Soy Protein Hydrolysis in Membrane Reactors

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ABSTRACT

An ultrafiltration (UF) based reactor system for continuous hydrolysis of proteins was developed to overcome limitations of the traditional batch process. A continuous stirred tank reactor was coupled to a hollow fiber module in a semiclosed loop configuration. Capacity of the reactor, defined as quantity of hydrolysate produced/time/weight of enzyme, was a sensitive function of enzyme concentration between 55 and 94% substrate conversion levels for the Pronase-Promine D system. Increasing flow rate also improved capacity, but substrate concentration and reactor volume had small effects on capacity within the levels of expected use. Productivity (defined as weight of hydrolysate/weight of enzyme) was at least 10-20 times greater for the continuous UF reactor than a batch reactor operating under otherwise identical conditions.

INTRODUCTION

Enzymatic hydrolysis of proteins is a convenient means of improving certain functional properties of proteins without diminishing nutritional values (1). Compared to acid or alkali hydrolysis methods of producing hydrolyzed vegetable protein (HVP), enzyme hydrolysis is milder and should result in little or no undesirable side reactions and toxic byproduct formation, and the relative specificity of various enzymes could be used to advantage in controlling the functionality of the end product (2). Traditional batch enzymatic hydrolysis methods, however, have their own disadvantages, such as the relatively high cost of enzymes (which are used only once) and their inherent inefficiency compared to continuous processes, resulting in low yields and productivity. In addition, the batch hydrolysate may consist of several fractions of varying molecular sizes which may make functional properties difficult to control, since they are closely related (3). Further, if the reaction is not carefully controlled, it could lead to excessive bitterness and off-flavors.

To overcome these problems, we have developed a continuous enzymatic hydrolysis process using semipermeable membranes (2,4). Although the "membrane reactor" concept has been known for several years (5), many workers used dead-end ultrafiltration (UF) cells or other flat-sheet configurations which were prone to fouling and rapid loss of reactor activity. Our studies indicated that the best process design would be: (a) physically to separate the reaction vessel and the ultrafiltration unit; (b) to use a UF module that would have high membrane area-to-volume ratio; and (c) to adjust operating conditions to attain and maintain a high level of conversion in the reactor system. Accordingly, we have developed the CSTR (continuous, stirred-tank, reactor)-UF reactor system (2,4) which is a significant improvement over previous methods. This paper reports on the effect of certain operating parameters on efficiency of conversion of protein to hydrolysate and the capacity of the reactor, and a comparison of the productivity of a batch reactor and the continuous membrane reactor.

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EXPERIMENTAL

Materials

The substrate used was a commercial soy protein isolate (Promine D, Central Soya Co., Fort Wayne, IN). It was made up to the required concentration in deionized water and heated at 100 C for 30 min, cooled to the required temperature (50 C), its pH adjusted to 8.0 and used as feed to the reaction vessel. The enzyme used was Pronase, a mixture of endo- and exo-peptidases from *Streptomyces griseus* (Calbiochem-Behring Corp., La Jolla, CA).

UF Reactor System

This is essentially a reaction vessel designed as a well mixed continuous stirred tank reactor, coupled in a semiclosed loop configuration with an ultrafiltration separation unit. The hollow fiber configuration was selected since it appeared to meet the criteria for our reactor adequately. Details of the set-up, operating conditions and reactor design aspects are available elsewhere (2,4,6,7). Experiments reported here were conducted at pH 8.0, 50 C and with the H1P10 hollow fiber module (10,000 molecular weight cut-off).

Independent variables in the experiments were enzyme concentration (E, mg/mL), substrate concentration (S_O, % w/v), reaction volume (V, mL) and flux, i.e., flow rate of substrate in or product out (J, mL/min). Measured variable was conversion (X), defined as nitrogen (N) concentration in the permeate (hydrolysate) divided by nitrogen concentration in the feed (substrate). Nitrogen concentrations were corrected for nonprotein nitrogen (NPN) in the feed (2).

