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Influence of whey protein hydrolysate addition to whey permeate batch fermentations for producing lactic acid

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Abstract

Addition of whey protein hydrolysate (WPH) to whey permeate fermentations, by *Lactobacillus helveticus*, to produce lactic acid was investigated. Three to four percent (w/w) supplementation with WPH solution (10% w/w protein) was required to obtain high lactose conversion and lactic acid yield in a fermentation time of 30–40 h. At this percentage supplementation, the nitrogen content of the media was 0.06–0.09% w/w. The bacteria used around 0.02% w/w nitrogen during fermentation, thus there was an abundance of unused nitrogen remaining at the end of fermentation. At 3–4% supplementation, it was calculated that around 36–47% of whey protein concentrate produced from whey would be required to supplement the whey permeate fermentation in the form of the hydrolysate.

Keywords: Whey protein hydrolysate; Lactic acid fermentation; Whey permeate

1. Introduction

Whey permeate consists mainly of lactose in solution with ash and soluble nitrogen, and is produced as a by-product from whey protein concentrate (WPC) [1], milk protein concentrate and casein production. There is continuing interest in ways of utilising this by-product. One alternative is the production of lactic acid by fermentation [2]. There is a major commercial push for producing lactic acid biodegradable plastics using lactic acid derived by fermentation [3]; however, lactic acid producers are mainly looking at producing the lactic acid from sucrose and dextrose-based media. Thus, there exists a challenge to make whey permeate a viable alternative substrate.

Some lactic acid bacteria, such as *Lactobacillus helveticus*, can convert homofermentatively the lactose in whey permeate to lactic acid. However, they require a nutrient supplement for complete conversion of lactose to lactic acid, otherwise the fermentation will proceed very slowly. Lactic acid bacteria have complex growth factor requirements including B vitamins, several amino acids, and purine and pyrimidine bases [4].

Whey protein hydrolysate (WPH) is a potential nutrient supplement which is readily useable by microbes [5,6]. It can be readily produced by dairy processors on-site, either by direct hydrolysis of whey, or by the hydrolysis of WPC as illustrated in Fig. 1. Leh and Charles [7] showed that peptides having an average molecular weight of about 720 were the most suitable for the growth of lactobacilli.

This paper investigates how the amount of WPH added to whey permeate affects:

- lactose conversion, lactic acid yield and fermentation time for *L. helveticus* fermentation;
- amount of nitrogen that remains unused at the end of fermentation;
- amount of WPC required to produce the hydrolysate.

In this work, there is an emphasis on evaluating how little WPH supplement is required to obtain high lactose conversion and lactic acid yield, because addition of nutrient supplement is a raw material cost and also adds to the residual impurities remaining after fermentation. In addition, these impurities may have to be removed by costly purification processes, such as in the production of high-purity polymer-grade lactic acid.

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2. Materials and methods

2.1. Fermentation medium

The whey permeate was made up by reconstituting whey permeate powder (supplied by Kerry Foods, Listowel, Ireland) in distilled water.

2.2. Preparation of whey protein hydrolysate

WPC powder (35% w/w protein) was obtained from Dairygold, Ireland. A WPC solution was made up to a concentration of 10% w/w protein by mixing WPC powder into de-ionised water. This solution was transferred to a 2 l stirred vessel equipped with temperature and pH control. The protease solution added to prepare the hydrolysate was Proteinase D5, EC 3.4.21.14 stabilised with glycerol (Rhodia Ltd., Chesire, England), which is a serine proteinase from *Bacillus licheniformis*. The protease was added at a protease solution/whey protein ratio of 0.05 w/w. Hydrolysis was carried out at 50 °C and the pH was maintained at pH 8 by addition of 4 M NaOH. The degree of hydrolysis (DH) was determined using the pH-stat technique [8], and the reaction was allowed to proceed until 20% DH was achieved. This corresponds to around 90% of the peptides having a molecular weight less than 1000. Hydrolysis was terminated by adding concentrated HCl to rapidly lower the pH to 4. After 30 min, 4 M NaOH was added to readjust the pH to 7. The hydrolysate was then frozen and used as required to supplement the fermentations.

2.3. Microorganism

L. helveticus (strain 02) was obtained from Chr. Hansen's Laboratory, Cork, Ireland, and was stored at −80 °C. A culture was defrosted under sterile conditions, and 0.1 ml of culture was added to 9 ml of sterile MRS broth and incubated for 14 h at the fermentation temperature. One millilitre of this broth was added to 100 ml of fermentation medium and incubated for a further 12 h. This was subsequently used to inoculate the fermentor at a level of 5% (v/v).

