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Malt combing nuts as a nutrient supplement to whey permeate for producing lactic by fermentation with *Lactobacillus casei*

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Abstract

Malt combing nuts (MCN) is a low value byproduct from the malting industry. It provides a cheap source of nitrogen and vitamins and has potential for being applied as a nutrient supplement in fermentations to produce lactic acid. This work investigates the supplementation of whey permeate with MCN to produce lactic acid by fermentation with *Lactobacillus casei*, and compares it with fermentations supplemented with yeast extract (YE). The results showed that MCN can be applied successfully as a nutrient supplement to produce lactic acid by fermentation, achieving complete sugar conversion and lactic acid yield similar to YE supplementation. 5% w/v MCN addition was required to achieve a fermentation time of around 55 h in whey permeate containing lactose at a concentration of 55 g/l. This was similar to fermentations with 0.3% w/v YE supplementation. The major advantage of using MCN is that its raw material cost for supplementing a fermentation is many times lower than YE for a comparable fermentation. However, on the other hand, the results showed that the levels of impurities remaining after fermentation are a lot higher. MCN adds much more ash to the fermentation and there is a lot more unused nitrogen remaining at the end of the fermentation. This is undesirable for the production of high purity lactic acid as it leads to increased separation costs.

Keywords: Malt combing nuts; Lactic acid fermentation; Whey permeate

1. Introduction

Whey permeate is a by-product of the production of whey protein concentrates from cheese whey, by ultrafiltration. It consists of water, lactose and small amounts of other soluble components [1]. The disposal of excess whey and whey permeate presents a problem, leading to research into whey and whey permeate utilization. One such possibility is the production of lactic acid from whey permeate by fermentation with lactic acid bacteria, such as *Lactobacillus casei* [2]. Lactic acid has long been of use in the pharmaceutical, chemical and food industries, primarily as a preservative and an acidulant. Currently, there are major commercial interests in the production of lactic acid for the production of lactic acid biodegradable plastics [3].

The fermentation of whey permeate with *L. casei* requires supplementary nutrients to obtain complete conversion of lactose and high lactic acid yields within reasonable fermentation times. In addition, lactic acid bacteria need specific minerals, B-vitamins, several amino acids, and purine and pyrimidine bases to ensure optimum growth [4]. However, this addition of supplements produces two problems. Nutrient supplementation is an extra raw material cost; furthermore the unused supplement is an impurity, which must be removed from the product stream in order to obtain high-purity lactic acid, and this will add to the purification cost [5]. It is therefore desirable to use as little supplement as possible, while still obtaining high lactose conversion and lactic acid yield within a reasonable fermentation time.

Yeast extract (YE) is commonly used in laboratory scale fermentations, but the cost of this raw material is high. There is consequently much research to find cheaper supplements, which could be used as alternatives to or in combination with YE. In this work the

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effect of malt combing nuts (MCN) is investigated. MCN are a waste material from the malting process of barley, consisting mainly of the shoots, roots and the husk of the cereal. Their commercial uses are limited, being mainly used in animal feeds, thus their cost is low. They provide a cheap source of nitrogen, amino acids and vitamins [6], and have potential for application in lactic acid fermentation to improve lactose conversion, lactic acid yield and fermentation time. MCN were added to the media firstly as the sole nutrient supplement, and then in conjunction with YE.

2. Materials and methods

2.1. Organism and inoculum

L. casei strain 01 used in this study was supplied by Chr. Hansen, Denmark. Stock cultures were maintained as direct vat set (DVS) frozen culture stored at −80 °C. Pellets were removed from the DVS storage carton using a sterile spatula, placed in a sterile McCarthy bottle and allowed to defrost. The defrosted culture of 0.1 ml were added to 9 ml of sterile MRS broth and incubated for 12 h at 38 °C. This broth of 1 ml was added to 100 ml of the fermentation medium and incubated for a further 12 h at 38 °C. This was subsequently used to inoculate the fermenter.

2.2. Fermentation media

The whey permeate powder (supplied by Kerry Foods plc., Listowel, Ireland) was dissolved in distilled water to a concentration of 6% w/v. All fermentations were supplemented with 0.03 g/l manganese sulphate ($MnSO_4 \cdot H_2O$), as *L. casei* has a requirement for manganese [7]. Manganese sulphate (AnalaR) was obtained from BDH Laboratory Supplies, Poole, England. YE was obtained from Oxoid Ltd, Basingstoke, Hampshire, England, and MCN from Malting Company of Ireland, Cork, Ireland.

