be up-to-date.

This book is likely to be a useful handbook for technologists whose primary language is German. There are two books in English with which I tried to compare the present work. They are: (1) *Handbook of Food Additives*, Furia (ed.), CRC Press, 1968, which has a chapter on antimicrobial food additives by Chichester and Tanner, and (2) *Disinfection, Sterilization and Preservation*, Block, (ed.), Lea & Febiger, 1977.

Block's book is much broader in scope than Lück's, but with respect to specific food additives. Luck's book is easier to use, and contains more information on specific food preservatives. Furia's book on the other hand is comparable in content of information, and readily accessible to the English reader.

M. KAREL

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Typewritten manuscripts in triplicate should be submitted to the editorial office. The typing should be double-spaced throughout with one-inch margins on all sides.

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HASSON, E. P. and LATIES, G. G. 1976. Separation and characterization of potato lipid acylhydrolases. Plant Physiol. *57*, 142–147.

ZABORSKY, O. 1973. *Immobilized Enzymes*, pp. 28–46, CRC Press, Cleveland, Ohio.

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Table 1. Activity of potato acyl-hydrolases on neutral lipids, galactolipids, and phospholipids

Description of experimental work or explanation of symbols should go below the table proper.

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