RESULTS AND DISCUSSION

Previous studies (6) had shown that the UF reactor system could be modeled as an ideal CSTR, provided the system was designed so that (a) the volume of the ultrafiltration module was small compared to the total volume, and (b) the recirculation rate (necessary to control concentration polarization and fouling) was much larger than the flux. The data could be adequately described by combining the simple Michaelis-Menten model for a single-substrate, uninhibited reaction with a mass balance for an ideal CSTR to result in the model shown below:

$$X + K_m X/S'_O\,(1 - X) = K_2\,\tau \qquad [1]$$

where X = fractional conversion = P'/S'_O; S'_O = initial substrate concentration corrected for NPN = $S_O - P_O$; P' = product (hydrolysate) concentration in permeate corrected for NPN = $P - P_O$; P_O = NPN in feed; K_m = apparent Michaelis constant; K_2 = reaction rate constant; and τ = modified space time parameter = $E.V/S'_O J$.

Space time (τ) was found to correlate the data better than the conventionally used residence time (V/J); it is also a more convenient parameter since all operating variables affecting the reactor can be conveniently grouped together in one term. Enzyme activity rather than enzyme concentration would be a better representation of space time, since then other variables affecting reactor performance could

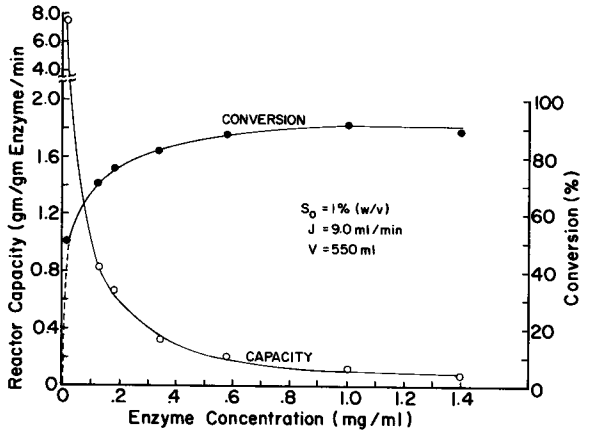

FIG. 1. Effect of enzyme concentration on conversion and capacity of UF reactor. Pronase-Promine D at pH 8.0, 50 C.

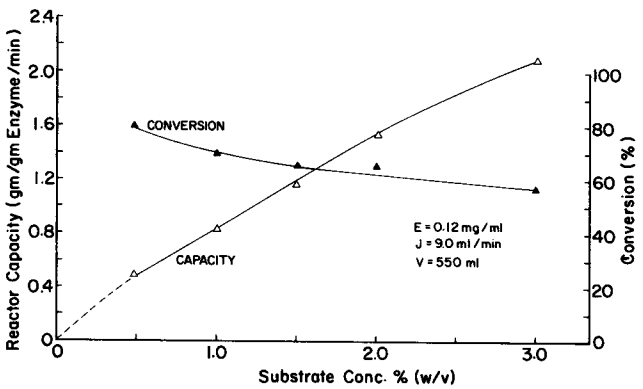

FIG. 2. Effect of substrate concentration on conversion and capacity of UF reactor.

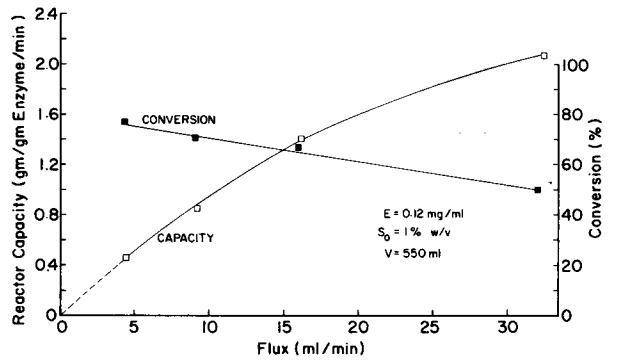

FIG. 3. Capacity and conversion of UF reactor as a function of flow rate through the system (flux).