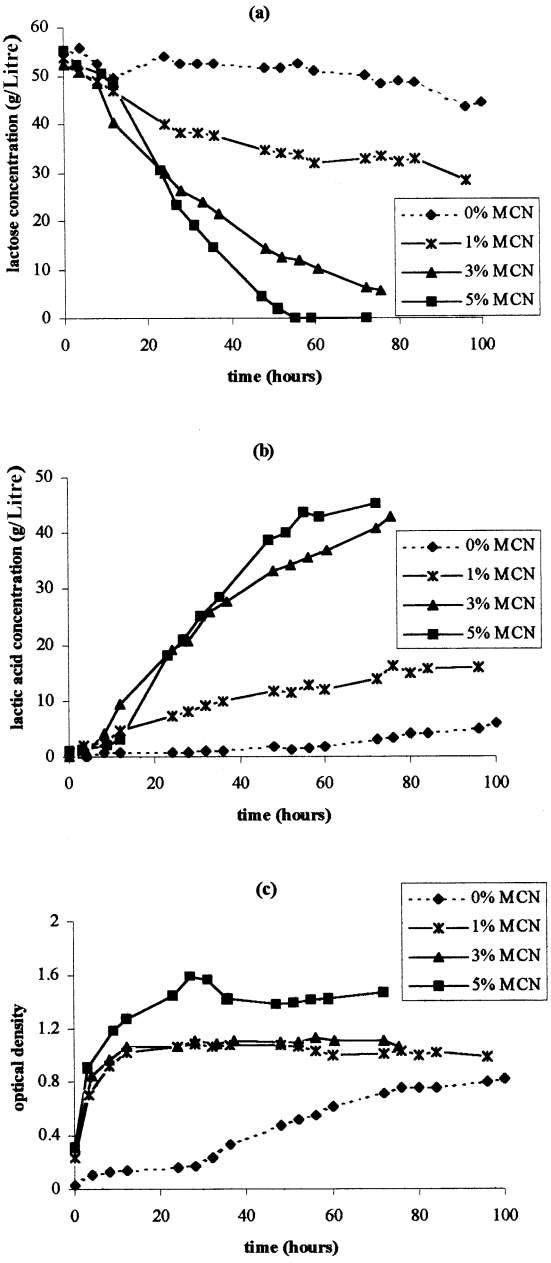

Fig. 1. Effect of malt combing nuts (% w/v MCN) addition on (a) lactose utilisation; (b) lactic acid production; and (c) cell concentration as measured by optical density at 620 nm (all fermentations were supplement with 0.03 g/l $MnSO_4 \cdot H_2O$).

Table 1
Effect of MCN addition on fermentation time and concentration of impurities

Malt combing nuts (% w/v)	Fermentation time (h)	Ash content[a] (% w/w)	Nitrogen at start (% w/w)	Nitrogen at end (% w/w)	Nitrogen used (% w/w)
0	> 144	0.28	0.020	0.016	0.004
1	> 96	0.30	0.036	0.033	0.003
3	> 76	0.39	0.062	0.058	0.004
5	55	0.49	0.092	0.077	0.015

All fermentations were supplemented with 0.03 g/l $MnSO_4 \cdot H_2O$.
[a] Ash content of the medium was measured at the start of fermentations.

2.3. Batch fermentation

Two fermenters with 2-l capacity were used. They consisted of a glass cylinder with rubber seals and stainless steal plates for the top and the bottom. The plates contained ports for heater, pH and thermometer probes, sampling port, a port for the base and a six-blade impeller. The culture medium was sterilized in the fermenter by autoclaving at 121 °C for 15 min at under a pressure of 15 psig. The pH probe (Schott Elektroden) was calibrated before each run, using standards of pH 4 and 7. The probe was connected to a peristaltic pump allowing pH regulation of the fermentation optimum of 5.4, by addition of 4 M NaOH. Temperature regulation was achieved using a platinum heating rod, and the temperature was thermostatically controlled at 38 °C (optimal for lactic acid production). Samples were taken throughout the fermentation for analysis.

