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Short Communication

The effect of whey protein hydrolyzate average molecular weight on the lactic acid fermentation

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SUMMARY

The batch fermentation of whey permeate to lactic acid was improved by supplementing the broth with enzyme-hydrolyzed whey protein. Hydrolyzates prepared with endoprotease were more stimulatory to acid production rates than were those prepared with exo/endo protease. The effect of hydrolyzate average molecular weight on acid production is presented. Results show that the hydrolyzate having an average molecular weight of 700 is the most stimulatory to acid production rates.

INTRODUCTION

Lactic acid production rates from batch fermentations of whey permeate are low, but can be increased by stimulating the growth of the fermentative microorganism, *Lactobacillus bulgaricus*.

Growth is stimulated by peptides and free amino acids used for protein synthesis. Although both are transported actively into the cell, the lactobacilli respond better to peptides [1,3,6,8,13]. For example, a peptide isolated from yeast extract stimulated *L. san francisco*, but a free amino-acid mixture approximating the peptide's composition did not [1].

Because peptides of certain sizes may be transported more readily than others, peptide effectiveness may depend on molecular weight and not just on sequence and/or composition. For example, the peptide fractions most stimulatory to *L. casei* contain, on average, five amino acids per peptide – independent of sequence or composition [13]. Also, *Streptococcus thermophilus* is stimulated by peptides having 5–12 amino acid residues but is inhibited by di- and tripeptides [3].

In this work, we determined the feasibility of improving lactic acid production from whey permeate by using growth-stimulating peptide fractions from enzyme-hydrolyzed whey protein.

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EXPERIMENTAL

Raw material

Reverse-osmosis (RO)-concentrated whey and RO-ultrafiltered whey permeate were obtained from Roy's Dairy, Monroe, WI. All concentrates were pH-adjusted (KOH) to 7.2, pasteurized and then frozen for subsequent use.

Enzyme screening

Takamine Bromelain (endo + exoprotease activity; from pine-apple), Takamine Fungal (endo + exo; from *Aspergillus oryzae*) and HT Proteolytic (endo only; from *Bacillus subtilis*) were used independently under supplier-recommended conditions (Miles Labs, Elkhart, IN) to hydrolyze pasteurized concentrates [16]. Hydrolyses were conducted in magnetically agitated flasks held in a thermo-stated water bath; pH was controlled by automatic addition of 5 N KOH or 5 N HCl [16].

Hydrolyses were terminated after 4 h by lowering the pH (with conc. HCl) to inactivate the enzymes (as per supplier recommendation). The hydrolyzates were stored at 5°C [6].

The hydrolyzates were evaluated for their effects on fermentation performance. Whey permeate (750 ml) supplemented with hydrolyzate (250 ml) was fermented. HT Proteolytic gave the most stimulatory hydrolyzate and was therefore used for all subsequent fermentation studies.

Fermentation studies

Pasteurized, RO-concentrated whey was hydrolyzed with 0.32 g/l of HT Proteolytic at 50°C and pH 7.2. Hydrolysis times of 5, 20, 170, 360 and 420 min gave peptide fractions having average molecular weights (AMW) of 4600, 2800, 1300, 1000, and 700 (at an HT concentration of 0.8 g/l), respectively [6]. The hydrolyses were conducted as noted above. AMWs were determined by the method of Myers et al. [11].

The unhydrolyzed controls (AMW = 30 000) were prepared by adding to pH 4, pasteurized RO-concentrate enzyme denatured by boiling for 5 min.

The AMW-200 hydrolyzate was prepared by adding 5 g/l of Sigma (St. Louis, MO) product L0375 to whey permeate, then pasteurizing. All hydrolyzates were stored frozen for subsequent use.

A 10% inoculum of *L. bulgaricus* was used for all fermentations [16]. Each medium was prepared by mixing 250 ml of the selected hydrolyzate with 750 ml of whey permeate. Duplicate 1 liter fermentations were conducted at 45°C, pH 5.5 (automatic addition of 18% aqua-ammonia). The fermentors were N_2-blanketed to insure anaerobiosis; agitation was provided by a magnetic stirrer. Total protein in all media was 4.0 g/l. Lactic acid concentrations were determined by HPLC [6,17].