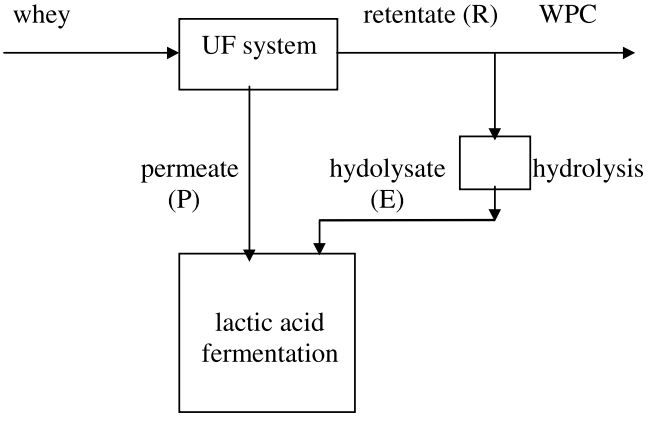

Fig. 1. Process flowsheet of a whey permeate fermentation supplemented with WPH obtained from hydrolysing a fraction of WPC produced from the ultrafiltration of whey.

2.4. Fermentation

The fermentor was a 2 l autoclavable glass vessel with disc turbine impeller and automatic pH and temperature control. The fermentation procedure consisted of adding the permeate and supplement into the glass vessel and autoclaving for 15 min at 121 °C. After cooling, the fermentation temperature was set to 42 °C and the fermentor was inoculated. The pH was set to pH 5.4 and controlled by addition of 4 M NaOH. Samples were taken throughout the fermentation for analysis.

2.5. Analysis

Lactose and lactic acid concentrations were measured by a HPLC system (Spectra series P100, USA) using an ion exclusion column (Aminex HPX-87H, BioRad, USA). Cell concentration is expressed as grams dry cell weight per litre (g/l). Cells were centrifuged, washed with distilled water and dried at 105 °C. Cell concentration was measured optically at 620 nm and converted to g/l using a calibration curve.

Nitrogen concentrations were measured by the Kjeldahl method using the Kjeltec system (Tecator, Sweden). The nitrogen values have a limited accuracy of $\pm 0.01\%$, which give an indication of how nutrient supplementation affects nitrogen content, and give an estimation of how much nitrogen is used during a fermentation.

Lactose conversion is defined as mass of lactose utilised during fermentation divided by mass of initial lactose. Lactic acid yield is defined as mass of lactic acid produced during fermentation divided by mass of lactose utilised.

3. Results and discussion

3.1. Effect of supplementation on conversion, yield and fermentation time

Fermentations were performed with no supplementation and with WPH supplementation in the range of 1–4% w/w. Supplementation had a beneficial effect on lactose utilisation and cell growth during fermentation, as illustrated in Fig. 2. The effect on lactose conversion and lactic acid yield is presented in Table 1, and this shows that supplementation at 3–4% WPH is required to obtain high lactose conversion and lactic acid yield.

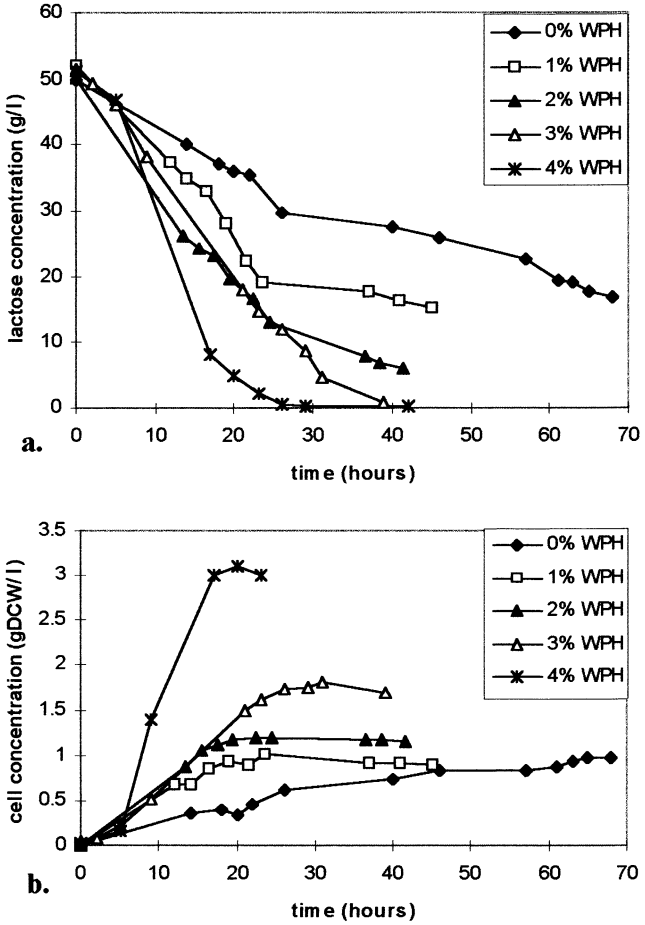

Fig. 2. Effect of WPH supplementation on the fermentation by *L. helveticus*, (a) lactose utilisation and (b) cell growth.