2.4. Analysis

Lactose, glucose, galactose and sodium lactate concentrations were measured with a HPLC system consisting of two isocratic pumps (Spectra series P100®, USA), a differential refractometer (RefractoMonitor® IV, thermo separation products, USA) and a satellite unit/integrator. For separation, a roa-organic acid column (Rezex 00H-0138-KO, Phenomenex UK Ltd, England) was used. Total nitrogen was measured by the Kjeldahl method using the Kjeltic system (1026 Distilling unit, Kjeltec System, Tecator, Sweden). The ash content of the medium was measured at the beginning of the fermentation using a fan oven (Balay) and a muffle furnace (Gallenkamp, Hotspot furnace). The cell density was measured at 620 nm using a direct reading spectrometer (Hach DR/200).

3. Results and discussion

3.1. Addition of MCN to the fermentation

Fermentations were performed to investigate the influence of the addition of MCN on fermentation performance and concentration of impurities. MCN were added at concentrations of 1, 3 and 5% w/v. The influence of MCN addition on lactose utilization, lactic acid production and cell concentration is illustrated in Fig. 1. The progress of the fermentation is very slow in whey permeate without MCN addition, and increasing the amount of MCN results in increasing lactic acid yield and sugar conversion. A fermentation time of 55 h was achieved with the addition of 5% w/v MCN. The fermentation results demonstrate that MCN is a useful nutrient supplement with which complete lactose conversion and lactic acid yields of around 90% can be

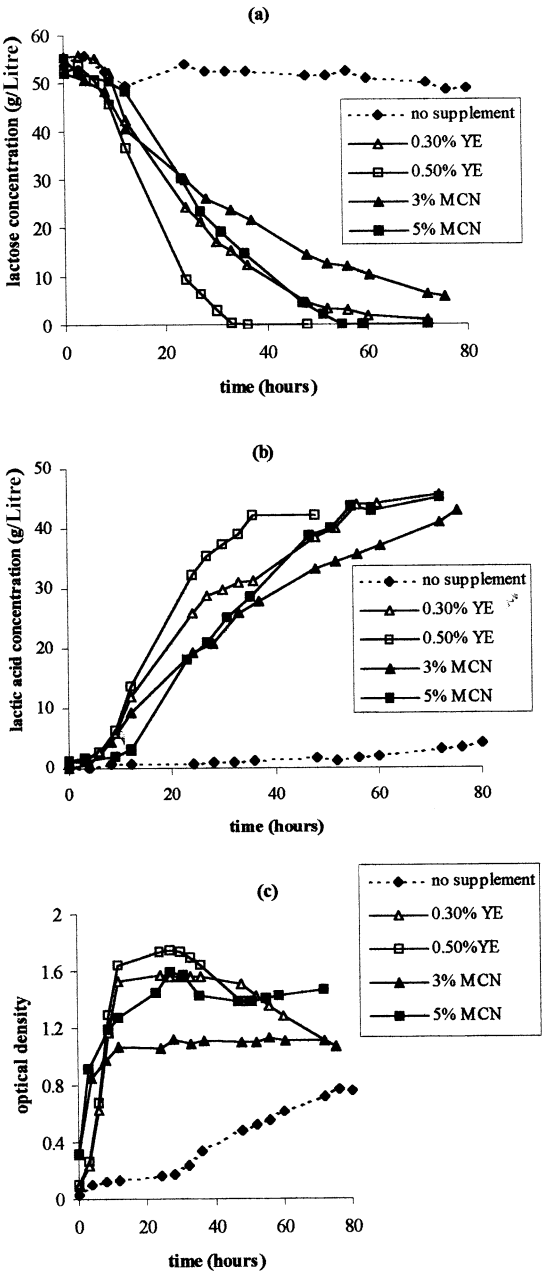

Fig. 2. Comparison of the effect of malt combing nuts (% w/v MCN) and yeast extract (% w/v YE) addition on (a) lactose utilisation; (b) lactic acid production; and (c) cell concentration as measured by optical density at 620 nm (all fermentations were supplement with 0.03 g/l $MnSO_4 \cdot H_2O$).

obtained (lactic acid yield is defined as mass of lactic acid produced divided by mass of sugars utilized expressed as a percentage).