RESULTS AND DISCUSSION

Enzyme screening

Fig. 1 shows that the hydrolyzate prepared with the bacterial protease HT Proteolytic stimulated acid production to the greatest extent. This is consistent with previously published results [1,3,5,8,13] in that HT Proteolytic is an endoprotease which yields relatively high (compared to exoproteases) molecular weight peptides known to stimulate the growth of lactobacilli; the other enzymes tested contained endo- and exoproteases which together yield a large number of less-stimulatory free amino acids.

AMW effect on acid production

Histories of acid concentration and instantaneous volumetric productivity were obtained for each hydrolyzate supplement fermented. Examples are

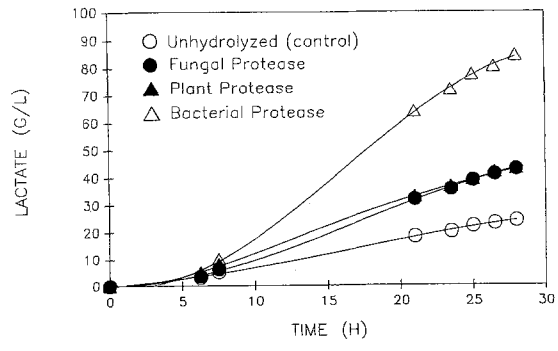

Fig. 1. Effect of protease type on lactic acid production.

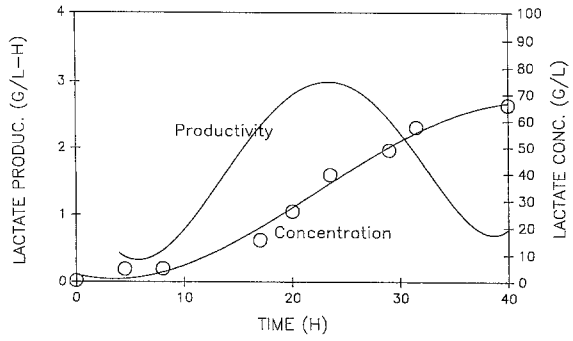

Fig. 2. Acid concentration and instantaneous productivity histories (AMW 200).

given in Fig. 2 for the hydrolyzate with an AMW of 200. Maximum instantaneous productivity is plotted in Fig. 3 as a function of AMW; the highest value was obtained at an AMW of approximately 700.

Overall volumetric productivity (total acid produced (g/l) divided by total fermentation time (h), which was 48 h or the time at which acid production ceased (whichever was shorter)) is plotted in Fig. 3 as a function of hydrolyzate AMW. Again, the highest value was obtained at a hydrolyzate AMW of about 700. Note that the differences in production rates cannot be attributed to a lag phase as cell growth was logarithmic from the outset of each fermentation [16].

These results show that the AMW-700 hydrolyzate which contains approximately six amino acid residues is the most stimulatory. This supports the

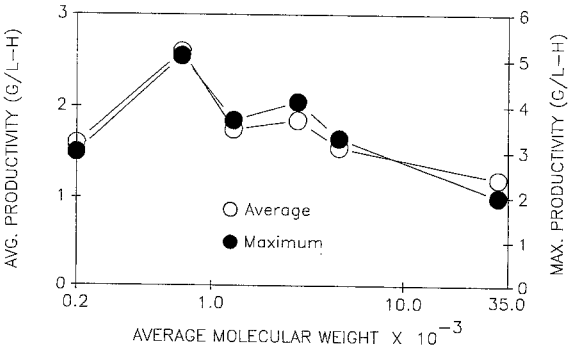

Fig. 3. Average and maximum instantaneous productivities as functions of AMW.

initial hypothesis that the AMW of the peptide supplement affects the acid productivity. In addition, the results appear consistent with the observations of other investigators: peptides having 5–10 amino acid residues are more stimulatory than those of higher or lower molecular weight [2,4,9,12,14,18].

CONCLUSIONS

(1) Whey protein hydrolyzates increase markedly the productivity of lactic acid in batch fermentations of whey permeate. (2) Hydrolyzates prepared with endoprotease are more stimulatory to lactic acid productivity in whey permeate fermentations than those prepared with exo/endo proteases. (3) Lactic acid production kinetics depend on hydrolyzate AMW; a hydrolyzate having an AMW of 700 appears to be most stimulatory to acid productivity.

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