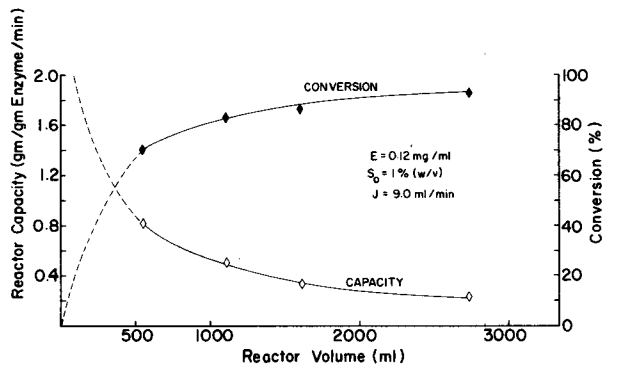

FIG. 4. Effect of reactor volume on UF reactor capacity and conversion.

also be incorporated into the model, such as pH, temperature, different enzymes, etc. However, for practical use of the model in deciding on an operating strategy for a particular application, the use of enzyme concentration is more convenient.

In choosing a desirable operating strategy on an industrial scale, an important consideration is the quantity of hydrolysate produced in a specified period of time, i.e., the "capacity" (C) of the reactor, defined as mass of product (i.e., weight of hydrolysate in permeate) per unit time per unit mass of enzyme:

$$C = P'J/EV = XS_0'J/EV \qquad [2]$$

Reactor capacity is usually calculated when X is at its maximum value and hence represents the maximum practical performance level of a particular reactor. It does not take into account any long-term instability of the system and is either useful only as a means of comparing reactors or the effects of a particular variable.

Figures 1-4 show how capacity is affected by operational parameters. Also shown for comparison purposes is the dependency of fractional conversion X on the same parameters. It can be seen that a particular variable has opposite effects on X and C, which appears to contradict the definition of capacity shown in Equation 2. However, it should be noted that X itself is a function of the four variables shown in Equation 2, and that a particular change in X does not necessarily mean a proportional change in C. Rather, X is controlled by the operating variables as described in Equation 1.

As expected from the data presented in previous papers (2,6,7) enzyme concentration has a very significant effect on capacity (Fig. 1), especially at the lower concentrations. For example, lowering the enzyme concentration from 0.1 mg/mL to 0.01 mg/mL only lowered the conversion from 70% to 50%, but there was a corresponding 900% increase in reactor capacity. Substrate concentration also has a significant effect on capacity. Because of the interaction between substrate concentration and other variables shown in Equation 1, increase in substrate concentration is not matched by a proportionate decrease in conversion. The net effect, as shown in Figure 2, is an increase in capacity with an increase in substrate concentration. This relationship is probably asymptotic and the C value would probably level off at concentrations higher than those considered here.

Figures 3 and 4 show effects of flux and volume on capacity. The effects of these variables on conversion are related to the residence time of substrate in the system. Higher volumes or lower flux increases the residence time, thus resulting in higher conversion, but results in lower capacity.

Productivity Comparisons

As mentioned earlier, capacity does not take into account the effect of long-term operation, since the only measured variable (X) in Equation 2 was measured at its maximum value. However, reactor activity is gradually declining with time, due to several factors such as enzyme leakage in the first few hours, loss of calcium ions that may be necessary for Pronase activity, thermal degradation and product inhibition (7). The relative magnitude of the decay can be controlled only to a limited extent; in fact, in certain cases, such measures may be counterproductive, e.g., reducing

TABLE I

Conversion (%) of Soy Isolate by Pronase in Batch Reactor at pH 8.0, 50 C

Time (min)	Substrate: Enzyme ratio 9	16
0	0	0
1	—	57.7
15	90.0	—
30	94.2	79.9
60	89.2	82.5
90	—	87.7
100	96.2	—
120	96.5	89.4

Substrate (≃1% w/v) was preheated as described in text.
Conversion determined as nitrogen soluble in 10% TCA divided by nitrogen in the substrate.