A potential disadvantage of MCN supplementation is the concentration of impurities remaining after fermentation. Table 1 shows how these impurities increase with MCN addition. The amounts of ash and nitrogen increased significantly with MCN addition. Most of the nitrogen in the media remained unused at the end of

Table 2
Comparison of malt combing nuts (MCN) and yeast extract (YE) addition on fermentation time, impurity concentrations and cost of supplementation

Supplement addition (% w/v)	Fermentation time (h)	Ash content[a] (% w/w)	Nitrogen at start (% w/w)	Nitrogen at end (% w/w)	Supplementation cost (€/m^3)[b]
No supplement	> 144	0.28	0.020	0.016	0
0.3% YE	75	0.31	0.049	0.038	16.5
0.5% YE	34	0.33	0.066	0.049	27.5
3% MCN	> 76	0.39	0.062	0.058	2
5% MCN	55	0.49	0.092	0.077	3

All fermentations were supplemented with 0.03 g/l $MnSO_4 \cdot H_2O$.
[a] Ash content of the medium was measured at the start of fermentations.
[b] Supplementation cost is the cost (in euros) required to supplement one m^3 of whey permeate. The supplement raw material costs used in the calculations were—malt combing nuts: 63 €/1000 kg (personal communication (2001) with Malting Company of Ireland), yeast extract: 5.5 €/kg (personal communication (2001) with Quest International, UK).

fermentation resulting in increased nitrogen containing impurities. At 5% w/v MCN addition, the unused nitrogen and ash content represent a large additional source of impurities that must be removed at a cost to produce high purity lactic acid. In addition, 5% w/v MCN represents a significant mass of supplement that must be added to each fermentation, and due to the solids nature of MCN, most of this must separated and disposed of after fermentation.

3.2. Comparison of fermentations supplemented with MCN and YE

Fermentations were carried out with YE supplementation to compare the fermentation performance of MCN with YE. Supplementation with YE was at concentrations of 0.30 and 0.50% w/v. The comparison of MCN and YE addition on lactose utilization, lactic acid production and cell concentration is illustrated in Fig. 2. The 5% MCN performed similarly to the 0.30% YE, and these levels of supplementation are necessary to obtain complete lactose utilization and high lactic acid yield in a reasonable fermentation time.

The concentrations of impurities present with MCN and YE supplementation are presented in Table 2. At 5% MCN addition, there is a large amount of ash added to the medium when compared to 0.3% YE addition. As there is around 0.28% w/w ash in the unsupplemented permeate, 5% MCN addition contributes an extra 0.21% w/w ash in comparison to 0.03% w/w ash for 0.3% YE addition. Likewise, 5% MCN addition results in more nitrogen being added. This leads to double the amount of unused nitrogen remaining at the end of fermentation when compared to 0.3% YE addition. Overall, the unused nitrogen and ash content are much greater for MCN than YE addition, and this represents a serious disadvantage for the application of MCN as a nutrient supplement for the production of high purity lactic acid.

The major advantage of MCN is low raw material cost, and this is presented also in Table 2. The MCN raw material cost at 5% w/v supplementation is less than one-fifth the cost of supplementation with 0.3% YE.

