LWT - Food Science and Technology 71 (2016) 125–129

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Production and stability of nisin in whey protein concentrate

Suja Senan [a, *], Hussein Abd El-aal [b], Rajiv Dave [c], Ashraf Hassan [d]

[a] Department of Dairy Science, South Dakota State University, SD 57006, USA
[b] Department of Food Science, Minia University, Minia, Egypt
[c] Nestle, Bakersfield, CA 93313, USA
[d] Daisy Brand, Garland, TX 75041, USA

ARTICLE INFO

Article history:
Received 13 August 2015
Received in revised form
10 February 2016
Accepted 16 March 2016
Available online 19 March 2016

Keywords:
Nisin
Whey protein concentrate
Stability
Activity

ABSTRACT

The objective of this research was to manufacture nisin-containing whey protein concentrate (WPC) and study its stability during storage. Pasteurized reconstituted (6% w/v) sweet whey powder was used before or after ultrafiltration (UF) as a growth medium for nisin-producing strains of *Lactococcus lactis* subsp. *lactis* (ATCC 7962 or BS10). The fermented medium was freeze-dried, stored in polyethylene bags protected from light at 4 °C or 30 °C for 3 months. There was no difference in nisin yields by both strains between the regular and UF whey. Yeast extract supplementation resulted in an increase in nisin activity in UF whey fermented with BS10 and ATCC 7962 from 183 to 167 IU/ml to 1617 and 1667 IU/ml respectively. During storage at 4 °C for 1 month, the freeze dried WPC powder lost 55–60% of the nisin activity. At 30 °C for the same period, a 65–70% reduction in nisin activity was observed. Samples reconstituted at pH 2 and then boiled for 5 min increased nisin activity ($P < 0.05$). The present study presents WPC as an attractive, clean label ingredient with antimicrobial activities.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Whey, a by-product of cheese manufacture poses the imminent challenge of an eco-friendly means of disposal. Whey proteins (WP) isolated from whey are excellent functional supplements enriched with essential amino acids and immunity boosting factors. They can be taken up by the muscles to repair and rebuild tissues that make them an effective ingredient in sports nutrition supplements. To further exploit the functionality of WP, whey is treated by various techniques such as diafiltration, ultrafiltration, electrodialysis and ion-exchange technologies to manufacture whey protein concentrate (WPC). The presence of thermophilic and mesophilic spores in dairy powders including WPC is a major concern for the dairy industry (Watterson, Kent, Boor, Wiedmann, & Martin, 2014). A recent study showed that sweet whey and nonfat dry milk contained higher counts of thermophilic and mesophilic spore formers than did acid whey and whey protein concentrate (Watterson et al., 2014). In an era of clean labeling, where consumers are seeking simple products with fewer, familiar and healthy ingredients, the use of a preservative or antimicrobial in food may not be a consumer friendly approach. Furthermore, preservatives are prohibited in some countries (Josefiak et al., 2013). Therefore, additives that possess antibacterial properties could become a desirable alternative for food manufacturers. Nisin is an anti-microbial peptide produced by *Lactococcus lactis* and consists of 34 amino acid residues. It is minimally toxic, odorless, colorless, and tasteless (Severina, Severin, & Tomasz, 1998) and affects the post-germination stages of bacterial spores by inhibiting pre-emergent swelling, out-growth, and formation of vegetative cells (Montville & Chen, 1998). It has been used as a preserver in pasteurized process cheese, liquid whole eggs, and salad dressing due to its inhibitory action on Gram-positive food-borne pathogens and spore-forming bacteria (McMullen and Stiles 1993). Factors such as bacterial strains, growth medium, temperature, aeration, pH, total sugar, nitrogen, phosphorous and buffer concentrations affect nisin production (Aasen, Moretro, Katla, Axelsson, & Storro, 2000; Guerra & Pastrana, 2001; Parente & Ricciardi, 1999). Although applications of nisin in foods have been extensively studied (Delves-Broughton, Blackburn, Evans, & Hugenholtz, 1996), limited information is available on the effect of drying and storage on its activity. Hence the present study focused on two main objectives (1) to investigate the suitability of ultrafiltered (UF) whey as a medium for nisin production as compared to regular whey and 2) to study

* Corresponding author.
E-mail addresses: suja.senan@sdstate.edu (S. Senan), huss_galil@yahoo.com (H.A. El-aal), Rajiv.Dave@rd.nestle.com (R. Dave), Ahassan@DaisyBrand.com (A. Hassan).

http://dx.doi.org/10.1016/j.lwt.2016.03.031
0023-6438/© 2016 Elsevier Ltd. All rights reserved.

stability of nisin during storage of WPC.

2. Material and methods

2.1. Bacterial strains

The two nisin producing strains used in this study were *L. lactis* subsp. *lactis* ATCC 7962 (American Type Culture Collection, Rockville, MD) and *L. lactis* subsp. *lactis* BS10 (Chr. Hansen Inc, Milwaukee, WI). Both cultures were propagated separately on M17 medium (Yu, Dunn, & Kim, 2002) at 30 °C. *Micrococcus luteus* ATCC 8166 was used as the sensitive indicator strain for the nisin bioactivity assay. *M. luteus* was grown on SI medium (Lv, Zhang, & Cong, 2005) containing 0.8% tryptone, 0.5% glucose, 0.5% yeast extract, 0.5% NaCl, 0.2% Na_2HPO_4, and 1.6% agar.