3.2. Effect of supplementation on unused nitrogen remaining after fermentation

Increasing the amount of supplement added increased the nitrogen content of the media as presented in Table 1; however, it also increased the amount of unused nitrogen remaining in the cell-free broth after fermentation. At 3–4% w/w supplementation with WPH, the nitrogen content of the media was around 0.06–0.09% w/w. The microbes used around 0.02% w/w nitrogen during fermentation, and around 0.04–0.07% w/w nitrogen remained unused in the cell-free broth at the end of the fermentation. Thus, there was an abundance of unused nitrogen remaining at the end of fermentation. Using the above numbers as an indication, this represents 67–78% of the nitrogen remaining unused. As a result, nitrogen appears not to be limiting, although it is possible that a specific nitrogen-containing component, such as a specific amino acid, may be limiting. Alternatively, it may be some other component, such as a vitamin or a mineral that may be limiting. Fitzpatrick et al. [9] showed how the addition of a trace of manganese ions could greatly reduce the yeast extract requirement for a *L. casei* fermentation.

The quantity of unused nitrogen represents an unused raw material cost. In addition, this is unsatisfactory for production of high-purity lactic acid because these unused components will have to be removed, which adds to separation process cost. Thus, it may be worthwhile determining what component is limiting and investigating combinations of supplements that can achieve high conversion and yield but with lower supplement cost and less unused components remaining after fermentation.

3.3. Estimation of the fraction of WPC produced from whey by ultrafiltration needed to supplement the fermentation

Mass balance calculations were performed on the system illustrated in Fig. 1, to estimate the fraction of WPC produced from ultrafiltration (UF) that would be required for supplementing the fermentation at a specified percentage. The composition of whey and the results of the mass balance calculations on the UF system are presented in Table 2. It was assumed that the protein was concentrated to 10% w/w, there was complete protein and fat recovery in the retentate and there was no partitioning of other components [1].

The percentage of WPC required to be hydrolysed to supplement the fermentation to a specified percentage of the fermentation was calculated as follows: Let R be the mass of retentate; P, the mass of permeate; E, the mass of retentate required to supplement the permeate as a hydrolysate to a specified percentage WPH (Fig. 1). The percentage WPH in the fermentation medium (%WPH) is defined as

Table 1
Effect of WPH addition on the fermentation by *L. helveticus*

WPH (% w/w)	Fermentation time (h)	Lactose conversion (%)	Lactic acid yield (%)	Nitrogen beginning (% w/w)	Nitrogen end (% w/w)	Nitrogen used (% w/w)
0	68	66	68			
1	45	70	74	0.04	0.036	0.004
2	42	88	78	0.053	0.042	0.011
3	39	98.5	96	0.057	0.039	0.018
4	29	99.5	96	0.088	0.071	0.017

Table 2
Mass balance calculations for UF fractionation of whey

	Whey	Retentate	Permeate
Total mass (kg)	100	8	92
Total solids (% w/w)	6.7	17.5	5.77
Composition (% w/w)			
Protein	0.8	10	0
Fat	0.2	2.5	0
Lactose	4.9	4.3	4.95
Minerals	0.5	0.44	0.51
Other small components	0.3	0.26	0.31

It is assumed that there is complete recovery of protein and milkfat in the retentate, and there is no partitioning of lactose, minerals and other small components.

$$\%\mathrm{WPH} = \frac{100\,E}{E+P}.$$

Re-arranging this equation gives

$$E = \frac{P(\%\mathrm{WPH})}{(100-\%\mathrm{WPH})}.$$

The percentage of WPC required to be hydrolysed to supplement the fermentation to a specified percentage of the fermentation is defined as $100E/R$. Substituting for E gives

$$100\frac{E}{R} = \frac{100\,P(\%\mathrm{WPH})}{R\,(100-\%\mathrm{WPH})}.$$

This equation can be applied directly to the experimental results because the protein concentration of the WPH solution used in the experimentation was 10% w/w, and the protein concentration of the retentate in the mass balance calculations was also 10% w/w. As mentioned above, 3–4% supplementation with WPH was required for *L. helveticus*. From applying the equation, this represents 36–47% of the WPC produced by UF, which would be needed to supplement the fermentation in the form of a hydrolysate. This shows that the amount of WPC produced from whey is more than sufficient to supplement the fermentation as a hydrolysate. However, 36–47% represents a significant portion of the WPC produced and the economic viability of using this as a supplement needs to be determined.

4. Conclusions

At least 3–4% w/w supplementation with WPH solution (10% w/w protein) was required to obtain high lactose conversion and lactic acid yield in a fermentation time of 30–40 h. This level of supplementation was estimated to represent 36–47% of WPC produced by UF of cheese whey.

A significant amount of the nitrogen was not utilised during fermentation, thus, there is scope for determining the limiting component, and for investigating combinations of WPH and small amounts of other nutrient supplements that may significantly reduce the amount of WPC required. This would make WPH supplementation more economically viable and would also reduce the amount of unused nutrients leaving the fermentation, which would reduce separation costs for the production of high-purity lactic acid.

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