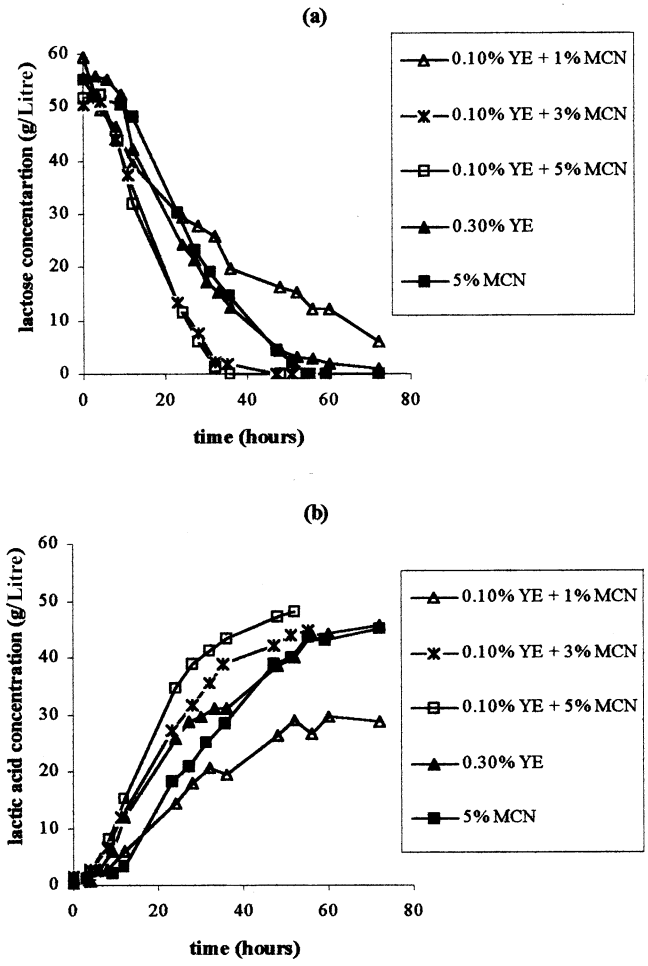

Fig. 3. Effect of combinations of malt combing nuts (% w/v MCN) and yeast extract (% w/v YE) addition on (a) lactose utilisation; and (b) lactic acid production (all fermentations were supplement with 0.03 g/l $MnSO_4 \cdot H_2O$).

Table 3
Effect of combinations of malt combing nuts (MCN) and yeast extract (YE) addition on fermentation time, impurity concentrations and cost of supplementation

Supplement addition (% w/v)	Fermentation time (h)	Ash content[a] (% w/w)	Nitrogen at start (% w/w)	Nitrogen at end (% w/w)	Supplementation cost (€/m^3)[a]
0.1% YE+1% MCN	>72	0.33	0.042	0.035	6.5
0.1% YE+3% MCN	40	0.42	0.076	0.065	7.5
0.1% YE+5% MCN	34	0.50	0.106	0.094	8.5
0.3% YE	75	0.31	0.020	0.016	16.5
0.5% YE	34	0.33	0.066	0.049	27.5
5% MCN	55	0.49	0.092	0.077	3

All fermentations were supplemented with 0.03 g/l $MnSO_4 \cdot H_2O$.
[a] See Table 2.

Nutrient supplement raw material cost has a major influence on fermentation cost [8], thus the fermentation cost with 5% MCN supplementation will be significantly lower than 0.3% YE. However, the higher concentrations of impurities produced with MCN supplementation will lead to higher separation costs for producing high purity lactic acid, and an overall process economic analysis needs to be performed to evaluate if MCN supplementation is more favourable to YE.

3.3. Combinations of MCN and YE

Further fermentations were carried out to investigate if it was more beneficial to use a combination of MCN and YE instead of the supplements on their own. Fermentations were performed with combinations of 0.1% YE and 1, 3, and 5% MCN, and the results are presented in Fig. 3 and Table 3. For the combination of 0.1% YE and 1% MCN, the progress of the fermentation was very slow and was not near completion after 75 h. The lactic acid yield was also lower than desired. The addition of 0.1% YE to the 5% MCN fermentation reduced the fermentation time by over 20 h, however it nearly triples the raw material cost and increases the unused nitrogen concentration.

The combination of 0.1% YE and 3% MCN appears more promising. The fermentation was faster than either the 0.3% YE or 5% MCN fermentations. The concentrations of ash and unused nitrogen were less than the 5% MCN fermentation, however the supplementation cost is nearly three-times greater. On the other hand, the supplementation cost is less than half that for the 0.3% YE fermentation, however the impurity concentrations were significantly higher. Once again, there is an economic trade-off between raw material cost and purification cost, therefore it is not strikingly obvious whether or not it is beneficial to use a combination of MCN and YE.

4. Conclusions

MCN can be applied successfully as a nutrient supplement to produce lactic acid by fermentation, achieving complete sugar conversion and lactic acid yields similar to YE supplementation. 5% w/v MCN addition is required to achieve a fermentation time of around 55 h in whey permeate containing lactose at a concentration of 55 g/l. This is similar to fermentations with 0.3% w/v YE supplementation, however it is significantly slower than fermentations with 0.5% YE which took about 34 h.

The main advantage of MCN is its low raw material cost, resulting in a supplementation cost of roughly one-fifth that of a similar fermentation supplemented with YE. On the other hand, MCN has disadvantages, the main one being the quantities of impurities remaining at the end of fermentation. MCN addition contributes a large amount of ash, which is undesirable. In addition, the amount of unused nitrogen is nearly double that of a comparable YE fermentation. Another disadvantage is the sheer mass of material that must be added to each fermentation, due to the solids nature of MCN, and subsequently, these solids needs to be separated and disposed of. Overall, the feasibility of using MCN as a nutrient supplement for producing high purity lactic acid depends on the economic trade-off between its low raw material cost and higher separation costs.

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