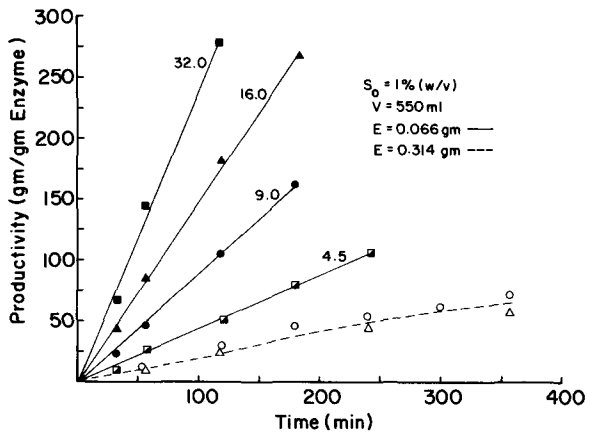

FIG. 5. Effect of flux and enzyme concentration on UF reactor productivity. E = weight of enzyme added to reaction vessel (flux at E = 0.314 g: 0 = 9 mL/min; Δ = 16 mL/min).

operating temperature from 50 C to 37 C increases enzyme half-life by 10-fold (7), but the absolute activity is lower and there may be more microbial problems in the system at 37 C than at 50 C. In any case, the reactor activity decay can be expressed with reasonable accuracy by the equation shown below:

$$X_t = Xe^{-k_d t} \quad [3]$$

where X_t is the conversion at any time t and k_d is a decay constant.

To take this reactor decay into account, another operational concept has been defined, called "productivity" (P), which is the mass of hydrolysate produced per unit mass of enzyme, and is expressed for the continuous UF reactor as (8,9):

$$P_{UF} = P_t\, Jt/EV \quad [4]$$

where P_t is the product concentration at any time t, or,

$$P_{UF} = X_t S_0' Jt/EV \quad [5]$$

For the batch reactor, productivity is calculated as

$$P_{BATCH} = X S_0'/E \quad [6]$$

Typical data for the batch hydrolysis of Promine-D by Pronase is shown in Table I under otherwise comparable conditions as the continuous UF reactor. At 85-90% conversion levels, P_{BATCH} would be ca. 13-15 g hydrolysate/g enzyme/batch (i.e., per volume replacement).

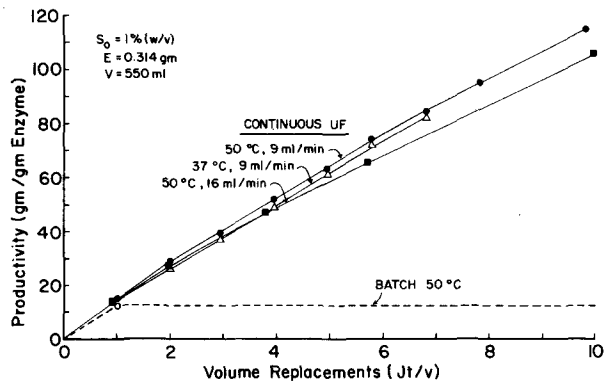

FIG. 6. Comparison of productivity of continuous UF reactor and batch reactor for the Promine D-Pronase system at pH 8.0. Volume replacement for the continuous UF reactor calculated as Jt/V.

Figure 5 shows the effect of flux on productivity as a function of operating time at two enzyme levels. This graph reemphasizes the points made earlier regarding capacity. Maximum productivity can be obtained by using the highest flux and the lowest enzyme concentration. Due to the continuous nature of the UF reactor, productivity levels are higher the longer the reactor operates before shutting down for cleaning/recharging, etc. This is shown in a clearer fashion in Figure 6 where the continuous and batch reactors have been compared on a volume replacement basis. For the batch process, every reactor volume processed requires the same amount of enzyme, substrate and volume and, assuming a consistent operation, will result in the same degree of conversion. Hence, the productivity of a batch reactor is constant with respect to volume replacements. The continuous process, however, requires only one charge of enzyme in the beginning. Hence, the longer the run, the greater the productivity and the larger the difference between continuous and batch systems.

The batch and continuous reactors shown in Figure 6 have been compared on a similar basis with respect to volume and substrate concentrations. However, what cannot be shown on the graph are some hidden advantages to the UF reactor continuous process. These are: (a) the product is of a more consistent and uniform molecular weight in the UF reactor process (2); (b) yields are much higher, typically ca. 90%, for the UF reactor process, whereas practical yields for the batch process are 65% (10); (c) the batch process requires the added expense and time to inactivate the enzyme, separate the solubles from the insolubles and clarify and filter the hydrolysate; and (d) the batch process requires more labor. The difference between the batch and continuous reactor would be greater if these factors had been taken into account, especially if Figure 6 had been plotted on a realistic time scale instead of in terms of volume replacements. If productivity is of prime importance, there is no question that the UF reactor is a better choice, regardless of the conversion levels attained during the run. However, if product characteristics and functional properties are of importance, then the strategy of operation of the UF reactor should be adjusted accordingly, since it appears that small changes in activity can have large effects on functionality of the hydrolysate (11).