2.2. Fermentation medium

Extra grade pasteurized sweet whey powder (Associated Milk Producers, Inc. New Ulm, Minnesota, USA) was rehydrated in distilled water to a final concentration of 6% (w/v). Whey was then UF to 5X volumetric concentration at 55 °C using a spiral wound membrane (Koch membrane systems Inc, Wilmington, MA). The concentrated whey was kept in the freezer until used. Yeast extract was added to the regular whey or the UF whey at 1% (w/v). The fermentation was carried out in a 4L fermentor (Bioflo 111, New Brunswick, Scientific Co., Inc. Edison, N.J., USA). The fermentor containing the whey medium was autoclaved at 121 °C for 20 min. The medium was inoculated at 1% (v/v) with an eight-hour old culture of *L. lactis* subsp. *lactis* ATCC 7962 or BS10 grown in M17 broth. During the first eight hours of fermentation at 30 °C with agitation at 100 rpm/min, the pH was maintained at 6.5 by automatic addition of 5 M NH_3OH (Yu et al., 2002). The pH was then allowed to drop freely via auto acidification. The combination of constant pH and auto acidification was reported to increase the nisin yield and decrease adsorption of nisin onto the bacterial cells (Pongtharangkul and Demirci, 2006). Fermented whey was freeze-dried using Labconco Freezone 12 (Marshall Scientific, Brentwood NH 03833). Sample was dispensed into freeze dryer flasks, frozen using liquid nitrogen and then loaded into the freeze dryer. Dried samples were packaged in polyethylene bags protected from light and stored at 4 °C or 30 °C for three months.

2.3. Nisin standards

A stock nisin solution (1000 IU ml^{-1}) prepared by dissolving 0.025 g of 10^6 IU g^{-1} commercial nisin (Sigma Chemical Co. St. Louis, MO) into 25 ml of sterile solution of 0.02 N HCl which was then heated in boiling water for 5 min and cooled rapidly to 20 °C. Concentrations ranging from 750 to 10 IU ml^{-1} (500, 250, 100, 50, 25, and 10 IU ml^{-1}) were prepared using the nisin stock solution and the activity was plotted against concentration to construct the standard curve.

2.4. Nisin activity determination

Nisin activity was determined in whey and WPC after rehydration to its original concentration (before drying). The pH of the samples was adjusted to 2 with 5 M HCl. Samples were then heated in a boiling water bath for 5 min, cooled to 20 °C, centrifuged at 670 × g for 15 min, and filtered through a 0.2 μm filter. Nisin assay in the supernatant was performed by the agar well diffusion method as described by Fowler, Jarvis, and Tramer (1975) and Pongtharangkul and Demirci (2006).

2.5. Lactose and lactic acid determination

Lactose and lactic acid were determined using a Waters HPLC System with an Aminex®HPX-87H column according to the method described by Va't Hul (1995). The column was operated at 65 °C with a 4 μM H_2SO_4 helium-degassed mobile phase at a flow rate of 0.6 ml/min. Peaks were detected using a refractive index detector and chromatographs were quantified using Waters Millennium integrating software (Milford, MA, USA). Standard aqueous solutions of 3 g/L were used to calibrate the integrator. Standard solutions consisted of lactose, glucose, succinic acid, lactic acid, glycerol, acetic acid, propionic acid and butyric acid.

2.6. Protein and moisture determination

Protein (Kjeldahl total nitrogen x 6.38) and total solids were determined according to AOAC (2005). The moisture content of WPC was determined using the Karl Fischer method (Hooi et al. 2004).

2.7. Statistical analysis

The SAS statistical analysis software package was used for ANOVA using the GLM procedure. Differences were considered significant at $P < 0.05$. All experiments were repeated three times.

3. Results

The rehydrated whey contained 60 g/L total solids, 7.10 g/L protein and 45.5 g/L lactose (Table 1). The ultrafiltration process increased the protein level and the protein: lactose ratio by about 3.7 times. Nutrient supplementation with yeast extract was found to have a significant effect on nisin production and yield. There was an approximately tenfold increase in nisin production by both ATCC 7962 and BS10 in UF whey as a result of yeast extract supplementation (Table 2). There were no significant differences ($P > 0.05$) in the level of nisin produced by the two commercial strains in UF whey (Table 2). The concentration of whey proteins did not affect nisin production by ATCC 7962 (Table 2). Boiling of cells prior to assay significantly ($P < 0.05$) increased nisin activity (Table 2). Higher lactose and lower lactic acid levels were found in the medium fermented by ATCC 7962 than in that fermented by BS10 (Table 3) although the amount of nisin did not differ (Table 2). Lactose consumption and lactic acid production were higher in the UF whey than in regular whey. Yeast extract supplementation increased ($P < 0.05$) the rate and quantity of acid production from lactose.

Stability of nisin during storage of WPC is shown in Tables 4 and 5. Same trend and almost identical data were observed in both cultures tested in this study. During storage at 4 °C for 1 month, the powder lost almost 55–60% of the nisin activity. Storage for the same period at 30 °C resulted in 65–70% reduction in activity. Boiling of whey adjusted to pH 2 prior to nisin assay significantly increased activity. In this case, powder lost only about 25% of the original activity after storage for one month at 4 °C. However, the

Table 1
Proximate composition (g/L) of regular whey and ultra-filtered whey.

Fermentation medium[1]	Protein	Total solid	Lactose
Whey 6%	$7.10^{b} \pm 0.01$	$60.00^{b} \pm 0.02$	$45.50^{a} \pm 0.06$
Ultra filtrated whey	$26.40^{a} \pm 0.24$	$78.90^{a} \pm 0.98$	$46.28^{a} \pm 0.42$

[a-b]Mean values followed by different letters in the same column are significantly different ($P \leq 0.05$).
[1]The fermentation medium contained 1% yeast extract.

Download English Version:

https://daneshyari.com/en/article/4563519

Download Persian Version:

https://daneshyari.com/article/4563519

Daneshyari.com