ACKNOWLEDGMENTS

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Investigation of 1-Decyne Formation in Cottonseed Oil Fried Foods[1]

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ABSTRACT

1-Decyne identified in oxidized cottonseed oil was previously thought to originate from oleic acid. However, we have demonstrated that 1-decyne is a degradative product from the photooxidation of cyclopropenoid fatty acids (CPFA) naturally present in cottonseed oil. Products containing photooxidized cottonseed oil have the distinct off-flavor of 1-decyne. Experiments were conducted to identify the factors involved in 1-decyne formation. Reactions were done under the following conditions: (a) in the dark or under light, (b) with or without removal of CPFA from cottonseed oil, (c) in the presence or absence of singlet oxygen quenchers, (d) in the presence or absence of a hydroperoxide-reducing agent (triphenylphosphine), and (e) with or without photosensitizers. Methyl sterculate was used as a substrate for studying 1-decyne formation under photosensitized oxidation conditions in a model system. We have concluded that 1-decyne is formed by the photooxidation of CPFA utilizing chlorophyll as a photosensitizer. A reaction mechanism for 1-decyne formation is proposed.

INTRODUCTION

Potato chips prepared with cottonseed oil and subsequently exposed to light develop a distinct off-flavor which is defined as "light-struck". Although this off-flavor is easily detected by sensory evaluation, the nature of this light-struck phenomena was unknown. Experiments were thus designed to investigate this problem with respect to the following questions. (a) What is the major compound responsible for "light-struck" off-flavor? (b) Is cottonseed oil the only chipping oil that exhibits "light-struck" aroma upon exposure to light? (c) Were there precursors? (d) Could we develop methods to destroy or remove the undesirable reactants from cottonseed oil? (e) What was the possible reaction mechanism?

EXPERIMENTAL PROCEDURES

Materials

Commercial cottonseed oil was purchased from Levelland Vegetable Oil Company (Lubbock, TX). *Sterculia foetida* seed oil was a gift from Dr. Randall Wood of Texas A & M University, College Station, TX. Methyl sterculate was obtained from Supelco, Inc. (Bellefonte, PA). Chlorophyll, oleic acid, and linoleic acid, were received from Sigma Chemical Company (St. Louis, MO). Aluminum silicate and absorption alumina were purchased from Fisher Chemical Company (Fairlawn, NJ). Triphenylphosphine was obtained from Alfa Division, and 1,4-diazabicyclo[2.2.2] octane (DABCO) was received from Aldrich Chemical Company (Milwaukee, WI).

Methods

The concentration of cyclopropenoid fatty acids (CPFA) was determined according to a modified Halphen procedure (1), using a standard curve of methyl sterculate under the same conditions. Chlorophyll was measured spectrophotometrically according to the AOAC method (2). Peroxide value was determined based on the AOAC method (3).

Headspace volatiles of oil or chip samples were collected by nitrogen purging into a trap containing Tenax-GC absorbent. The Tenax-trapped volatiles were then desorbed onto gas chromatographs of 10% SP-2100 column for analysis by gas chromatography-mass spectrometry (GC-MS) using the Hewlett-Packard model 5985B.

RESULTS AND DISCUSSION

1-Decyne is the Major Light-Struck Flavor

1-Decyne was identified by GC-MS and sensory evaluation as the major photodegradative off-flavor in cottonseed oil or cottonseed oil fried potato chips (Fig. 1).

Cottonseed Oil is Unique for 1-Decyne Formation

Among the common chipping oils (cottonseed, peanut, and soybean), cottonseed oil is the only oil which possessed the distinct off-flavor of 1-decyne after photooxidation (Table I). There were no detectable amounts of 1-decyne produced from peanut oil or soybean oil. Since 1-decyne was formed

[1]Presented at the 73rd AOCS annual meeting, Toronto, 1982.